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57th CROATIAN AND 17th INTERNATIONAL SYMPOSIUM ON AGRICULTURE

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00

Plenarna izlaganja
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01

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Agroecology, Ecological Agriculture and Environmental Protection

02

Agroekonomika i ruralni razvoj
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05

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06

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Fisheries, Game Management and Beekeeping

07
Stočarstvo
Animal Husbandry

08

Voćarstvo, vinogradarstvo i vinarstvo
Viticulture, Enology and Pomology

09

Poljoprivredna tehnika
Agricultural technics

10

Klima i poljoprivreda
Climate and Agriculture

Croatian 57 International Symposium on Agriculture 2022

Plenarna izlaganja

00

Plenary lectures

Suživot vinove loze i virusa uvijenosti lista vinove loze 3 u Hrvatskoj

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Sažetak
Bolest uvijenost lista vinove loze (GLD) jedna je od najznačajnijih virusnih bolesti vinove loze, a virus uvijenosti lista vinove loze 3 (GLRaV-3) jedan je od glavnih uzročnika te bolesti. Dosadašnja istraživanja vezana za GLRaV-3 u Hrvatskoj i svijetu uglavnom su bila usmjerena na proučavanje epidemiologije bolesti i razvoja pouzdanih metoda detekcije, dok su uloga različitih genomskih varijanti GLRaV-3 u etiologiji GLD i odgovor biljke na prisutnost ovog virusa u velikoj mjeri nepoznati. Jedan od glavnih ciljeva istraživačkog projekta GRAPE-PATH3 (u tijeku) podržanog od strane Hrvatske zaklade za znanost, je primjenom molekulranih metoda detekcije istražiti distribuciju ekonomski štetnih virusa vinove loze u autohtonim sortama jadranske Hrvatske, s posebnim osvrtom na GLRaV-3 i njegove genomske varijante. U cilju što boljeg razumijevanja promjena koje ovaj patogeni virus uzrokuje u osjetljivim domaćinskim biljkama, različite kombinacije genomskih varijanti GLRaV-3 u samostalnoj zarazi i koinfekciji s drugim ekonomski značajnim virusima, su biološki okarakterizirani na setu indikatorskih biljaka. Morfološkim, fiziološkim i biokemijskim metodama istražen je odgovor na zarazu pri čemu su mjerene promjene primarnog metabolizma, nutrienata i oksidativnog stresa.
Potvrđena je dominantna pojavnost GLRaV-3 u odnosu na ostale detektirane viruse i prisutnost genomskih varijatni I i II. Preliminarna analiza rane infekcije ukazuje na značajne razlike između zdravih biljaka, onih zaraženih GLRaV-3 u samostalnoj zarazi i u koinfekciji s drugim virusima. Odgovor loze varira ovisno o sorti i virusnom inokulumu. Ovi rezultati će omogućiti bolje razumijevanje patogenosti GLRaV-3 te posljedica koje ovaj virus ima na vinovu lozu u Hrvatskoj i šire, osim okom vidljivih simptoma uvijenosti lista, po kojemu je virus i dobio ime.

Ključne riječi: virusi uvijenosti lista, genotipovi GLRaV-3, koinfekcije, patogenost

Pojavnost virusa vinove loze i njihov značaj u vinogradarskoj proizvodnji

Vinova loza (*Vitis vinifera* L.) je jedna od najznačajnijih poljoprivrednih kultura u svijetu. Tijekom svog postojanja i kultivacije, koegzistirala je s brojnim patogenima te se smatra virozama najpodložnijom višegodišnjom kulturom s oko više od 100 identificiranih virusnih vrsta. Virusi su obligatni unutarstanični patogeni koji parazitiraju na vinovoj lozi, a nalazimo ih u vinogradima diljem svijeta gdje neki od njih izazivaju velike ekonomske štete i gubitke. Njihov prijenos unutar vinograda je uvjetovan prisustvom različitih vektora svojstvenih pojedinom virusu (nematode, štitaste uši, grinje…), dok je na veće udaljenosti prijenos primarno uvjetovan korištenjem zaraženog sadnog materijala. Virusi utječu na fenotip i fiziologiju vinove loze rezultirajući smanjenim prinosom i kvalitetom grožđa, neprikladnim sadržajem šećera i ukupnih kiselina, utječu na otpornost prema biotskom i abiotskom stresu

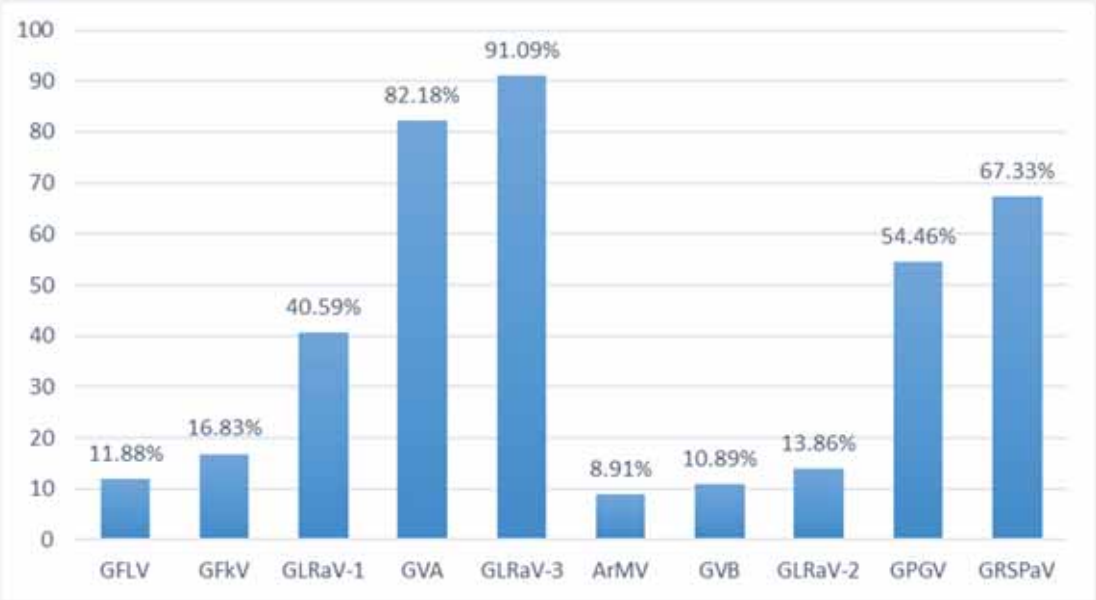
te u konačnici skraćuju životni vijek vinograda (Martelli, 1993.; Naidu i sur., 2015., Alabi i sur., 2016.). Različite studije se bave istraživanjima procjene ekonomskih gubitka uslijed zaraza virusima, pa je tako u SAD-u procijenjeno da je uslijed zaraze virusima uvijenosti lista u vinogradu starom 25 godina moguća kumulativna šteta od 40000 $/ha uz 50 % smanjenja prinosa te 10 % smanjene kvalitete. Europske studije također izvještavaju o gubitcima uslijed zaraze ovom skupinom virusa i virusa iz skupine infektivne degeneracije koji mogu uzrokovati smanjen prinos do 93 %, kod podloga smanjenu produkciju drveta do 64 %, slabije ukorjenjivanje do 60 % te smanjenu uspješnost cijepljenja na svega 10 do 26 % (Meng i sur., 2017.). Unatoč svim dosada poduzetim istraživanjima postoje brojne nejasnoće i nedosljednosti vezane za sam efekt virusa na vinovu lozu, pogotovo u vidu bioloških promjena i molekularnih mehanizama koji prate prisustvo virusa u domaćinskoj biljci.

Iako se neprekidno poduzimaju mjere za poboljšanje sanitarnog statusa vinove loze u zemljama uzgoja, postoji potreba za daljnjim kontroliranjem virusnog statusa radi pojavljivanja novih virusa te kako bi se proizvodnja bezvirusnih biljaka održala kao važna mjera nadzora širenja virusnih bolesti loze. Direktiva Europske komisije 2005/43/EC za sadni materijal vinove loze obuhvaća obaveznu kontrolu kompleksa infektivne degeneracije: virusa lepezastog lista vinove loze (GFLV), virusa mozaika gušarke (ArMV); uvijenosti lista vinove loze: virus uvijenosti lista vinove loze 1 (GLRaV-1), virus uvijenosti lista vinove loze 3 (GLRaV-3) te virusa pjegavosti vinove loze (GFkV) samo za podloge. Ukoliko se ovi virusi detektiraju u biljkama, one moraju biti isključene iz daljnje reprodukcije u matičnim nasadima.

Prvo izvješće o prisutnosti virusa na vinovoj lozi u Hrvatskoj dolazi od Šarić i Corte (1959.) koji su detektirali virus iz kompleksa infektivne degeneracije, a kasnijih godina se u više navrata izvještavalo o prisutnosti viroza na vinovoj lozi. Danas je potvrđeno da su virusi široko rasprostranjeni u komercijalnim vinogradima u Hrvatskoj (Karoglan Kontić i sur., 2009.; Poljuha i sur., 2010.; Vončina i sur., 2009., 2017. i 2019.) te kolekcijskim nasadima (Vončina i sur, 2011. i 2017.) pa se smatraju ekonomski važnim virusima u zemlji. Razvojem osjetljivijih metoda detekcije, sve veći broj virusa biva detektiran u sklopu ovakvih monitoringa, ostavljajući male šanse za pronalaženjem bezvirusnih biljaka vinove loze. U svim dosadašnjim istraživanjima u zemlji na ovu temu, GLRaV-3 virus je bio dominantno prisutan u odnosu na ostale viruse.

U okviru GRAPE-PATH3 projekta utvrdili smo vrlo slično stanje kao i prethodni autori, analizirajući 16 autohtonih sorti vinove loze iz kolekcijskog vinograda Instituta u Splitu (Hančević i sur., 2021.). Detaljnom obradom klonova sorte Plavac mali možemo zaključiti kako se takvo stanje u kojem nedvojbeno prevladava zaraza GLRaV-3, odražava i na našu najznačajniju crnu vinsku sortu Plavac mali (Čarija i sur., 2022.). Rezultati istraživanja rasprostranjenosti i učestalosti 10 ekonomski najvažnijih virusa upućuju na izrazito loše sanitarno stanje istraživanih sorti u kolekcijskom nasadu, dominaciju GLRaV-3 i A virusa vinove loze (GVA; Slika 1) potpuno odsustvo bezvirusnih jedinki u kolekciji te dominantnu istovremenu prisutnost više virusa po biljci. Najviše trsova imalo je barem 4 virusa (28,71 %). Nešto manje bilo ih je s 5 (25,74 %) i 3 virusa (16,83 %), dok je najmanja zastupljenost bila onih trsova sa 7 virusa (0,99 %).

Trenutno bez "lijeka" za zaražene biljke, svaki aktivni pristup za smanjenje utjecaja virusnih bolesti i njihovog širenja je od velike koristi. Prepoznavanje simptoma virusne zaraze, pouzdana laboratorijska detekcija virusa, te eliminacija zaraženih biljaka u daljnjoj reprodukciji predstavlja temelj za kontroliranje virusnih bolesti loze.



GFLV: virus lepezastog lista vinove loze; GFkV: virus pjegavosti vinove loze; GLRaV-1:virus uvijenosti lista vinove loze 1; GVA: A virus vinove loze; GLRaV-3: virus uvijenosti lista vinove loze 3, ArMV: virus mozaika gušarke; GVB: B virus vinove loze; GLRaV-2: virus uvijenosti lista vinove loze 2; GPGV: virus Pinota sivog; GRSPaV: virus naboranosti drva podloge Rupestris du Lot

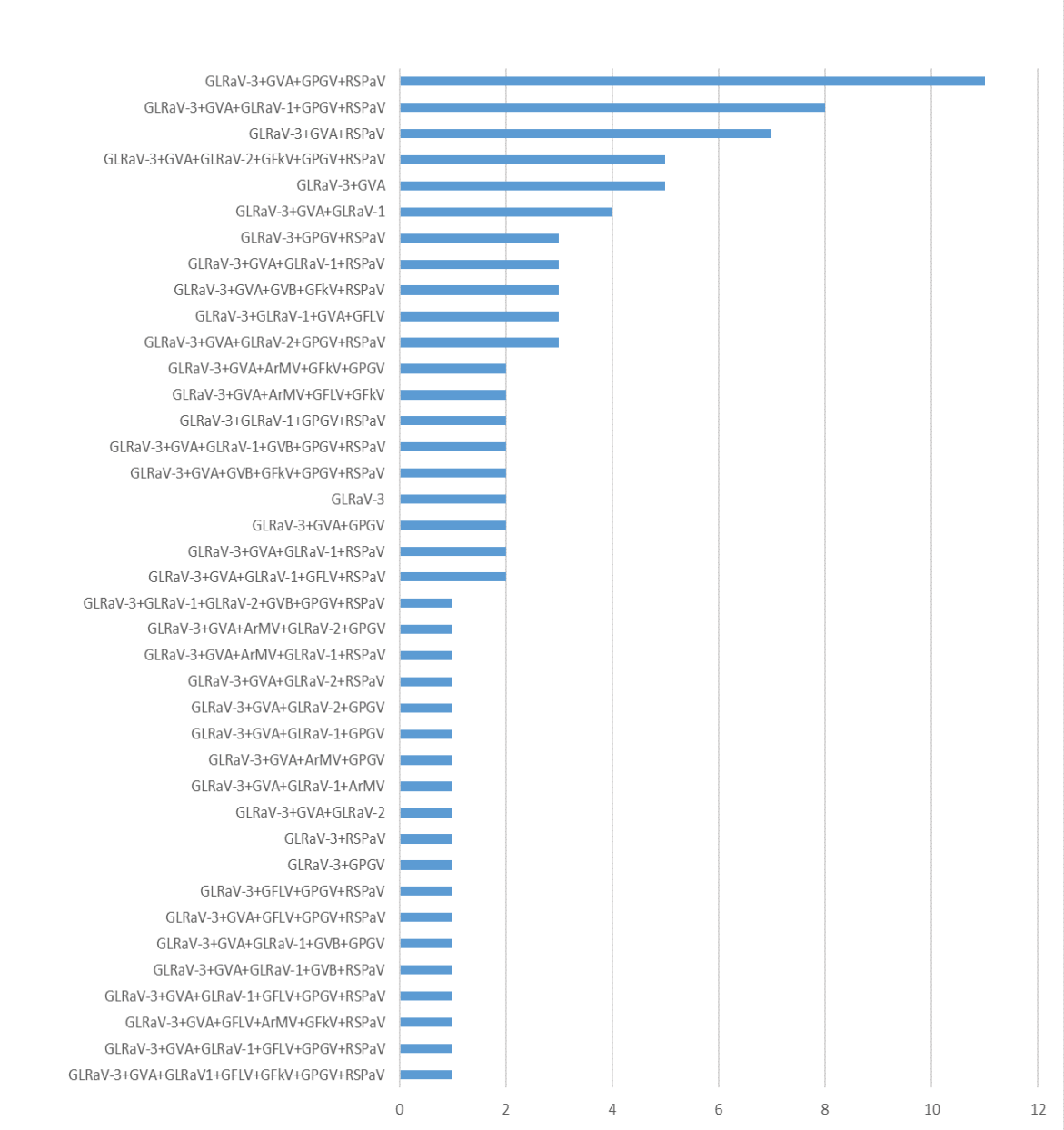
Slika 1.Postotak zastupljenosti virusa u kolekcijskom nasadu Instituta

Virusi uvijenosti lista vinove loze s naglaskom na GLRaV-3 i kako prepoznati simptome

Prošlost otkrivanja virusa i njegova prisutnost u vinovoj lozi sastojala se od opisivanja simptoma virusne infekcije na vinovoj lozi kao prvih zapisa opažanja bolesti. Najraniji zapisi o simptomatologiji bolesti zapisani su u talijanskoj i francuskoj literaturi (Maree i sur. 2013.) a datiraju iz početka 20. stoljeća. Neposredno prije početka 20. stoljeća, prikupljeni i očuvani herbarijski primjerci vinove loze sa Sicilije, poslužili su kao uvid u virološku situaciju 1880-ih godina. Martelli i Piro (1975.) su opisali karakteristike herbarijskog materijala koji je ukazivao na prisutnost simptoma povezanih s bolešću vinove loze. Istraživanja u prošlosti su bila usmjerena prema otkrivanju uzroka same bolesti što je nesumnjivo potvrđeno otkrivenjem virusa u vinovoj lozi elektroskopskom mikroskopijom kada su u tankom presjeku floema pronađeni virioni nalik closterovirusima (Namba i sur. 1979.). Od tada su istraživanja većinom fokusirana na karakteristike samog virusa što je dovelo do otkrivanja različitih virusnih izolata i sve obuhvatnije spoznaje o genetičkoj raznolikosti virusa.

Nekoliko virusa iz familije *Closteroviridae* se povezuje s bolešću uvijenosti lista vinove loze (GLD), ali GLRaV-3 (rod *Ampelovirus*) je najrašireniji zahvaljujući visokoj stopi replikacije (Velasco i sur., 2014.) i glavni je uzročnik bolesti uvijenosti lista (Maree i sur., 2013.). Danas je to najvažnija bolest vinove loze, a usporediva je s nekim gljivičnim oboljenjima (Armijo i sur. 2016.). Karakteristični simptomi zaraze GLRaV- 3 virusom pa tako i ostalim iz skupine virusa uvijenosti lista javljaju se u ljeto te se najbolje očituju kod crnih sorti pojavom crvenila u međužilnom prostoru lista, dok usko područje oko glavnih žila ostaje zelene boje. Odmakom vegetacije crvenilo postaje sve izraženije te dolazi do postupnog uvijanja lista prema naličju. Kod bijelih sorti simptomi su diskretniji, naizgled nepostojani u početku, ali s odmakom vegetacije dolazi do uvijanja listova prema naličju kao i kod crnih sorti, ali uz pojavu žućenja. S vremenom zaražena biljka fiziološki slabi, zaostaje u razvoju te je smanjenog prinosa i kvalitete grožđa (Martelli, 2009.) što ovaj virus čini značajnom prijetnjom za vinogradarsku proizvodnju. Otežavajuća okolnost u prepoznavanju ovog

patogena može biti ukoliko je loza istovremeno zaražena i drugim virusima ili drugim patogenima, kada se komplicira slika simptoma. U prirodi su česte koinfekcije s više različitih virusa što smo potvrdili i istraživanjem na GRAPE-PATH3 projektu. Najčešće koinfekcije GLRaV-3 s drugim virusima koje smo detektirali istražujući loze iz kolekcijskog nasada vidljive su iz slike 2. U nekim slučajevima simptomi nedostatka pojedinog hranidbenog elementa kod vinove loze mogu nalikovati simptomima zaraze virusima uvijenosti lista, dok u drugim, zaražene biljke mogu biti asimptomatske.



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Slika 2. Broj trsova vinove loze zaraženih virusom uvijenosti lista vinove loze 3, samostalno i u koinfekciji s drugim virusima u kolekcijskom nasadu

Molekularna raznolikost GLRaV-3

Koinfekcije različitim virusima kao i različiti virusni genotipovi tj. varijante unutar izolata, neovisno o njihovoj populacijskog zastupljenosti, mogu imati važan utjecaj na razvoj simptoma bolesti što je utvrđeno kod nekih predstavnika porodice *Closteroviridae* (Černi *i* sur., 2008.). Dosada su u bazi *GenBank* objavljeni nukleotidni sljedovi čitavog genoma za 41 izolat virusa GLRaV-3, a koji su na temelju filogenetskih analiza svrstani u osam osnovnih filogenetskih skupina (I, II, III, V, VI, VII, IX, X; Diaz-Lara i sur., 2018.). Populacije RNA virusa pa tako i GLRaV-3 su kompleksi višestrukih genetskih varijanti koji proizlaze iz procesa replikacije koji je sklon pogreškama zbog odsutnosti mehanizma korekcije. Kao posljedica nastaju nove varijante/mutante koje mogu biti nepovoljne za sami virus, ali i rezultirati povećanjem patogenosti, što može imati značajne ekonomske posljedice. U okviru GRAPE-PATH3, kako bi razjasnili efekt pojedinačnih genomskih varijanti GLRaV-3 virusa na domaćinske biljke, ali i učinak najčešćih GLRaV-3 koinfekcija, ispitana je populacijska struktura GLRaV-3 virusa u kolekcijskom nasadu Instituta u Splitu. Genomske varijante homologa proteina toplinskog šoka 70 (HSP70h) identificirane su jednolančanim konformacijskim polimorfizmom (SSCP) i sekvencirane. Analiza sekvenci potvrdila je njihovo grupiranje u filogenetske skupine I i II, pojedinačno ili u kombinaciji (Čarija i sur., 2022.; Hančević i sur., 2021.) s većom zastupljenosti grupe II. Iako je kod drugih virusa iz porodice *Closteroviridae* dokazana povezanost između zaraze specifičnom varijantom virusa i odgovora domaćina (Černi i sur., 2008.; Hančević i sur., 2013.), kod GLRaV-3 do sada nisu rađena sustavna istraživanja o djelovanju genomskih varijanti GLRaV-3 na domaćinsku biljku (Meng i sur., 2017., Maree i sur., 2013.).

Razumijevanje odgovora biljke na virusnu infekciju GLRaV-3 i njegovih koinfekcija

Mehanizmi uključeni u promjene koje ovaj virus uzrokuje u biljkama s obzirom na morfologiju, fiziologiju i biokemiju nisu dobro poznati, isto kao što značaj i utjecaj genomske raznolikosti GLRaV-3 na različite domaćinske biljke s biološke perspektive nije dosada bio opisan. Asimptomatične ili s vidljivim simptomima, virusne infekcije djeluju na brojne metaboličke i fiziološke procese u domaćinskim biljkama, koji za posljedicu mogu ometati diferencijaciju i razvoj biljaka. Kako bi se opisalo *in vitro* ponašanje zaraženih biljaka Plavca malog koji imaju različit virusni sastav, proliferacija rasta *in vitro* uspoređena je među biljkama s obzirom na različit sastav virusa. Slično kao i kod eliminacije virusa, rast biljaka *in vitro* ovisio je o kompoziciji virusa u biljci (Hančević i sur., 2015.). Dosadašnja istraživanja na ovu temu su pokazala da su neki od učinaka GLRaV-3 uglavnom povezani s poremećajem u fotosintetskoj aktivnosti, sadržajem klorofila i karotenoida, te provodljivosti puči. U fiziologiji i biokemiji zaraženih biljaka vinove loze, nailazimo i na promjene u antioksidacijskim odgovorima biljke, u koncentraciji stresnih enzima (Sgherri i sur., 2013.) i fitohormona (Cui i sur., 2016.). Geni kod kojih dolazi do promijenjene aktivnosti uslijed virusne infekcije GLRaV-3 su povezani s transportom šećera, biosintezom antocijana, hormonima te procesom senescencije pa stoga i ne čude fenotipske i biokemijske promjene povezane upravo sa spomenutim genima. Vrlo često oprečne rezultate u literaturi pripisujemo činjenici da odgovor loze u ovakvim aspektima istraživanja može varirati, ovisno o virusnom izolatu, sorti, podlozi, sezoni vegetacije i okolišnim faktorima (Naidu i sur., 2014.).

Kako bi istražili točne efekte pojedinih genomskih varijanti GLRaV-3 i njihovih kombinacija te efekte GLRaV-3 koinfekcija na zdravlje vinove loze, naš tim je postavio pokus s ukupno 280 biljaka uključujući i bezvirusne kontrolne tretmane za svaku sortu u konroliranim uvjetima staklenika. Tri bezvirusne indikatorske vrste: Cabernet franc, Merlot i Pinot noir koje smo uvezli kao certificirani sadni materijal smo ukorijenili i cijepili na spavajući pup s različitim genomskim varijantama virusa GLRaV-3, samostalno i u

koinfekciji s drugim najčešćim virusima prisutnim u kolekciji. Drvenaste reznice Cabernet Sauvignona zaražene samo jednom varijantom virusa tj. monofiletskim izolatima GLRaV-3 smo dobili ljubaznošću dr. Rachelle Bester sa Stellenbosch Sveučilišta u Južnoafričkoj Republici. Analiza prisutnosti virusa i genozipizacija sojeva GLRaV-3 je ranije odrađena u Stellenboschu, a sekvence su deponirane u GeneBank (Diaz-Lara i sur., 2018.; Bester i sur., 2012.).

Naknadno smo reznice Cabernet Sauvignona testirali multipkeks RT-PCR reakcijom (Gambino i Gribaudo, 2006.) na prisutnost 10 virusa te je potvrđeno da donorski materijal ne sadrži druge viruse već samo GLRaV-3. Na isti način je provjeren bezvirusni status uvezenih reznica standardnih indikatora. Set indikatorskih biljaka smo zarazili monofiletskim varijantama (I,II,III), njihovim kombinacijama (I+II, I+III, II+III, I+II+III) te dodatnim monofiletskim izolatima (VI, VII). Pripremljena su i četiri tretmana kojima smo također zarazili biljke a sastojala su se od prirodnih izolata pronađenih u kolekciji (X,Y,Q,Z) u kojima su pored GLRaV-3 bili prisutni i drugi virusi u različitim kombinacijama (GVA, GLRaV-1, GLRaV-2, GPGV, GRSPaV, GFkV).

Spomenuti indikatori su svjetski poznate vinske sorte. U eksperiment smo uključili i autohtonu sortu Tribidrag, inače prisutnu u brojnim svjetskim vinogradima pod sinonimima Primitivo (Italija) i Zinfandel (California, SAD), kako bi ispitali odgovor naše domaće sorte na zarazu ovim virusom. Izvor bezvirusnih biljaka Tribidraga koji je tretiran na isti način kao ostale indikatorske sorte, je kolekcija bezvirusnih autohntonih sorti vinove loze u Institutu za jadranske kulture.

Rezultati analiza provedenih u fazi rane virusne infekcije (6 mjeseci od inokulacije) ukazuju na različit odgovor domaćina tj osjetljivost na zarazu ovisno o tipu domaćina i o tipu inokuluma. Iako su ELISA test i klasični PCR uglavnom dali negativne rezultate na prisutnost virusa, imajući na umu kratko vrijeme od zaraze do testiranja, kao i metodu prijenosa tj inokulacije, ovakvi rezultati nisu iznenadili. Osjetljivije metode detekcije kao što su dvostruki PCR (nested PCR) i PCR u realnom vremenu (qPCR) potvrdile su prijenos virusa na svim testiranim uzorcima. Osim različitog titra virusa u ovoj fazi eksperimenta, što indirektno zaključujemo iz nedovoljne osjetljivosti pojedinih metoda detekcije, zaražene biljke su pokazale jasne razlike u odnosu na kontrolne. Preliminarnom analizom morfoloških, fizioloških i biokemijskih parametara utvđene su razlike u mineralnom sastavu, u fotosintetskim i kolorimetrijskim parametrima te parametrima oksidativnog stresa. U ovoj fazi ispitivanja najveće razlike su izmjerene između zaraženih i kontrolnih sorti Merlota i Tribidraga. Različita osjetljivost sorti na zarazu potvrđena je i razvojem različitih simptoma i njihovog intenziteta na biljkama domaćinima. Najizraženije vizualne simptome u prvoj vegetacijskoj sezoni od inokulacije je razvila sorta Merlot (Slika 3).

Kod Tribidraga su izostali simptomi ali su registrirane intezivne promjene fiziološke i biokemijske naravi što, kada uzmemo u obzir da se nisu razvili simptomi, sugerira poželjan odgovor Tribidraga na ranu zarazu. Također su reakcije ovisile o tipu virusnog inokuluma pa su tako reakcije domaćina na GLRaV-3 u koinfekciji s drugim virusima bile brojnije u odnosu na GLRaV-3 u samostalnoj infekciji, iako nisu zabilježeni virusni inokulumi kod kojih odskaču specifični parametri u ovoj fazi zaraze kako kod prirodnih izolata iz kolekcije tako i samostalne GLRaV-3 infekcije. Određene genomske varijante GLRaV-3 u samostalnoj GLRaV-3 infekciji su pokazale različit potencijal da mijenjaju biološki odgovor domaćina na zarazu. Kod njih nije postignut kumunlativni efekt zaraze, već su samostalne monofiletske varijante uzrokovale jaći odgovor biljke na zarazu. Budući da smo promjene bilježili 6 mjeseci od same zaraze, preostaje nam utvrditi sve parametre i naredne vegetacijske sezone kako bismo bolje i detaljnije spoznali interakcije virusa i biljke.

Slika 3. Tipični simptomi virusa uvijenosti lista vinove loze 3 na sorti Merlot

Zaključak

Projektni tim se usredotočio na istraživanje GLRaV-3, koji je široko prisutan u svijetu, kao i u hrvatskim vinogradima u priobalnom području te je od iznimne ekonomske važnosti. Osim sustavnih informacija o prisutnosti različitih virusa vinove loze u autohtonim sortama vinove loze koje su dobivene kroz GRAPE-PATH3 projekt, rezultati ukazuju na širu sliku GLRaV-3 infekcije, uključujući njegovu genomsku varijabilnost i utjecaj na morfologiju, fiziologiju i biokemijske procese domaćina. Prvi rezultati utjecaja pojedinih GLRaV-3 varijanti i njihovih kombinacija s drugim virusima, kojima smo inficirali različite vrste domaćinskih biljaka vinove loze, pružaju informacije o osjetljivosti domaćina i cjelovitom rasponu učinka virusa -od vrlo blagih do onih vrlo intenzivnih reakcija na zarazu. Pretpostavljamo da osim efekta sorte i virusnog inokuluma, duljina trajanja zaraze će imati utjecaj na promatrane aspekte ove interakcije.

Napomena

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Literatura

Alabi OJ., Casassa L.F., Gutha L.R., Larsen R.C., Henick-Kling T., Harbertson J.F., Naidu, R.A. (2016). Impacts of Grapevine Leafroll Disease on Fruit Yield and Grape and Wine Chemistry in a Wine Grape (Vitis vinifera L.) Cultivar. PLoS ONE. 11(2): 1-18.

Armijo G., Schlechter R., Agurto M., Muñoz D, Nuñez C., Arce-Johnson P. (2016). Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. Frontiers in Plant Science. 7: 382.

Bester R., Maree H.J., Burger J.T. (2012). Complete nucleotide sequence of a new strain of grapevine leafroll-associated virus 3 in South Africa. Archives of virology. 157(9): 1815-1819.

Cui Z.H,. Bi W.L., Hao X.Y., Xu Y Li PM., Walker M.A., Wang Q.C. (2016). Responses of In vitro-Grown Plantlets (Vitis vinifera) to Grapevine leafroll-associated virus-3 and PEG-Induced Drought Stress. Frontiers in Physiology. 7: 203.

Čarija M.. Radić T., Černi S., Mucalo A., Zdunić G., Vončina D., Jagunić M., Hančević K. (2022). Prevalence of Virus Infections and GLRaV-3 Genetic Diversity in Selected Clones of Croatian Indigenous Grapevine Cultivar Plavac Mali. Pathogens. 11: 176.

Černi S., Ruscić J., Nolasco G., Gatin Z., Krajacić M., Skorić D. (2008). Stem pitting and seedling yellows symptoms of Citrus tristeza virus infection may be determined by minor sequence variants. Virus Genes. 36(1): 241-249.

Diaz-Lara A., Klaassen V., Stevens K., Sudarshana M. R., Rowhani A., Maree H. J., Chooi K. M., Blouin A. G., Habili N., Song Y., Aram K., Arnold K., Cooper M. L., Wunderlich L., Battany M. C., Bettiga L. J., Smith R. J., Bester R., Xiao H., Meng B., Preece J. E., Golino D., Al Rwahnih M. (2018). Characterization of grapevine leafroll-associated virus 3 genetic variants and application towards RT-qPCR assay design', PLoS ONE. 13(12).e0208862.

Gambino G. i Gribaudo I. (2006). Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with coamplification of a plant RNA as internal control. Phytopathology. 96(11): 1223–1229.

Hančević K., Černi S., Nolasco G., Djelouah K., Radić T., Škorić D. (2013). Biological characterization of Citrus tristeza virus monophyletic isolates with respect to p25 gene. Physiological and Molecular Plant Pathology. 8: 45-53.

Hančević K., Zdunić G., Vončina D., Radić T. (2015). Virus composition influences virus elimination success and in vitro growth characteristic of the grapevine cv. Plavac mali. Journal of plant pathology. 97(1): 159-162.

Hančević K., Saldarelli P., Čarija M., Černi S., Zdunić G., Mucalo A., Radić, T. (2021). Predominance and Diversity of GLRaV-3 in Native Vines of Mediterranean Croatia. Plants. 10(1): 17.

Karoglan Kontić J., Preiner D., Šimon S., Zdunić G., Poljuha D., Maletić E. (2009). Sanitary status of Croatian native grapevine varieties. Agriculturae Conspectus Scientificus. 74: 99-103.

Maree H.J, Almeida R.P.P., Bester R., Chooi K.M., Cohen D., Dolja V.V., Fuchs M.F., Golino D.A., Jooste A.E.C., Martelli G.P., Naidu R.A., Rowhani A., Saldarelli P., Burger J.T. (2013). Grapevine leafroll-associated virus 3. Frontiers in Microbiology. 4: 1-21.

Martelli G.P. (1993). Graft-transmissible diseases of grapevines. Handbook for detection and diagnosis. FAO Publication Division, Rome.

Martelli G.P. (2009). Grapevine virology highlights 2006-2009. In extended abstract of 16 meeting of ICVG, Dion, France, pp. 15-23.

Martelli G.P., Piro, G. (1975). Virus diseases in a Sicilian herbarium of the past century. Vitis. 13: 329-335.

Meng B., Martelli GP., Golino DA., Fuchs, M. (2017). Grapevine Viruses: Molecular Biology, Diagnostics and Management.1st ed.;Springer: Cham, Switzerland. pp. 167-195.

Naidu R., Rowhani A., Fuchs M., Golino D., Martelli GP. (2014). Grapevine leafroll: A complex viral disease affecting a high-value fruit crop. Plant disease. 98(9): 1172-1185.

Naidu R. A., Maree H. J. and Burger J. T. (2015). Grapevine Leafroll Disease and Associated Viruses: A Unique Pathosystem', Annual Review of Phytopathology. 53(1): 613–634.

Namba S., Yamashita S., Doi Y., Yora K., Terai Y., Yano R. (1979). Grapevine leafroll virus, a possible member of closteroviruses. Annals of the Phytopathological Society of Japan. 45: 497-502.

Poljuha D., Sladonja B., Bubola M. (2010). Incidence of viruses infecting grapevine varieties in Istria (Croatia). Journal of food, agriculture & environment. 8: 166-169.

Sgherri C., Ranieri A., Quartacci M.F. (2013). Antioxidative responses in Vitis vinifera infected by grapevine fanleaf virus. Journal of Plant Physiology. 15; 170(2): 121-128.

Šarić A., Corte A. (1959) Simptomatologia della degenerazione infetiva della vite in Istria. Atti Ist. Bot. Lab. Crit. Universita degli studii di Pavia. 17: 3-8.

Velasco L., Bota J., Montero R., Cretazzo E. (2014). Differences of three ampeloviruses multiplication in plant contribute to explain their incidences in vineyards. Plant Disease. 98: 395–400.

Vončina D., Đermić E., Cvjetković B., Maletić E., Pejić I., Karoglan Kontić J. (2009). Occurrence of grapevine leafroll-associated virus-1 and 3 in Croatian autochthonous grapevine varieties from Dalmatia. Extended abstracts 16th Meeting of ICVG, Boudon-Padieu E. (ed.), 116-117. Dijon, France: Le Progres Agricole et Viticole.

Vončina D., Badurin, D., Preiner D., Vjetkovic B., Maletic E., Kontic J.K.(2011). Incidence of virus infections in grapevines from Croatian collection plantations. Phytopathologia Mediterranea. 50: 316–326.

Vončina D., Rwahnih M.A., Rowhani A., Gouran M., Rodrigo P.P. Almeida. (2017). Viral diversity in autochtonous Croatian grapevine cultivars. Plant Disease.101(7): 1230-1235.

Vončina D., Preiner D., Šimon S., Cvjetković B., Maletić E., Pejić I., Kontić J. (2019). Distribution of nine viruses in croatian autochthonous grapevine (Vitis vinifera L.) cultivars from dalmatian region included in clonal selection. Journal of Central European Agriculture. 20(1): 262–273.

Coexistence of grapevine plants and grapevine leafroll-associated virus 3 in Croatia

Abstract

Grapevine leafroll disease (GLD) is one of the most important viral diseases of grapevine, and grapevine leafroll-associated virus 3 (GLRaV-3) is one of the main causal agents of this disease. Research related to GLRaV-3 is mainly focused on studying the epidemiology of the disease and developing reliable detection methods, while the role of the different GLRaV-3 genomic variants in GLD etiology and plant response to the presence of this virus is still largely unknown. One of the main objectives of the research project GRAPE-PATH3 (ongoing), supported by the Croatian Science Foundation, is to examine the presence of economically damaging grapevine viruses, with the particular reference to GLRaV-3 and its genomic variants by using molecular methods. Further objective was to describe plant-virus interaction by inoculating a set of indicator plants with GLRaV-3, either individal or in coinfections with other economically important viruses. Morphological, biochemical and physiological parameters were meassured to determine changes in primary metabolism, nutritive status and oxidative stress response. Results showed that GLRaV-3 was the most abundant virus found in tested vines and the presence of genomic variants I and II was confirmed. Preliminary analysis of virus infection point to a significant differences between healthy control plants, only GLRaV-3 infected and those plants infected with GLRaV-3 in combination with other viruses. Plant response varies depending on virus inoculum composition and the type of indicator used.

The obtained results should enable a better understanding of the pathogenicity of GLRaV-3 alone and in combination with other viruses, and the consequences that this virus has on grapevine, apart from the visible symptoms of leafrolling, after which the virus got its name.

Keywords: leafroll viruses, GLRaV-3 genotypes, coinfections, pathogenicity

Intercropping of walunt orchard and arable crops as an innovative approach in agroecosystems

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Abstract
Intercropping involves combining more plant species on the same parcel of land at the same time. The significance of intercropping is in the cultivation of plant species in systems that are less susceptible to different stress conditions. The aim of our research is to investigate yields of arable crops (wheat, buckwheat, barley and maize) intercropped between tree rows of grafted walnuts. The field trial was establishedat at a site in Đakovo, eastern Croatia, walnuts were eleven years old. Crop yields per cropped area did not differ much between the intercropped system and sole cropped system. However, crop yields per total area were lower by 30-40% in the intercropped system compared to the sole cropped control site. Stabile and high crop yields per cropped area indicate that there is no potential negative, allelopathic relationships of plants in such systems. Although yields per total area were lower, land equivalent ratio calculations have shown that the productivity of such intercopped orchard increases with introducing an intercrop. In conclusion, growing crops under the shade of trees results in lower crop yields but higher productivity of the area and at the same time increases the ecological aspect of the field by increasing biodiversity.

Key words: alley cropping, barley, buckwheat, maize, wheat

Introduction

Due to the recent climate changes, agriculture systems need to be more resilient to stress and less vulnerable to large fluctuations in temperature and humidity. One of the approaches that is gaining more and more attention is using rows of trees on arable land in order to reduce large microclimatic extremes. Such systems are agroforestry intercropped systems referred to as *alley cropping* where the agricultural crop is sawn in between tree rows. The incorporation of trees into arable land can have a positive effect on intercrops by creating microclimate conditions that improve plant resistance to stress conditions (Gosme et al., 2016), protection against plant diseases, pests, and weeds (Pumari et al., 2015), and it can positively influence soil fertility (Zake et al., 2015), soil carbon sequestration (Ivezić et al., 2022), increase soil biodiversity (Puškarić et al., 2021) as well as water and nutrients availability (Zhu et al., 2020). Therefore, we can argue that such systems, that combine permanent and perennial species, can reduce the negative effects caused by intensive agriculture (Quinkenstein et al., 2009).

Alley cropping systems are the most common agricultural practice in a part of the world with degraded soil (Africa, Asia, and Central America). However, due to changes caused by various factors such as a decrease of organic matter in the soil, reduced biological and microbiological activity, soil density, poor water-air capacity range and climate changes, scientific interest for such systems is growing in Europe as well (Ivezić et al., 2021). In France and Belgium crops are grown in walnut orchards (Dupraz, 1994; Mary et al., 1999; Mulia and Dupraz, 2006; Dufour et al., 2013; Cardinael et.al., 2015; Artru et al., 2017) and poplar plantations (Graves et al., 2010). Intercropping of agricultural crops with poplar trees has been investigated in England (Burgess et al., 2004; Graves et al., 2010), and

intercropping of agricultural crops with poplar, willow and black locust in Germany (Quinkenstein et al., 2009). Therefore the most common tree species used in Europe are: Poplar (*Populus spp.*), willow (*Salix spp.*), walnut (*Juglans spp.*) and black locust (*Robinia pseudoacacia*). The choice of tree species mainly depends on the purpose of such systems. If the goal is biomass production for bioenergy, poplars and willows are used, if the goal is diversification of production walnuts are used and if the goal is an improvement of degraded soil, black locust is used.

Although there are numerous ecological benefits of alley cropping, such systems create competition between crops and trees for light, water, and nutrients which can affect crop growth and profitability of growing crops in such systems. These problems can be solved by the appropriate design of alley cropping. Negative allelopathy is often associated with walnut trees due to the toxic substance it produces, juglone. However, studies showed that juglone secretion is not intensive in young, grafted orchards and that its activity has no significant toxic effect on intercrops during the first 15 years (Scott & Sullivan, 2007). The suitability of walnuts for intercropping with arable crops is manifested in its late leafing and early leaf fall, which reduces the competition for light, especially with winter crops due to the very short overlap of crop and walnut growing seasons. It is predicted that in the first six years of walnut orchard intercropping is profitable (Žalac et al., 2021).

The productivity of the alley cropping system can be evaluated by the *land equivalent ratio* (LER) equation. The land equivalent ratio is defined as the ratio of the area under monoculture production to the area under intercropping needed to give equal yields at the same management level (Ong & Kho, 2015). However, the question is how profitable or productive intercropped system can be i.e. how would such combined cultivation affect the yields of arable crops?

Given that the agroecological influence on agricultural production varies between climates, carrying out this kind of research on regional scales is inevitable. The aim of this research is to explore the possibilities of combining walnut orchards and arable crops as an answer to increasingly negative agroecological influences and identify the influence of microclimatic conditions of such systems on yields and LER of different arable crops..

Material and methods

The experiment was set up in Osijek-Baranja County (Đakovo) where in the year 2018 the orchard was 11 years old. The results obtained are applicable to a wider area of the Danube River basin. The experimental site applied the principles of organic agriculture. Data and samples were collected from 3 parcels: 1.) arable land (crop-control), 2.) permanent walnut orchard with intercropping between walnut rows (crop/wlanut), and 3.) permanent walnut orchard without intercropping (walnut-control). Observed permanent orchards are with grafted walnuts. The walnut orchard has 10 rows of walnuts out of which the first 5 rows always had a lower walnut yield. Walnut yield of the first five rows was always around 30% of the total yield, while the last five rows had around 70% of the total walnut yield. We have decided to sow crops in the 4 alleys in between these first five rows to increase the productivity of this low-productive area (Figure 1).

The crop rotation on control arable land as well as in orchard was uniform. The collected data consisted of agro-climatologic data, soil samples, and plant material. Climatic parameters were monitored during the vegetation period. After the harvest, soil samples were collected and analyzed for each system for pH, organic matter, carbonates, hydrolytic acidity, available P, and available K. Yields of barley, wheat, and buckwheat were determined by harvesting a 1m^2 area (8 replicates from intercropped system and 8 from crop control site), separating and weighing the grain, and calculating the grain weight per 1ha area to obtain yields per cropped area in t/ha. From the same 1m^2 frame number of plants

were counted to express plant density in each system. The yield of maize was determined based on the yield from the maize row of 14,3 m length (4 replicates from intercropped system and 4 from crop control site) and then recalculated on 1 ha to obtained yields per cropped area. To obtain yields per total area, space occupied by tree rows was taken in consideration, so the cropped yields were reduced by 25% as 25% of the 1 ha area was occupied by tree rows. Yield components were also measured by sampling random plants for each system. From the crop and walnut fruit yields, the land equivalent ratio (LER) was estimated (Eq 1).

Table 1. Soil properties

ĐAKOVO						
	Treatment	n	Mean	StDev	Min	Max
pH H_2O	intercropped	128	6.3	0.5	5.6	8.4
	arable ctrl	138	5.6	0.7	4.8	8.1
	orchard ctrl	114	6.2	0.5	5.1	7.9
Humus	intercropped	128	2.0	1.2	0.9	14.6
	arable ctrl	138	1.6	0.2	0.9	2.1
	orchard ctrl	114	1.9	0.5	1.1	3.3
AL - P	intercropped	128	8.7	2.7	4.9	19.6
	arable ctrl	138	9.7	5.5	4.3	41.1
	orchard ctrl	114	8.9	5.3	3.2	46.4
AL - K	intercropped	128	13.8	2.7	8.8	28.0
	arable ctrl	138	12.1	2.1	5.5	18.3
	orchard ctrl	114	16.9	7.7	8.7	57.9

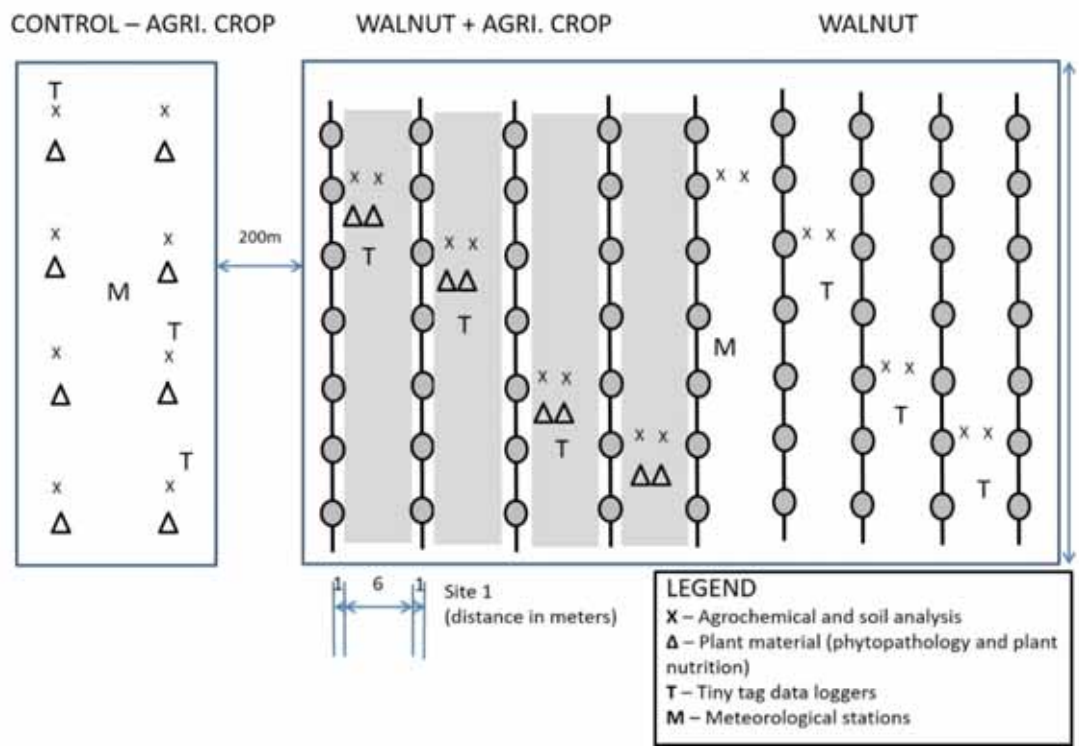

Figure 1. Filed trial scheme

Crop rotation:
YEAR 1. 10.2017. - 07.2018. – Wheat
08.2018. - 04.2019. – Green manure (Oilseed rape)
YEAR 2. 05.2019. - 09.2019. – Buckwheat
YEAR 3. 09.2019. - 06.2020. – Barley
07.2020. - 10.2020. – Green manure (Buckwheat)
YEAR 4. 04.2021. - 10. 2021. – Maize

Land equivalent ratio
The land equivalent ratio is defined as the ratio of the area under monoculture production to the area under intercropping needed to give equal yields at the same management level (Ong & Kho, 2015). It is calculated as the ratio of tree yield from intercropped system to the tree monoculture yield plus the ratio of crop yield from the intercropped system to the crop monoculture yield, as shown in Equation 1:

eq. 1 $$LER = \frac{\text{walnut intercropped } yield}{\text{walnut pure orchard } yield} + \frac{buckwheat \text{ intercropped } yield}{buckwheat \text{ monculture } yield}$$

When LER ≤ 1, there is no agronomic advantage of intercropping over sole cropping, but when LER is >1, production in the intercropped system is higher than in the separate sole crops or sole orchard.

Statistical analysis was carried out using Minitab statistical program and included analysis of variance, descriptive statistics (averages, standard error, ranges), and testing differences between the average of treatments using analysis of variance between the observed properties.

Results and discussion
The significance of intercropping is in the cultivation of plant species in a system that is less susceptible to different stress conditions. The main benefits of such systems are a better use of the production area, positive influence on soil fertility, diversity of production in one vegetation, protection against plant disease, pests, and weeds, better use of nutrients and water in the soil as well as increased biodiversity and consequently higher overall yields per ha (Quinkenstein et al., 2009; Tsonkova et al., 2012). Our results have shown statistical differences between arable and intercropped systems in yields (Table 2)..

Table 2. crop yields in sole crop and intercropped systems (per cropped area and per total area)

	Yield per cropped area (t/ha) – control	Yield per cropped area (t/ha) – intercropped orchard	Yield per total area (t/ha) – control	Yield per total area (t/ha) –intercropped orchard
Wheat 2018	6.7^{a}	6.0^{b}	6.7^{a}	4.5^{b}
Buckwheat 2019	2.5^{a}	1.8^{b}	2.5^{a}	1.4^{b}
Barley 2020	7.5^{ns}	7.2^{ns}	7.5^{a}	5.4^{b}
Maize 2021	8.4^{ns}	8.0^{ns}	8.4^{a}	6.0^{b}

ns indicates no significant differences between treatmens, letters a and b indicate significant differences

Table 3. crop and walnut yields, and LER

Site and crop rotation	Crop		Walnut		LER
	Arable (control)	Intercropped walnut orchard	Orchard (control)	Intercropped walnut orchard	
	t/ha		kg/ha		
ĐAKOVO					
Wheat -2018	6.7	4.5	752	441	1.26
Buckwheat - 2019	2.3	1.35	746	378	1.09
Barley - 2020	7.52	5.41	2625	2136	1.53
Maize - 2021	8.4	6	2560	1490	1.30

crop yields refer to t/ha per total area

Wheat

In our first year of observation, on the wheat control plot, the yield was 6.7 t/ha while yield of wheat intercropped with the walnut was 6.0 t/ha per cropped area. However, given that only 75% of the intercropped area was covered with wheat (25% of the area occupied the rows of walnuts), yield per total area was lower, i.e. 4.5 t/ha which is 33% lower than the control. Yet, the intercropped area in addition to wheat yield also had a walnut yield of 441 kg/ha which was almost half of the yield of walnuts in rows that were not intercropped (not intercropped rows of walnuts had a walnut yield of 752 kg/ha) (Table 3.). Therefore, the first five rows of walnut gave 67% of wheat yield from the wheat control plot and 59% of walnut yield from the walnut control plot. Altogether it comes out that intercropped plot had a land equivalent ratio (LER) of 1.26 (eq. 2) which means that by intercropping wheat in these rows of walnut of low productivity we have increased its production by 26% (Table 3).

Equation 2. $LER = \frac{441\ kg/ha}{752\ kg/ha} + \frac{4.5\ t/ha}{6.7\ t/ha} = 0.59 + 0.67 = \mathbf{1.26}$

Picture 1. Intercropped wheat

Buckwheat

Growing buckwheat in the alleys of first five tree rows the system had walnut yield of 378 kg/ha and buckwheat yield 1.8 t/ha per cropped area. Since only 75% (6m out of 8m) of the area in alleys was covered with buckwheat (the rest 25% was walnut rows) the actual buckwheat yield per ha in the intercropped system was actually 1.35 t/ha per total area. Walnut control plot had a walnut yield of 746 kg/ha and the buckwheat control plot had a buckwheat yield of 2.3 t/ha. In relative numbers, the walnut yield in the intercropped system was 51% (0.51) of the walnut yield in the walnut control plot and buckwheat yield was 58% (0.58) of the buckwheat yield in the buckwheat control plot. Altogether, it comes out that

intercropped plot had a land equivalent ratio (LER) of 1.09 (eq. 3) which means that by intercropping buckwheat in between these low productivity rows of walnut we have increased the production of this area by 9% in comparison to the high productive area (last five rows of walnut trees) or control buckwheat plot.

Equation 3. $LER = \frac{378\ kg/ha}{746\ kg/ha} + \frac{1.35\ t/ha}{2.3\ t/ha} = 0.51 + 0.58 = \mathbf{1.09}$

Barley

Barley yield, on control arable plot was 7.52 t ha^{-1} and in intercropped orchard 7.21 t ha^{-1} per cropped area. However, barley yield per total area per ha in the intercropped system was 5.41 t ha^{-1}. At the same time, walnut fruit yield in the intercropped orchard was 2136 kg ha^{-1} and in the sole orchard 2625 kg ha^{-1}. The sum of relative intercropped yields for walnut and barley gave an LER value of 1.53 (Eq. 4), which means that intercropping arable crops in walnut orchard increased productivity of that area by 53% in regards to sole crop arable systems or sole walnut system.

Equation 4. $LER = \frac{2136\ kg/ha}{2625\ kg/ha} + \frac{5.41\ t/ha}{7.52\ t/ha} = 0.81 + 0.72 = \mathbf{1.53}$

Even though the total barley yield and plant density were reduced in intercropped system, all of the market-relevant yield components: the number of fertile spikelets, the length of spikes, and the weight of 1000 grains were significantly higher in the intercropped orchard suggesting better ecological conditions for plant growth.

Maize

In alleys of intercropped systems maize yield was 8 t/ha per cropped area which is just slightly lower than on the control plot (8.4 t/ha). However, when we consider the total area, taking into consideration area under walnut, then maize yield in the intercropped system is 6 t/ha which is 29% lower than in the control arable plot. Walnut yields in 2021 were 2560 kg/ha in the sole orchard and 1490 kg/ha in the intercropped orchard, meaning the intercropped orchard had 42% lower walnut fruit yields. The sum of relative intercropped yields for walnut and maize gave an LER value of 1.30 (Eq. 5), which means that intercropping arable crops in walnut orchard increased productivity of that area by 30% in regards to sole arable crop systems or sole walnut system.

Equation 5. $LER = \frac{1490\ kg/ha}{2560\ kg/ha} + \frac{6.00\ t/ha}{8.4\ t/ha} = 0.58 + 0.72 = \mathbf{1.30}$

Therefore, the results show that some crops, intercropped in orchards, can substantialy increase productivity and provide a potential additional source of profit which can be of special importance on the low productive plots. Barley and maize yields per cropped area showed no significant differences between intercopped system and sole cropped system. High yields in intercropped systems indicate that there was no effect of allelopathic relationships between plants. In intercropping systems with walnut, it is desirable to choose orchards with grafted plants since the walnut tree secretes juglone that may have a toxic effect on the germination of other plants. In the orchards of walnuts with grafted trees, the secretion of juglone is not so intense and the activity of juglone in such orchards has no significant toxic effect in the first 15 years (Scott and Sullivan, 2007). Furthermore, an introduction of organic matter or organic fertilizers is recommended because it promotes

microbiological activity that enhances juglone decomposition (Schmidt, 1988). All of the above-mentioned resulted in no negative effect on crop growth within walnut alleys.
We have observed somewhat better results with winter crops (wheat and barley), as there was no overlapping of vegetation with walnut and lower rates of weed occurrence. Summer crops required weed control to maintain stable yields. Several authors indicate that the light and the shading effect are more important than comepetition for nutrients (Dupraz et al., 2005; Dufour et al., 2013; Talbot et al. 2014) which supports our findings regarding the winter crops. From agroecological aspect, winter crops show higher potential as the soil is covered during the winter months, there is a lower occurrence of weeds and by the time walnut starts to leaf the crop has already covered the area within the alleys.

Conclusions

Yields of barley and maize in intercropped systems with walnuts have shown no reduction in yields per cropped area, while wheat showed some minor reduction. However, if we consider observing yields per total area, yields for all the crops were lower by 30-40%. Stabile and high crop yields per cropped area confirm that there is no potential negative, allelopathic relationships of plants in such systems. Land equivalent ratio calculations have shown that the productivity of such orchard increases with introducing an intercrop. In conclusion, growing crops under the shade of trees results in lower crop yields per total area but higher productivity of the area and at the same time increases the ecological aspect of the field by increasing biodiversity.

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References

Artru S., Garre S., Dupraz C., Hiel M.P., Blitz-Frayret C., Lassois L. (2017). Impact of spatio-temporal shade dynamics on wheat growth and yield, perspectives for temperate agroforestry. European Journal of Agronomy. 82: 60-70.

Burgess PJ, Incoll LD, Corry DT, Beaton A, Hart BJ (2004) Poplar (Populus spp) growth and crop yields in a silvoarable experiment at three lowland sites in England. Agroforestry Systems. 63: 157–169.

Cardinael R., Chevallier T., Barthès B.G., Saby N.P.A., Parent T., Dupraz C., Bernoux M., Chenu C. (2015). Impact of alley cropping agroforestry on stocks, forms and spatial distribution of soil organic carbon — A case study in a Mediterranean context. Geoderma. 259–260: 288–299.

Dufour L., Metay A., Talbot G., Dupraz C. (2013). Assessing Light Competition for Cereal Production in Temperate Agroforestry Systems using Experimentation and Crop Modelling. Journal of Agronomy and Crop Science. 199: 217–227.

Dupraz C. (1994). Prospects for easing land tenure conflicts with agroforestry in Mediterranean France: a research approach for intercropped timber orchards. Agroforestry Systems. 25: 181 - 192

Dupraz C., Burgess P., Gavaland A., Graves A., Herzog F., Incoll L., Jackson N., Keesman K., Lawson G., Lecomte I., Liagr, F., Mantzanas K., Mayus M., Moreno G., Palma J., Papanastasis V., Paris P., Pilbeam D., Reisner Y., Vincent G., Werf Van der W. (2005). Synthesis of the Silvoarable Agroforestry For Europe project. INRA-UMR System Editions, Montpellier, 254 p.

Graves A.R., Burgess P.J., Palma J., Keesman K.J., Van der Werf W., Dupraz C., van Keulen H., Herzog F., Mayus M. (2010). Implementation and calibration of the parameter-sparse Yield-SAFE model to predict production and land equivalent ratio in mixed tree and crop systems under two contrasting production situations in Europe. Ecological Modelling. 221: 1744–1756

Gosme M., Dufour L., Inurreta-Aguirre H., Dupraz C. (2016). Microclimatic effect of agroforestry on diurnal temperature cycle. *3rd European Agroforestry Conference (May)*, pp. 183–186.

Ivezić V., Yu Y., Van der Werf W. (2021). Crop Yields in European Agroforestry Systems: A Meta-Analysis. Frontiers in Sustainable Food Systems. 606631: 1-13.

Ivezić V., Lorenz K., Lal R. (2022). Soil Organic Carbon in Alley Cropping Systems: A Meta-Analysis. Sustainability. 14(3): 1296.

Mary F., Dupraz C., Delannoy E. and Liagre F. (1999). Incorporating agroforestry practices in the management of walnut plantations in Dauphiné, France: an analysis of farmers' motivations. Agroforestry Systems. 43: 243–256

Mulia R. i Dupraz C. (2006). Unusual fine root distributions of two deciduous tree species in southern France: What consequences for modelling of tree root dynamics? Plant and Soil. 281: 71–85

Ong C.K., Kho R.M. (2015). A framework for quantifying the various effects of tree-crop interactions. Tree-Crop Interactions: Agroforestry in a Changing Climate. 1–23.

Pumari L., Midega C., Jonsson M., Sileshi G.W., Gripenberg S., Kaartinen R., Barrios E., Muchane M.N. (2015). Effects of agroforestry on pest, disease and weed control: A meta-analysis. Basic and Applied Ecology. 16(7): 573–582.

Puškarić J., Jović J., Ivezić V., Popović B., Paponja I., Brmež M. (2021). The Communities of the Nematodes, Bacteria, and Fungi and the Soil's Organic Matter in an Agroforestry Ecosystem in Croatia. Poljoprivreda/Agriculture. 27(1): 66-74.

Quinkenstein A., Wöllecke J., Böhm C., Grünewald H., Freese D., Schneider B.U., Hüttl R.F. (2009). Ecological benefits of the alley cropping agroforestry system in sensitive regions of Europe. Environmental Science & Policy.. 12: 1112-1121.

Schmidt S.K. (1988). Degradation of juglone by soil bacteria. Journal of Chemical Ecology. 14(7): 1561–1571.

Scott R. i Sullivan W.C. (2007). A review of suitable companion crops for black walnut. Agroforest Syst. 71: 185–193

Talbot G, Roux S, Graves A, Dupraz C., Marrou H. (2014). Relative yield decomposition: A method for understanding the behaviour of complex crop models. Environmental Modelling & Software. 51: 136–148.

Tsonkova P., Böhm C., Quinkenstein A., Freese D. (2012). Ecological benefits provided by alley cropping systems for production of woody biomass in the temperate region: A review. Agroforestry Systems. 85(1): 133–152.

Zake J., Pietsch S.A., Friedel J.K., Zechmeister-Boltenstern S. (2015). Can agroforestry improve soil fertility and carbon storage in smallholder banana farming systems? Journal of Plant Nutrition and Soil Science. 178(2): 237–249.

Zhu X., Liu W., Chen J., Bruijnzeel L.A., Mao Z., Yang X., Cardinel R., Meng F.R., Sidle R.C., Seitz S., Nair V.D., Nanko K., Zou X., Chen C., Jiang X.J. (2020). Reductions in water, soil and nutrient losses and pesticide pollution in agroforestry practices: a review of evidence and processes. Plant and Soil, 453.

Žalac H., Burgess P., Graves A., Giannitsopoulos M., Paponja I., Popović B., Ivezić V. (2021). Modelling the yield and profitability of intercropped walnut systems in Croatia. Agroforestry Systems. 2021.

Konsocijacijski sustavi oraha i ratarskih kultura kao inovativni pristup u agroekosustavima

Sažetak
Konsocijacija podrazumijeva kombiniranje više biljnih vrsta na istoj površini u isto vrijeme. Značaj usijavanja ratarskih kultura među redove drvenastih vrsta očituje se u stabilnosti takvih sustava i otpornosti na stresne uvjete. Cilj našeg istraživanja je bio odrediti prinose ratarskih kultura (pšenice, heljde, ječma i kukuruza) usijanih između redova oraha i usporediti ih s prinosima istih kultura na oranici. Pokus je postavljen na lokalitetu u Đakovu, u voćnjaku starosti jedanaest godina. Prinosi usjeva po usijanoj površini nisu se mnogo razlikovali između konsocijacijskog sustava i oranice. Međutim, prinosi usjeva po ukupnoj površini bili su niži za 30-40% u konsocijaciji u usporedbi s prinosima na oranici. Stabilni i visoki prinosi usjeva po usijanoj površini ukazuju na to da nema potencijalnih negativnih, alelopatskih odnosa između ratarskih kultura i oraha. Unatoč smanjenu prinosa oraha i ratarskih kultura po ukupnoj površini, *Land Equivalent Ratio* (LER), izračun produktivnosti, pokazao je da istraživani konsocijacijski sustav ima veću produktivnost po jedinici površine od samog voćnjaka oraha ili od oranice. Zaključno, uzgoj usjeva u sjeni drveća rezultira nižim prinosima usjeva, ali većom produktivnošću površine te istovremeno poboljšava ekološki aspekt povećanjem biološke raznolikosti.

Ključne riječi: međuredno usijavanje, ječam, heljda, kukuruz, pšenica

Upgraded nutritional value of maize - the beneficial effect of phytochemicals

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Abstract
In nature, animals select feeds that contain both nutrients and phytochemicals, compounds that have a beneficial effect on animal and human health. In the sustainable production of farm animals, value-added feeds meet nutrient requirements, and maintain digestive efficiency and metabolic health. Due to high content of phytochemicals such as carotenoids, resistant starch, tocols and phenolics, maize grain is such a feed. These compounds have a beneficial effect on the health and well-being of animals and, if not used for this purpose, are deposited in the tissues. In this way, they improve the quality of meat and egg yolk, but also become sources of phytochemicals in human nutrition. This paper presents the content of phytochemicals in maize grain and their beneficial effects on farm animals.

Key words: maize, carotenoids, resistant starch, phenolics, tocols

Introduction

The challenges in food production system today are to provide an adequate amount of food rich in nutrients and bioactive compounds, meet the demands of the consumers, and at the same time reduce the negative impact on the environment (Viola and Marinelli, 2016). Therefore, feed production and animal feeding are directed towards sustainable animal diets. One of the aspects of sustainable nutrition of farm animals is the use of feeds that meet more than one nutritional requirement of the animals. Although the primary role of diet is to provide the amount of nutrients needed for animal production, it can also affect animal health, behaviour and well-being. In that regard, the main focus is on the adverse effects on animal production, health and well-being due to the presence of pathogens and compounds such as mycotoxins, heavy metals and pesticides, but feeds also contain compounds that have positive effects. These compounds are phytochemicals, bioactive chemical compounds that occur naturally in plants and provide health benefits to animals and humans. A wide range of phytochemicals have been identified in plants; some of the compounds include phenolics, terpenes, carotenoids, alkaloids, fatty acids and polysaccharides (Campos-Vega and Oomah, 2013).

Maize grain is the primary energy source in the diets of farm animals worldwide and its proportion in the diet can be as high as 70%. Among the cereals used in animal nutrition, maize has the highest values of useful energy due to its high starch and oil content (740 and 43 g kg^{-1} DM, respectively) and low content of fiber (neutral detergent fiber, 12 g kg^{-1} DM) and anti-nutritional factors (Eeckhout and De Paepe, 1994; Sauvant et al., 2004). Maize grain also contains a variety of bioactive compounds such as carotenoids, phenolics, tocols and resistant starch (Liu et al., 2007; Siyuan et al., 2018), most of which are in the highest content among cereals. The content of these compounds varies among genotypes, reflecting their different beneficial potential. Due to the high proportion of maize grain in diets of farm animals, these compounds significantly contribute to animal health and affect production performance and quality of animal products.

Of the aforementioned phytochemicals that have been shown to affect animal performance, health and well-being, as well as on the quality of animal products (Mahfuz et al., 2021),

only carotenoids and resistant starch have been thoroughly studied in maize. Therefore, this article presents the latest findings on the beneficial effects of carotenoids and resistant starch of maize, while for other phytochemicals, their content and potential beneficial effects are explained. Furthermore, it has to be noted that the content and effect of phytochemicals is presented for minimally processed maize grain, i.e., dried after harvest and milled. Further processing, such as hydrothermal treatment or ensiling, which is often used in the preparation of maize for animal diets, could lead to degradation of phytochemicals, affect their bioavailability, and alter them (Suri and Tanumihardjo et al., 2016).

Carotenoids

Carotenoids are a large group of natural yellow, orange and red pigments that are soluble in lipids and, in addition to their pigmenting properties, possess antioxidant and provitamin A activity. The latter activity is primarily related to the hydrocarbon carotenes, while the pigmenting activity is related primarily to the xanthophylls, oxygenated derivatives of carotenes. The carotenoids present in maize grain are lutein, zeaxanthin, α- and β-cryptoxanthin and β-carotene (Kljak and Grbeša, 2015), and the range of total carotenoid content in commercial maize hybrids is between 14.48 and 32.61 μg g^{-1} DM (Zurak et al., 2021a). With 84% of the total content, lutein and zeaxanthin, also known as macular pigments, are the predominant carotenoids.

The best known role of carotenoids in animal nutrition is the pigmentation of egg yolk, meat, skin and fat in poultry, whereas these products become a source of carotenoids in human nutrition. In commercial poultry production, maize carotenoids are neglected and pigment additives, usually of synthetic origin, are added to diet. The addition of pigments is most common in egg production, where producers follow consumer preference for intensive yolk colour. However, due to the increasing concern in healthy nutrition, the addition of synthetic pigments has become an issue, and due to the high pigmenting ability of such pigments, the higher colour intensity of the yolk does not imply the higher yolk carotenoid content (Kljak et al., 2021a). As a result, maize regained attention as a natural source of carotenoids in hen nutrition. There is increasing focus on the production of carotenoid-enriched maize, which has been shown to have strong yolk pigmentation ability (Moreno et al., 2020; Ortiz et al., 2021), but Kljak et al. (2021b) showed that commercial maize hybrids could be the only source of carotenoids in the hen diet. In the latter study, the authors evaluated five Croatian maize hybrids that, at a 60% level in hen diet, resulted in yolk colour scores of 10-11 according to the DSM Yolk colour fan scale (Table 1), which are acceptable by consumers in most EU countries (Grashorn, 2016).

In addition, commercial maize hybrids could be used as the only source of carotenoids for pigmentation of breast meat and skin, shank skin and abdominal fat in chicken diets (Kljak et al., 2018). Besides the effect on pigmentation, maize carotenoids have an evident impact on poultry health. Nogareda et al. (2016) showed that chickens infected with *Eimeria tenella* had higher weight, only mild disease symptoms and lower faecal oocyst counts when fed high-carotenoid maize than when fed low carotenoid maize. The authors concluded that high-carotenoid maize could be used as a complementary strategy to boost resistance to coccidiosis.

The carotenoid content of maize represents only a potential that animals can absorb and use for biological functions. Therefore, a prerequisite for the utilisation of maize carotenoids is that they are released from the feed matrix during digestion. Recent findings by Zurak et al. (2021a) have shown that the bioaccessibility of carotenoids differs among different hybrids. Furthermore, in a subsequent study, Zurak et al. (2021b) showed that hybrids with the highest kernel hardness had the highest carotenoid digestibility, implying that kernel structure affects carotenoid digestibility. Therefore, selecting maize hybrids with increased

carotenoid digestibility provides a basis for increased carotenoid utilisation. In addition, the use of maize as a carotenoid source in poultry diets would simplify diet preparation and reduce egg production costs due to lower pigment additive costs.

Table 1. The effect of maize hybrid on egg yolk colour and total carotenoid content (Kljak et al., 2021b)

Dietary treatment containing hybrid	TC content in the diet	TC content in egg yolk	YCF Colour
	μg g^{-1} DM	μg g^{-1}	
Bc 572	17.13	25.99	11
Kekec	13.45	24.47	10
Mejaš	14.78	25.47	10
Riđan	13.86	25.49	10
Pajdaš	14.04	21.97	10

TC – total carotenoids; YCF – DSM Yolk Colour Fan

Maize carotenoids with provitamin A function are β-cryptoxanthin and β-carotene, and although their content is up to 15-fold lower than that of lutein and zeaxanthin (Kljak and Grbeša, 2015), there are hybrids with increased content. Such hybrids contribute to the vitamin A status of farm animals. Feeding chickens with high-carotenoid maize hybrids resulted in accumulation or increased accumulation of retinol in the liver and increased retinol serum concentrations compared to chickens fed low-carotenoid maize (Díaz-Gómez et al., 2017; Nogareda et al., 2016). Furthermore, piglets born to vitamin A-deficient sows fed high-carotenoid maize continuously during lactation were found to have increased accumulation of retinol in the liver compared with sows given retinyl palmitate orally (Heying et al., 2013).

In ruminants, the effect of maize carotenoids has not been studied because maize has a much lower carotenoid content than forages (Pickworth et al., 2012). However, maize carotenoids contribute to carotenoid concentrations in plasma, liver and subcutaneous fat, especially in rations high in maize. Cattle have a higher capacity to absorb carotenoids compared to sheep and goats (Yang et al., 1992), and bovine milk is the only one that contains appreciable levels of carotenoids (Álvarez et al., 2015), resulting in pigmentation of dairy products. For example, 95% of β-carotene and 64% of xanthophylls, initially present in milk fat, were found in cheese fat (Nozière et al., 2006).

Resistant starch

Based on nutritional properties, i.e., the extent to which starch is digestible, starch is classified into digestible and resistant starch in monogastric animals (RS; Sajilata et al., 2006). Digestible starch is further subdivided into rapidly and slowly digestible starch (RDS and SDS, respectively; Englyst et al., 1992), which differ in the rate of starch digestibility, although both fractions are digested in the small intestine. The RS fraction escapes digestion in the small intestine and could be fermented by the resident microbiota in the colon. Thus, RS is considered as dietary fiber and acts as a prebiotic. On the other hand, RS in ruminants is the starch fraction that is not fermented in the rumen and is digested in the small intestine of the animal.

Maize, like other cereals, contains RS_1-type starch, i.e., physically protected RS, which is a result of inaccessibility of starch to amylolytic and digestive enzymes; this starch can be digested in the small intestine only if it is thoroughly milled (Raigond et al., 2015; Sajilata et al., 2006). The digestibility of starch in maize is influenced by numerous intrinsic factors that include amylose content, amylose-to-amylopectin ratio, starch granule size, starch and

non-starch lipid content, and zein content (Kljak et al., 2019; Zurak et al., 2020). The results of these studies show that the starch digestibility rate in maize decreases with increasing content of zein, amylose, starch, and non-starch lipids and with decreasing granule size. These properties negatively affect the intestinal digestibility rate of starch and contribute to an increase in SDS and RS content. Furthermore, these properties differ among maize genotypes, resulting in a wide range of content of starch fractions (Table 2). The content of RS in maize also varies depending on the determination method; it can be determined using an analytical method or estimated using *in vitro* procedure for starch digestibility. The latter method, originally proposed for humans (Englyst et al., 1992), was tested for poultry, and Weurding (2002) showed that digestion coefficients after 2 and 4 hours of incubation in the *in vitro* digestibility procedure could be estimated for starch digested to the posterior jejunum (i.e., RDS) and posterior ileum (i.e., SDS), respectively, whereas RS can be calculated as the difference from 100% starch. However, it should be noted that the analytical method could result in lower values because it is not based on the gastrointestinal digestion of starch, thus excluding a possible interaction between the cereal matrix and intestinal enzymes.

Table 2. Range of contents of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) in commercial maize hybrids estimated from starch digestion coefficients in *in vitro* procedure for poultry using equations by Weurding (2002)

Starch fraction	Hybrids used in study by Zurak et al. (2020)	Hybrids used in study by Kljak et al. (2019)
	g kg^{-1} DM	
RDS	306.2 – 594.8	486.3 – 527.5
SDS	24.5 – 256.5	90.1 – 124.5
RS	81.4 – 148.3	103.7 – 145.4

In monogastric animals, fermentation of RS in the colon leads to the production of short-chain fatty acids (SCFA), which lower intestinal pH that suppresses the growth of pathogenic microorganisms but enhances the growth of beneficial microbes (Tan et al., 2021). In addition, RS modulates the gut microbiota and enhances the animal immune response. However, SCFA are a less efficient energy source than glucose from the small intestine. An excessive amount of RS in the diet, for example, above 30% in finishing pigs, may reduce feed intake (Tan et al., 2021). Inclusion of high-amylose maize, maize abundant in RS, in the diet of pigs alters fermentation in the colon and results in higher production of propionate and butyrate. Butyrate is the most beneficial, it serves as an energy source for normal colonocytes and has a beneficial effect on reducing the risk of diseases such as colorectal neoplasia (Bird et al., 2007). In addition, He et al. (2017) have shown in an *in vitro* experiment that increasing the content of maize RS reduces protein fermentation in both the cecum and colon, thereby reducing ammonia production.

For poultry, even lower levels of RS are required in the diet to avoid negative effects on animal production. This is most likely due to the shorter colon and the reduced possibility of utilisation of RS by the gut microbiota. Liu et al. (2020) have shown that a level greater than 4.18% of maize RS results in lower nutrient retention, weight gain and feed efficiency in broilers. However, a possible health effect is more than evident; for example, the addition of 5 g kg^{-1} high-amylose maize in the diet provided protection similar to antibiotics in chickens suffering from necrotic enteritis (when inoculated with sporulated oocysts of each of *Eimeria maxima*, *Eimeria acervulina*, and *Eimeria brunetti* at 9 days of age and *Clostridium perfringens* at 10 days of age) without negatively affecting feed intake and weight gain in broilers (M'Sadeq et al., 2015). In addition, maize RS at up to 12% in the diet reduced the abundance of caecal Firmicutes which decreased fatty acid synthesis in the liver

and suppressed abdominal fat deposition in broilers (Zhang et al., 2020). Excessive fat deposition decreases feed efficiency and waste during meat processing.
In ruminants, since RS is digested in the small intestine, it reduces the availability of starch for microbial degradation in the rumen and helps prevent rumen acidosis. Compared to the rumen, where fermentation of starch leads to the production of SCFA, digestion in the small intestine leads to a direct supply of glucose that can be used more efficiently by the animal compared to hepatic gluconeogenesis of propionate (Deckardt et al., 2013). However, rumen degradable starch is required for normal rumen conditions, digestion and animal production. For example, Matthé et al. (2001) recommended feeding no more than 1.3-1.8 kg RS per cow per day to avoid reducing starch utilisation efficiency. Furthermore, the introduction of maize high in RS into ruminant diets, as Wang et el. (2016) showed in goats, shifted the fermentation of starch in the rumen toward increased production of acetate, butyrate and isobutyrate and decreased production of propionate and total SCFA. In addition, the increase in starch flow into the small intestine has positive effects on metabolic health status in terms of haematocrit and levels of albumin, C-reactive protein, growth hormone, interleukin-2, oxaloacetate transaminase and creatine kinase in the blood.

Other phytochemicals present in maize

Phenolics are the largest natural phytochemicals whose structure consists of an aromatic ring with one or more hydroxyl substituents; they can range from simple phenolic molecules to highly polymerized compounds (Vuolo et al., 2019). Phenolics are potent antioxidants, and their beneficial effects include anti-inflammatory, antidiabetic, cardioprotective, neuroprotective, antitumor and anti-ageing properties. Cereals are among the richest sources of phenolics, with compounds from the phenolic acid and flavonoid subgroups. They can be present in free and conjugated forms and are mainly found in aleurone. Grbeša and Kljak (2017) showed that Croatian maize hybrids contain between 3735 and 4715 mg/kg of galic acid equivalents of phenolics, of which on average 81% are bound. Furthermore, about 40% of maize phenolics are released during digestion in the small intestine and can be absorbed, while the remaining phenolics enter the colon, where they are released by fermentation and can counteract the prooxidants (Kljak et al., 2009).
Due to their antioxidant, antimicrobial and anti-inflammatory properties, phenolics are considered a natural alternative to antibiotics in animal production. Numerous studies have been conducted so far on a wide range of herbs and plant extracts rich in phenolics from different subgroups (Hashemzadeh-Cigari et al., 2014; Mahfuz et al., 2021; Maggiolino et al., 2021). Thus, maize phenolics could contribute to the health status and well-being of animals. In addition, farm animals can deposit phenolics in meat and egg yolks, where they improve quality by improving oxidative stability. This effect has also been demonstrated for maize. Duvnjak et al. (2018) demonstrated that maize phenolics reduce the formation of malondialdehyde in yolks exposed to induced lipid oxidation.
Naturally occurring vitamin E (tocols) consists of eight vitamers (α-, β-, γ-, and δ-tocopherols and their corresponding unsaturated tocotrienols). Of them, α-tocopherol has the highest vitamin E activity, which is why the term vitamin E is used synonymously for this tocol. The activity of the other tocols compared to α-tocopherol is 50% for β-tocopherol, 10% for γ-tocopherol, 3% for δ-tocopherol, 30% for α-tocotrienol, 8% for γ-tocotrienol, and 5% for β-tocotrienol, while it has not been demonstrated for δ-tocotrienol (Szewczyk et al., 2021). Maize contains α-, γ- and δ-tocopherol and α- and γ-tocotrienol, and their average contents in 105 commercial maize hybrids were 4.1, 25.0, 0.7, 0.9 and 2.2 μg g^{-1} DM, respectively (Zurak et al., 2021). Additionally, increased tocol content is accompanied by increased content of unsaturated fatty acids in grain (Goffman and Böhme, 2001).

Tocols have anti-inflammatory and antioxidant functions, while their non-antioxidant functions are possible through specific interactions with enzymes, structural proteins, structural lipids, and transcription factors (Szewczyk et al., 2021). In addition, the antioxidant activity of individual tocols varies. In some biological processes, tocotrienols are more effective than tocopherols, while, compared to α-tocopherol, γ-tocopherol, the most abundant tocol in maize, has the ability to scavenge reactive forms of nitrogen (Jiang, 2014; Sen et al., 2006). In addition, tocols can be further metabolized, and the resulting metabolites affect the activity of transcription factors (Szewczyk et al., 2021). The combination of supplemental vitamin E and selenium has been shown to ameliorate oxidative stress in animals exposed to heat stress or contamination with mycotoxins (He et al., 2013; Zhang et al., 2020). Apart from health effects, tocols can also be deposited in meat and egg yolk and improve their quality (Zhang et al., 2020). Although maize has moderate tocol content, it can undoubtedly strengthen the immune system of farm animal and improve the quality of their products.

Conclusions

Maize is a rich source of phytochemicals in animal nutrition. While some of them have been studied and shown to have beneficial effects, others contribute significantly to animal health status and production performance due to the high proportion of maize grain in the diet. In addition, all maize phytochemicals could have additive and synergistic effects and improve animal health status to a greater extent than any single compound (Siyuan et al., 2018). Moreover, if these phytochemicals are not used to improve the health status of animals, they are deposited in the tissues and improve the quality of meat and egg yolk. These products also become their sources in the human diet. Therefore, maize should not be considered only as a source of energy in the diet of farm animals, and further studies on its beneficial effects are needed. In addition, breeding maize hybrids should also improve the content of phytochemicals, not only yield and pest resistance, so that the potential of maize to enhance animal health and well-being could be used to reduce the cost of additives in compound feed production.

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References

Álvarez R., Meléndez-Martínez A. J., Vicario I. M., Alcalde M. J. (2015). Carotenoid and vitamin A contents in biological fluids and tissues of animals as an effect of the diet: A review. Food Reviews International. 31(4): 319-340.

Bird A. R., Vuaran M., Brown I., Topping D. L. (2007). Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs. British Journal of Nutrition. 97(1): 134-144.

Campos-Vega R., Oomah B. D. (2013). Chemistry and classification of phytochemicals. In: *Handbook of plant food phytochemicals: Sources, stability and extraction*, Tiwari B. K., Brunton N. P., Brennan C. S. (eds.), 5-48. Oxford, UK: John Wiley & Sons, Ltd.

Deckardt K., Khol-Parisini A., Zebeli Q. (2013). Peculiarities of enhancing resistant starch in ruminants using chemical methods: opportunities and challenges. Nutrients 5(6): 1970-1988.

Díaz-Gómez J., Moreno J. A., Angulo E., Sandmann G., Zhu C., Ramos A. J., Capell T., Christou P., Nogareda, C. (2017). High-carotenoid biofortified maize is an alternative to color additives in poultry feed. Animal Feed Science and Technology. 231: 38-46.

Duvnjak M., Kljak K., Palačić E., Gorupić M., Pintar J., Janječić Z., Grbeša D. (2018). Effect of maize hybrid antioxidant potential on egg yolk oxidative stability – correlation analysis. Proceedings of the XVth European Poultry Conference, Prukner-Radovčić E., Medić H. (eds.), 465. Zagreb, Croatia: Croatian Branch of the World's Poultry Science Association.

Eeckhout W., De Paepe, M. (1994). Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal feed Science and Technology. 47(1-2): 19-29.

Englyst H. N., Kingman S. M., Cummings J. H. (1992). Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition. 46: S33-50.

Grashorn M. (2016). Feed additives for influencing chicken meat and egg yolk color. In: *Handbook on Natural Pigments in Food and Beverage*, Carle R., Schweiggert R. (eds.), 283-302. Duxford, UK: Woodhead Publishing.

Goffman F. D., Böhme T. (2001). Relationship between fatty acid profile and vitamin E content in maize hybrids (*Zea mays* L.). Journal of Agricultural and Food Chemistry. 49(10): 4990-4994.

Grbeša D., Kljak K. (2017). Content and antioxidant activity of phenolic compounds from Bc maize hybrids. *Book of Abstracts of the 24th International conference "Krmiva 2017"*, Lulić S., Modrić M. (eds.), 7-8. Zagreb, Croatia: Krmiva.

Hashemzadeh-Cigari F., Khorvash M., Ghorbani G. R., Kadivar M., Riasi A., Zebeli Q. (2014). Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. Journal of Dairy Science. 97(12): 7487-7497.

He X., Sun W., Ge T., Mu C., Zhu W. (2017). An increase in corn resistant starch decreases protein fermentation and modulates gut microbiota during in vitro cultivation of pig large intestinal inocula. Animal Nutrition. 3(3): 219-224.

He J., Zhang K. Y., Chen D. W., Ding X. M., Feng G. D., Ao X. (2013). Effects of vitamin E and selenium yeast on growth performance and immune function in ducks fed maize naturally contaminated with aflatoxin B_1. Livestock Science: 152(2-3): 200-207.

Heying E. K., Grahn M., Pixley K. V., Rocheford T., Tanumihardjo S. A. (2013). High-provitamin A carotenoid (orange) maize increases hepatic vitamin A reserves of offspring in a vitamin A-depleted sow-piglet model during lactation. The Journal of Nutrition. 143(7): 1141-1146.

Jiang Q. (2014). Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radical Biology and Medicine. 72: 76-90.

Kljak K., Carović-Stanko K., Kos I., Janječić Z., Kiš G., Duvnjak M., Safner T., Bedeković, D. (2021a). Plant carotenoids as pigment sources in laying hen diets: Effect on yolk color, carotenoid content, oxidative stability and sensory properties of eggs. Foods. 10(4): 721.

Kljak K., Duvnjak M., Bedeković D., Kiš G., Janječić Z., Grbeša D. (2021b). Commercial corn hybrids as a single source of dietary carotenoids: Effect on egg yolk carotenoid profile and pigmentation. Sustainability. 13(21): 12287.

Kljak K., Duvnjak M., Grbeša D. (2019). Effect of starch properties and zein content of commercial maize hybrids on kinetics of starch digestibility in an *in vitro* poultry model. Journal of the Science of Food and Agriculture. 99(14): 6372-6379.

Kljak K., Grbeša D. (2015). Carotenoid content and antioxidant activity of hexane extracts from selected Croatian corn hybrids. Food Chemistry. 167: 402-408.

Kljak K., Kiš G., Grbeša D. (2009). *In vitro* digestibility of phenolics in grain of maize hybrids. Italian Journal of Animal Science. 8(sup3): 166-168.

Kljak K., Madjeruh M., Makar A., Janječić Z., Grbeša D. (2018). Broiler chicken pigmentation is influenced by maize hybrid as the only source of carotenoids. *Proceedings of the XVth European Poultry Conference*, Prukner-Radovčić E., Medić H. (eds.), 326. Zagreb, Croatia: Croatian Branch of the World's Poultry Science Association.

Liu R. H. (2007). Whole grain phytochemicals and health. Journal of Cereal Science. 46(3): 207-219.

Liu Y. S., Zhang Y. Y., Li J. L., Wang X. F., Xing T., Zhu X. D., Zhang L., Gao F. (2020). Growth performance, carcass traits and digestive function of broiler chickens fed diets with graded levels of corn resistant starch. British Poultry Science. 61(2): 146-155.

Mahfuz S., Shang Q., Piao X. (2021). Phenolic compounds as natural feed additives in poultry and swine diets: A review. Journal of Animal Science and Biotechnology. 12(1): 1-18.

Maggiolino A., Bragaglio A., Salzano A., Rufrano D., Claps S., Sepe L., Damiano S., Ciarcia R., Dinardo, F. R., Hopkins, D. L., Neglia, G., De Palo P. (2021). Dietary supplementation of suckling lambs with anthocyanins: Effects on growth, carcass, oxidative and meat quality traits. Animal Feed Science and Technology. 276: 114925.

Matthé A., Lebzien P., Hric I., Flachowsky G., Sommer A. (2001). Effect of starch application into the proximal duodenum of ruminants on starch digestibility in the small and total intestine. Archives of Animal Nutrition. 55(4): 351-369.

Moreno J. A., Díaz-Gómez J., Fuentes-Font L., Angulo E., Gosálvez L. F., Sandmann G., Portero-Otin, M., Capell T., Zhu C., Christou P., Nogareda, C. (2020). Poultry diets containing (keto)carotenoid-enriched maize improve egg yolk color and maintain quality. Animal Feed Science and Technology. 260: 114334.

M'Sadeq S. A., Wu S. B., Swick R. A., Choct M. (2015). Dietary acylated starch improves performance and gut health in necrotic enteritis challenged broilers. Poultry Science. 94(10): 2434-2444.

Nogareda C., Moreno J. A., Angulo E., Sandmann G., Portero M., Capell T., Zhu C., Christou, P. (2016). Carotenoid-enriched transgenic corn delivers bioavailable carotenoids to poultry and protects them against coccidiosis. Plant Biotechnology Journal. 14(1): 160-168.

Nozière P., Graulet B., Lucas A., Martin B., Grolier P., Doreau M. (2006). Carotenoids for ruminants: From forages to dairy products. Animal Feed Science and Technology. 131(3-4): 418-450.

Ortiz D., Lawson T., Jarrett R., Ring A., Scoles K. L., Hoverman L., Rocheford E., Karcher D. M., Rocheford, T. (2021). Biofortified orange corn increases xanthophyll density and yolk pigmentation in egg yolks from laying hens. Poultry Science. 100(7): 101117.

Pickworth C. L., Loerch S. C., Kopec R. E., Schwartz S. J., Fluharty F. L. (2012). Concentration of pro-vitamin A carotenoids in common beef cattle feedstuffs. Journal of Animal Science. 90(5): 1553-1561.

Raigond P., Ezekiel R., Raigond, B. (2015). Resistant starch in food: a review. Journal of the Science of Food and Agriculture. 95(10): 1968-1978.

Sajilata M. G., Singha R. S., Kulkarni P. R. (2006). Resistant starch – a review. Comprehensive Reviews in Food Science and Food Safety. 5(1): 1-17.

Sauvant D., Perez J. M. Tran, G. (2004). Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen, Netherlands: Wageningen Academic Publishers.

Sen C. K., Khanna S., Roy, S. (2006). Tocotrienols: Vitamin E beyond tocopherols. Life Sciences. 78(18): 2088-2098.

Siyuan S., Tong L., Liu R. (2018). Corn phytochemicals and their health benefits. Food Science and Human Wellness. 7(3): 185-195.

Suri D. J., Tanumihardjo S. A. (2016). Effects of different processing methods on the micronutrient and phytochemical contents of maize: from A to Z. Comprehensive Reviews in Food Science and Food Safety. 15(5): 912-926.

Szewczyk K., Chojnacka A., Górnicka, M. (2021). Tocopherols and tocotrienols – Bioactive dietary compounds; what is certain, what is doubt? International Journal of Molecular Sciences. 22(12): 6222.

Tan F. P., Beltranena E., Zijlstra R. T. (2021). Resistant starch: Implications of dietary inclusion on gut health and growth in pigs: a review. Journal of Animal Science and Biotechnology. 12(1): 1-15.

Viola I., Marinelli A. (2016). Life Cycle Assessment and environmental sustainability in the food system. Agriculture and Agricultural Science Procedia. 8: 317-323.

Vuolo M. M., Lima V. S., Junior M. R. M. (2019). Phenolic compounds: Structure, classification, and antioxidant power. In *Bioactive compounds*, Segura Campos M. R. (ed.), 33-50. Duxford, United Kingdom: Woodhead Publishing.

Wang S. P., Wang W. J., Tan Z. L. (2016). Effects of dietary starch types on rumen fermentation and blood profile in goats. Czech Journal of Animal Science. 61(1): 32-41.

Weurding R. E. (2002). Kinetics of starch digestion and performance of broiler chickens. Doctoral thesis. Wageningen, Netherlands: Wageningen University and Research.

Yang A., Larsen T. W. Tume, R. K. (1992). Carotenoid and retinol concentrations in serum, adipose tissue and liver and carotenoid transport in sheep, goats and cattle. Australian Journal of Agricultural Research. 43(8): 1809-1817.

Zhang M., Dunshea F. R., Warner R. D., DiGiacomo K., Osei-Amponsah R., Chauhan, S. S. (2020). Impacts of heat stress on meat quality and strategies for amelioration: A review. International Journal of Biometeorology. 64(9): 1613-1628.

Zhang Y., Liu Y., Li J., Xing T., Jiang Y., Zhang L., Gao F. (2020). Dietary corn-resistant starch suppresses broiler abdominal fat deposition associated with the reduced cecal Firmicutes. Poultry Science. 99(11): 5827-5837.

Zurak D., Grbeša D., Duvnjak M., Kiš G., Međimurec T., Kljak K. (2021a). Carotenoid content and bioaccessibility in commercial maize hybrids. Agriculture. 11(7): 586.

Zurak D., Duvnjak M., Grbeša D., Kiš G., Kljak K. (2021b). Kernel hardness affects carotenoid digestibility in commercial maize hybrids. *Proceedings of the 1st Virtual Conference on Carotenoids*, 47. Cleveland, Oh, USA: International Carotenoid Society and Case Western University.

Zurak D., Duvnjak M., Kiš G., Grbeša D., Kljak K. (2021). Bioaccessibility of tocols as a prerequisite for their utilization from maize grain. *Book of Abstracts of the 56th Croatian and 16th International Symposium on Agriculture*, Rozman V., Antunović Z. (eds.), 271-272. Osijek, Croatia: Faculty of Agrobiotechnical Sciences Osijek University Josip Juraj Strossmayer in Osijek.

Zurak D., Kljak K., Grbeša, D. (2020). The composition of floury and vitreous endosperm affects starch digestibility kinetics of the whole maize kernel. Journal of Cereal Science. 95: 103079.

Unaprijeđena hranidbena vrijednost kukuruza - blagotvorni utjecaj fitokemikalija

Sažetak
U prirodi, životinje biraju krmiva koja sadrže i hranjive tvari i fitokemikalije, spojeve koji imaju blagotvoran učinak na zdravlje životinja i ljudi. U održivoj proizvodnji domaćih životinja, krmiva dodane vrijednosti zadovoljavaju potrebe za hranjivim tvarima i održavaju učinkovitu probavu i metaboličko zdravlje. Zbog visokog sadržaja fitokemikalija poput karotenoida, rezistentnog škroba, tokola i fenola, zrno kukuruza je takvo krmivo. Ove fitokemikalije imaju blagotvoran učinak na zdravlje i dobrobit životinja, a ukoliko se ne iskoriste za tu svrhu, životinje će ih deponirati u svoja tkiva. Na taj način poboljšavaju kvalitetu mesa i žutanjka jajeta, ali i postaju izvori fitokemikalija u prehrani ljudi. U ovom radu prikazan je sadržaj fitokemikalija zrna kukuruza te predstavljen njihov potencijalan blagotvoran učinak na domaće životinje.

Ključne riječi: kukuruz, karotenoidi, rezistentni škrob, fenoli, tokoli

There is no place like home - importance of breeding locally adapted honey bees (*Apis mellifera*)

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Abstract
The honey bee (*Apis mellifera*) provides pollination services for many plant species. Over the last decade, major losses of honey bee colonies have been recorded worldwide, mainly as a consequence of diseases, climate changes, agricultural and beekeeping practices. In order to improve the condition of their apiaries, beekeepers must adapt by applying new management strategies or introducing a new concept of colonies protection against diseases. One of the steps that can be taken is to keep and breed honey bees of local origin, bees that are adapted to the specific environment they inhabit. In this review article, the importance of local adaptation and current status of Carniolan honey bee in Croatia has been presented.

Key words: *Apis mellifera,* Carniolan, local adaptation, breeding

Introduction

The honey bee (*Apis mellifera* L.) is economically one of the most important insects in the agroecosystem (Klein et al., 2007), which by its pollination activities, increases the worldwide annual crop production value between 235 and 577 billion of US $ (Lautenbach et al., 2012). This value greatly exceeds the value of the production of the beekeeping sector. However, during the last decade beekeepers are facing serious problems with reduced yields of honey and high colony losses, caused mainly by diseases (Brodschneider et al., 2018), intensive agriculture (Chmiel et al., 2020; Muñoz-Colmenero et al., 2020; Tomé et al., 2020) and climate changes (Le Conte and Navajas, 2008; Flores et al., 2019). The direct economic cost of winter honey bee colony losses often exceeds the values of subsidies provided to beekeepers (Popovska Stojanov et al., 2021). To mitigate the overall negative stress of the environment on honey bees, beekeepers need to adapt their management strategies and gain new knowledge and skills, often recognised as a major problem in the apicultural sector (Jacques et al., 2017). This could be achieved by implementing novel management strategies in protecting honey bee colonies from ectoparasitic mite *Varroa destructor* (Büchler et al., 2020) and by breeding locally adopted honey bees with better resistance mechanisms towards Varroa (Kovačić et al., 2020; Mondet et al., 2020).

The honey bees naturally inhabit the area of Africa, Europe, Western and Central Asia, from where it was widespread throughout the world. The taxonomy of the honey bee is determined by morphological characteristics, and 28 subspecies have been described to date (Ruttner, 1988; Sheppard et al., 1997; Sheppard and Meixner, 2003; Meixner et al., 2013; Chen et al., 2016). Based on morphological, ecological, behavioural, genetic and physical parameters, four evolutionary lineages were determined: African branch (A), northern and western European branch (M), southeaster European branch (C) and Middle Eastern branch (O) (Ruttner, 1988.). In Western and Northern Europe there are naturally subspecies of honey bees belonging to the M evolutionary branch (*A. m. mellifera and A. m. iberiensis*), and in Central and South-Eastern Europe, subspecies of the C evolutionary branch (*A. m. ligustica*, *A. m. carnica*, *A. m. macedonica* and *A. m. cecropia*). Today, due to its desired traits such as

gentleness and high honey production, Carniolan honey bee (*A. m. carnica*) is a one of the most widespread and commercially used subspecies in the world (Lodesani and Costa, 2003; Meixner et al., 2010). In Croatia, Carniolan bee is an autochthonous honey bee with three recognised ecotypes: subalpine (mountain), Pannonian and Mediterranean (Ruttner, 1988., Kezić et al., 2001; Muñoz et al., 2009; Bouga et al., 2011, Puškadija et al., 2020).
In the following paragraphs, we will clarify the importance of keeping and breeding locally adapted honey bees on their survival and on the production, behavioural and disease resistance traits. Further, we will show evidence on the selection progress when all aspects of the breeding cycle are included (Uzunov et al., 2017). Finally, we will present the current situation of the Carniolan bee in Croatia and its possible improvement in the future.

Importance of local adaptation

The local adaptation and its importance have been studied since the beginning of understanding the process of evolution. Its importance is reflected in the fact that locally adapted individuals usually show better fitness in their local area than individuals from elsewhere (Williams, 1966). The same is true and demonstrated for the honey bees as well. The importance and relevance of the local adaptation were in the study's essence, shortly named GEI (Genotype Environment Interactions, Costa et al., 2012., www.beebreeding.net). A Europe-wide experiment for assessing the impact of GEI on the vitality of honey bee colonies was conducted from 2009. to 2012. when more than 600 colonies were established at 21 apiaries across 11 European countries from Scandinavia to the Mediterranean (Figure 1, Meixner et al., 2015.). Using standardised management and evaluation methodology, the colonies originated from five honey bee subspecies (*A. m. carnica, A. m. ligustica, A. m. siciliana, A. m. mellifera, and A. m. macedonica*), were compared for their performance as well as vitality and survivability against the non-local colonies. After the initial treatment for Varroa mite (*Varroa destructor*) control, the colonies were managed without additional chemical treatment. However, highly infested colonies were removed from the study and considered dead. The Varroa infestation level was regularly monitored, and samples were collected to identify the presence of *Nosema spp*. and two honey bee viruses (ABPV and DWV).

Figure 1. Distribution of 16 genotypes (abbreviations in the right top legend) from 5 different honey bee subspecies across 21 locations in Europe (Meixner et al., 2015).

The field study and the comparison of the colonies terminated after 912 days (30 months) when only around 15% of the colonies survived. In the end, there were significant differences in the survival between the colonies from local and non-local origins. Across all locations, the local colonies, on average, survived for 553 days, which was 83±23 ($p<0.001$) days longer than the colonies headed with queens from the non-local origin (Figure 2, Büchler et al., 2014).

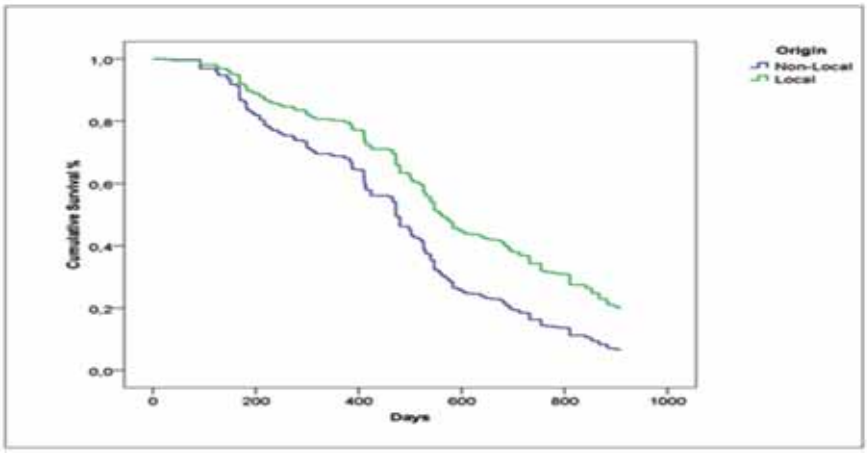

Figure 2. Colony survival for the origin of queens (local vs. non-local) during the experiment. (Büchler et al., 2014).

The Varroa infestation and infections with ABPV and DWV were strongly influenced by location (apiary) compared to the effect of the origin. Still, in some apiaries, the colonies from local origin had lower loads of pathogens (Francis et al., 2014). Interestingly, in the GEI study, the lines found by genetic analysis to be hybridised expressed significantly higher defensive behaviour, i.e., were less gentle (Uzunov et al., 2014; Francis et al., 2014). This finding was in line with the results from other authors (Ruttner, 1988; Fresnaye and Lavie, 1976). A similar trend was also observed in the case of honey yield when the colonies from local origin produced more honey than the non-native, although the difference was not significant (Hatjina et al., 2014).

The outcomes of the GEI study show that in future, more efforts need to be devoted and invested in the conservation and genetic improvement of the locally adapted honey bee populations. In fact, this conclusion was the leading idea throughout the EU founded SMARTBEES project (www.smartbees.eu) implemented from 2014 to 2018. The Work Package 6 "Testing and selection of honey bee populations" significantly improved understating and the importance of the local adaptation in honey bees (Büchler and Uzunov, 2016). Therefore, by applying the breeding concept "conservation by utilisation" and use of standardised protocols, based on the experience in Central Europe, SMARTBEES initiated or rebooted breeding initiatives for genetic improvement and conservation of many populations from A. *m. ruttneri, A. m. macedonica, A. m. siciliana, A. m. carnica, A. m. iberiensis, A. m. mellifera, A. m. cecropia* (Uzunov et al., 2015 and 2017). In addition, the SMARTBEES consortium developed a honey bee subspecies diagnostic tool that enables the beekeepers, breeders, honey bee experts, extensionists and researchers to monitor the hybridisation rate of the colonies and certify the subspecies origin or to control queen trade (Momeni et al., 2021).

Kovačić et al. (2020) studied the consequence of selective breeding and local adaptation by comparing queens of local origin with 5 generation of selection (MS) with queens of local origin without any organised selection history (NS) and queens of foreign origin (Germany) with high selection history (HS). Manageability traits such as defensive behaviour, calmness on the comb and swarming drive (DB, CAL and SWA in the Table 1) are under clear influence of selection and change of environment didn't show to have an effect on their expression. However, when parameters related to the colony's fitness are considered, that is colony strength, development and success of overwintering (bees, brood and OI in the Table 1), foreign (HS) group showed clear signs of maladaptation.

Table 1. Differences between groups for measured parameters: number of bees (Bees), number of brood cells (Brood), overwintering index (OI), defensive behavior (DB), calmness on the comb (CAL), swarming behavior (SWA), honey extraction (HE), adult bee infestation with *V. destructor* (BI), percentage of non-reproducing mites (SMR), recapping of brood cells (REC) and hygienic behavior (HYG). The color and direction of the arrows shows significant differences within the column. Significant differences are between red and green arrows, while blue arrows present no significant difference. Direction of arrows show which trait is better in which group (Kovačić et al., 2020).

Group	Bees	Brood	OI	DB	CAL	SWA	HE	BI	SMR	REC	HYG
NS	↓	↓	↑	↓	↓	↓	→	→	→	↑	→
MS	↑	↑	↑	→	↑	↑	→	→	→	→	→
HS	↓	↓	↓	↑	→	↑	→	→	→	↓	→

The biggest experiment conducted on honey bees so far in the frame of the EurBeST project (Büchler et al., 2022), was performed on 3565 colonies in 7 European countries. The aim was to compare how genotypes selected for increased Varroa resistance traits (such as hygienic behaviour, VSH, SMR and recapping) express these traits in different environments, comparing to the local bees. In addition, all traits relevant to selection and breeding were measured (Uzunov et al., 2021). As in the previous studies, colonies with queens of the local origin showed higher honey production and were better in strength than those with the foreign queens. However, when it comes to the Varroa resistance traits, no influence of local adaptation was found but instead, an effect of selection on Varroa resistance was observed, and colonies headed by queens coming from breeding programs (including varroa resistance traits) showed significantly lower infestation with mites in the end of the trial (Figure 3).

Honey bees are adapted to the local environment not only by brood cycle during the year (Strange et al. 2007; Dražić et al., 2014), but also at the metabolic level (Parker et al., 2010, Salmela et al., 2015) and locally adapted bees show higher resistance to the local pathogens (Francis et al. 2014, Hatjina et al., 2014). Furthermore, microbiota is likely to play a role in local adaptation, as it is known that the environment is one of the major factors that influence the shape of microbiota (Muñoz-Colmenero et al., 2020).

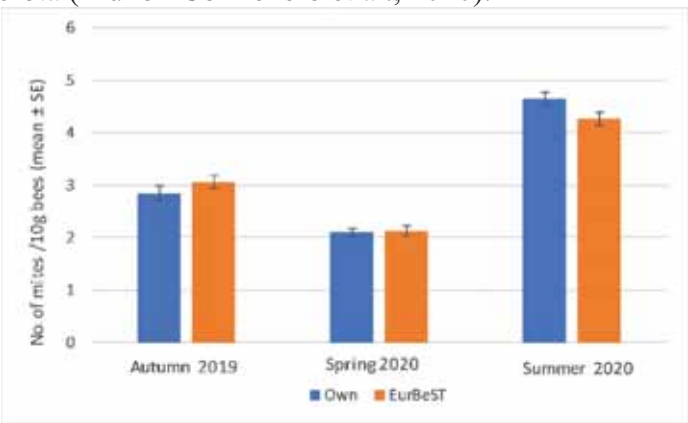

Figure 3. The average infestation of colonies with Varroa mite in the EurBeST study, comparing beekeeper's own stock (blue) to the preselected EurBeST stock (orange) in autumn 2019, spring 2020 and summer 2020 (Büchler et al., 2022).

The game of drones

With the discovery that the queen mates with several drones (Woyke, 1955, Ruttner, 1956), the understanding on the importance of controlled mating of queens also began. Without controlled mating, a little or no gain is made in selection progress (Plate et al., 2019). To find a mating partner, queens and drones may fly up to 5 km (Ruttner and Ruttner, 1972) to so called "drone congregation areas" where queen mates with 10-20 drones (Neumann et al., 1999; Tarpy et al., 2015; Withrow and Tarpy, 2018).

Hybridisation may cause the mixture of two subspecies if they find themselves close to each other. There is a plenty of studies on the hybridisation of native bees with imported ones (Nazzi, 1992; Soland-Reckeweg et al., 2009; Parejo et al., 2016; Oleksa et al., 2011; Calfee et al., 2020; Oleksa et al., 2021) which are mainly the results of beekeeping activities such as colony migrations and trade of swarms and queen bees. In the worst case scenario this may lead to the loss of genetic diversity and hybridisation (Jensen et al., 2005.; Oleksa et al., 2011; Pinto et al., 2015; Parejo et al., 2016).

In the frame of the "BeeConSel" project (Honey bee conservation and selection, www.beeconsel.eu) different types of mating control concepts are test and assess in Croatia, Macedonia and Slovenia using field observations and molecular tools. Thus, in the project, isolated mountain valleys, glades in the woods or plateau in the hills, then, saturation of the mating station with a large number of drones of the desired origin, instrumental insemination and the novel "moonshine mating" method are under scrutiny. The results of the project will show which approach in controlled mating from a practical point of view can be recommended to queen breeders in different areas.

Think globally, act locally

The different environment that the Carniolan honey bee naturally inhabits, from the Alps through Pannonian valley to the Adriatic sea, leads to the adaptation of this honey bee to different regions (Ruttner, 1992). Possible morphological and genetical differences were the subject of research by several scientific groups. Bubalo et al. (1997) measured the cubital index on the wings of worker bees collected from different regions and didn't find significant differences between regions. However, the study on the measurements on the hind legs of the worker bees by Dražić et al. (1998) showed some differentiation of samples from Dalmatia. Further, the study of Muñoz et al. (2009) using microsatellite and mitochondrial markers showed differentiation of north Adriatic subpopulations from the south Adriatic. However, the greatest difference between ecotypes could be noticed when measuring the brood cycle. Dražić et al. (2014) compared bees from Alps (Austria) with bees from the Pannonian valley in both regions and found that bees transferred to the other region follow brood cycle regime from their area of origin.

The study of Sušnik et al. (2004) on microsatellites and mitochondrial DNA using samples of worker bees from Slovenia and Croatia showed that there is no sign of the possible introduction of different subspecies in the studied area. Puškadija et al. (2020.) collected samples of worker bees from 160 apiaries from all regions in Croatia and analysed them using wing geometric morphometrics. The samples were collected from non-migratory apiaries and beekeepers that do not buy queens and swarms to get as much local honey bee as possible. The analysis showed that out of 160 samples, 1 was not related to Carniolan bee for sure, and for additional four was assumed to be hybrid between Carniolan and some other subspecies. All other samples were classified as *A. m. carnica*, which present almost 97%, meaning that Carniolan honey bee is still quite pure in Croatia. Although hybridisation has been found in a small number of samples, the main hybridisation zones are located at the endpoints where Carniolan bee resides. On the southeast region of Serbia there is a hybridisation with *A. m. macedonica* (Nedić et al., 2014) in Poland with *A. m. mellifera*

(Pentek-Zakar et al., 2015) and with *A.m.ligustica* in the southwest parts of Slovenia (Sušnik et al., 2004; Kozmus, 2011).
In Croatia in 1997. the breeding program was established with the aim of selection and protection of autochthones Carniolan honey bee (Dominiković et al., 1997). The breeding program is implemented by the Association of breeders of selected queen bees in Croatia. Around 50 registered breeders work on selection and breeding on their local stock and rear about 50.000 of queens per year.

Conclusions

Carniolan honey bee is still present and quite pure in Croatia, although hybridisation with other subspecies in a few places is found. Because of the specific mating behaviour, importation of queens from other subspecies in Croatia would lead to admixture and hybridisation of bees in the wider area. The hybridisation of native bees with imported ones, which is evident in several European countries, leads to the expensive conservation program and establishment of controlled mating to preserve and genetically improve the local honey bee. Further, several research groups confirmed that locally adapted honey bees show better performance than imported ones for most of the traits, especially survival. For these reasons, it is now urgent to organise the cooperation of all relevant stakeholders to preserve our local varieties of Carniolan honey bee from hybridisation with other subspecies and define strategy for its protection and conservation. If hybridisation occurs, we would need to invest much more time, effort and money in to the conservation of our native pure honey bee.

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References

Bouga M., Alaux C., Bienkowska M., Büchler R., Carreck N., Cauia E., Chlebo R., Dahle B., Dall'Olio R., De la Rúa P., Gregorc A., Ivanova E., Kence A., Kence M., Kezic N., Kiprijanovska H., Kozmus P., Kryger P., Le Conte Y., Lodesani M., Murilhas A.M., Siceanu A., Soland G., Uzunov A., Wilde J. (2011). A review of methods for discrimination of honey bee populations as applied to European beekeeping. Journal of Apicultural Research. 50(1): 51-84.

Brodschneider R., Gray A., Adjlane N., Ballis A., Brusbardis V., Charriere J-D., Cheblo R., Coffey, M.F., Dahle B., de Graaf D.C., Dražić, M.M., Evans G., Fedoriak M., Forsythe I., Gregorc A., Grzeda U., Hetzroni A., Kauko L., Kristiansen P., Martikkala M., Martin-Hernandez R., Medina-Flores C.A., Mutinelli F., Raudments A., Ryzhikov V.A., Simon-Delso N., Stevanović J., Uzunov A., Vajsnaes F., Woehl S., Zammit-Mangion M., Danihlik J. (2018). Multi-country loss rates of honey bee colonies during winter 2016/2017 from the COLOSS survey. Journal of Apicultural Research. 57 (3): 452-457.

Bubalo D., Dražić M., Odak M., Kezić N. (1997). The biological diversity of the Carniolan bee (*Apis mellifera carnica* Pollmann, 1879) in Croatia and neighbouring countries. In *Proceedings of 3. Colloquium Entomofauna of Croatia and Adjoining Countries*, Croatia, 9–10 December, pp. 19–20.

Büchler R., Uzunov A., Kovačić M., Prešern J., Pietropaoli M., Hatjina F., Pavlov B., Charistos L., Formato G., Galarza E., Gerula D., Gregorc A., Malagnini V., Meixner M.D., Nedić N., Puškadija Z., Rivera-Gomis J., Rogelj Jenko M., Smodiš Škerl M.I.,

Vallon J., Vojt D., Wilde J., Nanetti A. (2020). Summer brood interruption as integrated management strategy for effective Varroa control in Europe. Journal of Apicultural Research. 59 (5): 764–773.

Büchler R., Uzunov A., Costa C., Meixner M., Le Conte, Y., Mondet F., Kovačić M., Andonov S., Carreck N.L., Dimitrov L., Basso B., Bienkowska M., Dall'Olio R., Hatjina F., Wirtz U. (2022). EurBeST — A Pilot Study Testing Varroa-resistant Bees Under Commercial Beekeeping Conditions. American Bee Journal. 2: 213-215.

Calfee E., Agra M.N., Palacio M.A., Ramírez S.R., Coop G. (2020). Selection and hybridisation shaped the rapid spread of African honey bee ancestry in the Americas. PLoS Genetic. 16 (10): e1009038.

Chen C., Liu Z., Pan Q., Chen X., Wang H., Guo H., Liu S., Lu H., Tian S., Li R., Shi W. (2016). Genomic Analyses Reveal Demographic History and Temperate Adaptation of the Newly Discovered Honey Bee Subspecies *Apis mellifera sinisxinyuan* n. ssp. Molecular Biology and Evolution. 33(5):1337-1348.

Costa C., Büchler R., Berg S., Bienkowska M., Bouga M., Bubalo D., Charistow L., Le Conte Y., Drazic M., Dyrba W., Filipi J., Hatjina F., Ivanova E., Kezic N., Kiprijanovska H., Kokinis M., Korpela S., Kryger P., Lodesani M., Meixner M., Panasiuk B., Pechhacker H., Petrov P., Oliveri E., Ruottinen L., Uzunov A., Vaccari G., Wilde J. (2012). A Europe-wide experiment for assessing the impact of genotype–environment interactions on the vitality and performance of honey bee colonies: experimental design and trait evaluation. Journal of Apicultural Sciences. 56 (1): 147–158.

Chmiel J.A., Daisley, B.A., Pitek, A.P., Thompson, G.J., Reid, G. (2020). Understanding the Effects of Sublethal Pesticide Exposure on Honey Bees: A Role for Probiotics as Mediators of Environmental Stress. Frontiers in Ecology and Evolution 8.

Dominiković Z., Brence-Lazarus T., Bubalo D., Dražić M., Kezić N. (1997). Program gojidbenog stvaranja pčela u Republici Hrvatskoj. Hrvatski stočarsko selekcijski centar.

Dražić M., Bubalo D., Kezić D., Odak M., Grbić D., Kezić N. (1998). Morfometric differences of Mediterranean ecotype of Carniolan bee (*A. m. carnica*). In *Proceedings of XXXIV Znanstveni Skup Hrvatskih Agronoma.* Opatija.

Dražić M.M., Filipi J., Prđun S., Bubalo D., Špehar M., Cvitković D., Kezić D., Pechacker H., Kezić N. (2014). Colony development of two Carniolan genotypes (*Apis mellifera carnica*) in relation to environment. Journal of Apicultural Research. 53(2): 261-268.

Fresnaye J., Lavie P. (1976). Selection et hybridation de 1'abeille en France. *Symp. Génétique, selection et reproduction de 1'abeille.* Bulletin Technique Apicole OPIDA 3 15-20.

Francis R.M., Amiri E, Meixner M., Kryger P, Gajda A., Andonov S., Uzunov A., Topolska G., Charistos L., Costa C., Berg S., Bienkowska M., Bouga M., Buchler R., Dyrba W., Hatjina F., Ivanova E., Kezić N., Korpela S., Le Conte Y., Panasiuk B., Pechaker H., Tsoktouridis G., Wilde J. (2014). Effect of genotype and environment on parasite and pathogen levels in one apiary - a case study. Journal of Apicultural Research. 53(2): 230-232.

Hatjina F., Costa C., Büchler R., Uzunov A., Drazic M., Filipi J., Charistos L., Ruottinen L., Andonov S., Meixner M.D., Bienkowska M., Dariusz G., Panasiuk B., Le Conte Y., Wilde J., Berg S., Bouga M., Dyrba W., Kiprijanovska H., Korpela S., Kryger P., Lodesani M., Pechhacker H., Petrov P., Kezic N. (2014). Population dynamics of European honey bee genotypes under different environmental conditions. Journal of Apicultural Research. 53(2): 233-247.

Jacques A., Laurent M., EPILOBEE Consortium, Ribière-Chabert M., Saussac M., Bougeard S., Budge G.E., Hendrikx P., Chauzat M-P. (2017). A pan-European

epidemiological study reveals honey bee colony survival depends on beekeeper education and disease control. PLOS ONE. 12(3): e0172591.

Jensen A.B., Palmer K.A., Boomsma J.J., Pedersen B.V. (2005). Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the black honeybee, *Apis mellifera mellifera*, in northwest Europe. Molecular Ecology. 14: 93–106.

Flores J.M., Gil-Lebrero S., Gámiz V., Rodríguez M.I., Ortiz M.A., Quiles F.J. (2019). Effect of the climate change on honey bee colonies in a temperate Mediterranean zone assessed through remote hive weight monitoring system in conjunction with exhaustive colonies assessment. Science of The Total Environment. 653: 1111-1119.

Kezić N., Dražić M., Bubalo D., Mustapić Z. (2001). Breeding and selection of carniolan bees (*A. m. carnica*) in Croatia. *Abstracts of the 37th International Apicultural Congress - Apimondia 2001.* Apimondia Press (ur.). - Durban: Apimondia Press.

Klein A.M., Vaissiere B.E., Cane J.H., Steffan-Dewenter I., Cunningham S.A., Kremen C., Tscharntke T. (2007). Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences. 274: 303–313.

Kovačić M., Puškadija Z., Dražić M.M., Uzunov A., Meixner M.D., Büchler R. (2020): Effects of selection and local adaptation on resilience and economic suitability in *Apis mellifera carnica*. Apidologie. 51: 1062-1073.

Kozmus P. (2011): Population of Carniolan bees (*Apis mellifera carnica Pollman*) in Slovenia and its hybridisation with other subspecies based on colour of abdomen. Acta agriculturae Slovenica. 98(2):159-166.

Lautenbach S., Seppelt R., Liebscher J., Dormann C. F. (2012). Spatial and temporal trends of global pollination benefit. PLoS One. 7: e35954.

Le Conte Y., Navajas M. (2008). Climate change: impact on honey bee populations and diseases. Revue scientifique et technique. 27(2): 499-510.

Lodesani M., Costa C. (2003). Bee breeding and genetics in Europe. Bee World. 84: 69–85.

Meixner M., Costa C., Kryger P., Hatjina F., Bouga M., Ivanova E., Buchler R. (2010). Conserving diversity and vitality for honey bee breeding. Journal of Apicultural Research. 49 (1): 85–92.

Meixner M.D., Pinto M.A., Bouga M., Kryger P., Ivanova E., Fuchs S. (2013). Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. Journal of Apicultural Research. 52(4): 1-28.

Meixner M., Büchler R., Costa C., Andonov S., Bienkowska M., Bouga M., Filipi J., Hatjina F., Ivanova E., Kezić N., Kryger P., Le Conte Y., Panasiuk B., Petrov P., Ruottinen L., Uzunov A., Wilde J. (2015). Looking for "the Best Bee" - An experiment about interactions between origin and environment of honey bee strains in Europe. American Bee Journal. 155(6): 663-666.

Momeni J., Parejo M., Nielsen R.O., Langa J., Montes I., Papoutsus L., Farajzadeh L., Bendixen C., Cauia E., Charriere J-D., Coffey M.F., Costa C., Dall'Olio R., De la Rua P., Dražić M.M., Filipi J., Galea T., Golubovski M., Gregorc A,, Grigoryan K., Hatjina F., Ilyasov R., Ivanova E., Janashia I., Kandemir I., Aikaterini K., Kekecoglu M., Kezić N., Matray E.Sz., Mifsud D., Moosbeckhofer R., Nikolenko A.G., Papachristoforou A., Patrov P., Pinto M.A., Poskryakov A.V., Sharipov A.Y., Siceanu A., Soysal M.I., Uzunov A., Zammit-Magnion M., Vingborg R., Bouga M., Kryger P., Meixner M.D., Esonba A. (2021). Authoritative subspecies diagnosis tool for European honey bees based on ancestry informative SNPs. BMC Genomics. 22: 101.

Mondet F., Parejo M., Meixner M. D., Costa C., Kryger P., Andonov S., Büchler R. (2020). Evaluation of Suppressed Mite Reproduction (SMR) Reveals Potential for Varroa Resistance in European Honey Bees (*Apis mellifera* L.). Insects. 11(9): 595.

Muñoz-Colmenero M., Baroja-Careaga I., Kovačić M., Filipi J., Puškadija Z., Kezić N., Andone E., Büchler R., Zarraonaindia I. (2020). Differences in honey bee bacterial diversity and composition in agricultural and pristine environments – a field study. Apidologie. 51: 1018–1037

Muñoz I., Dall'Olio R., Lodesani M., De la Rua P. (2009). Population genetic structure of coastal Croatian honeybees (*Apis mellifera carnica*). Apidologie. 40(6): 617–626.

Nazzi F. (1992). Morphometric analysis of honeybees from an area racial hybridisation in northeastern Italy. Apidologie. 23: 89–96.

Nedić N., Francis R. M., Stanisavljević L., Pihler I., Kezić N., Bendixen C., Kryger P. (2014). Detecting population admixture in honey bees of Serbia. Journal of Apicultural Research. 53(2): 303–313.

Neumann P., Moritz R.F.A., van Praagh J.P. (1999). Queen mating frequency in different types of honey bee mating apiaries. Journal of Apicultural Research. 38: 11–18.

Oleksa A., Chybicki I., Tofilski A., Burczyk J. (2011). Nuclear and mitochondrial patterns of introgression into native dark bees (*Apis mellifera mellifera*) in Poland. Journal of Apicultural Research. 50: 116–129.

Oleksa A., Kusza Sz., Tofilski A. (2021). Mitochondrial DNA Suggests the Introduction of Honeybees of African Ancestry to East-Central Europe. Insects. 12: 410.

Parejo M., Wragg D., Gauthier L., Vignal A., Neumann P., Neuditschko M. (2016). Using Whole-Genome Sequence Information to Foster Conservation Efforts for the European Dark Honey Bee, *Apis mellifera mellifera*. Frontiers in Ecology and Evolution. 4.

Parker R., Melathopoulos A.P., White R., Pernal S.F., Guarna M.M., Foster L.J. (2010). Ecological adaptation of diverse honey bee (*Apis mellifera*) populations. PLoS ONE. 5(6): e11096.

Péntek-Zakar E., Oleksa A., Borowik T., Kusza S. (2015). Population structure of honey bees in the Carpathian Basin (Hungary) confirms introgression from surrounding subspecies. Ecology and Evolution. 5(23): 5456–5467.

Pinto M.A., Henriques D., Chávez-Galarza J., Kryger P., Garnery L., van der Zee R., Dahle B., Soland-Reckeweg G., de la Rua P., Dall'Olio R., Carreck N.L., Johnston J.S. (2015). Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: A genome-wide assessment using SNPs and mtDNA sequence data. Journal of Apicultural Research. 53: 269–278.

Plate M., Bernstein R., Hoppe A., Bienefeld K. (2019). The importance of controlled mating in honeybee breeding. Genetics Selection Evolution. 51: 74.

Popovska Stojanov D., Dimitrov L., Danihlík J., Uzunov A., Golubovski M., Andonov S.; Brodschneider R. (2021). Direct Economic Impact Assessment of Winter Honeybee Colony Losses in Three European Countries. Agriculture. 11: 398.

Puškadija Z., Kovačić M., Raguž N., Lukić B., Prešern J., Tofilski, A. (2020): Morphological Diversity of Carniolan Honey Bee (*Apis mellifera carnica*) in Croatia and Slovenia. Journal of Apicultural Research. 60: 326–336,

Soland-Reckeweg G., Heckel G., Neumann P., Fluri P., Excoffier L. (2009). Gene flow in admixed populations and implications for the conservation of the Western honeybee, *Apis mellifera*. Journal of Insect Conservation. 13: 317–328.

Ruttner F. (1956). The mating of the honeybee. Bee World. 37: 3–15.

Ruttner F. (1988). Biogeography and Taxonomy of Honeybees. Springer Verlag, Berlin.

Ruttner H. and Ruttner F. (1972). Untersuchungen fiber die Elugaktivitlt und das Paarungsverhalten der Drohnen. Apidologie. 3: 303-232.

Ruttner F. (1992). Naturgeschichte der Honigbienen. Ehrenwirth Verlag, München.

Salmela H., Amdam G.V., Freitak D. (2015). Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. PLoS Pathogens. 11(7): e1005015.

Sheppard W.S., Arias M.C., Greech A., Meixner M.D. (1997). *Apis mellifera ruttneri*, a new honey bee sub-species from Malta. Apidologie. 28: 287-293.
Sheppard W.S., Meixner M.D. (2003). *Apis mellifera pomonella*, a new honey bee sub-species from Central Asia. Apidologie. 34: 367-375.
Strange J.P., Garnery L., Sheppard W.S. (2007). Persistence of the Landes ecotype of *Apis mellifera mellifera* in southwest France: confirmation of a locally adaptive annual brood cycle trait. Apidologie. 38(3): 259-267.
Sušnik S., Kozmus P., Poklukar J., Meglič V. (2004). Molecular characterisation of indigenous *Apis mellifera carnica* in Slovenia. Apidologie. 35: 623–636
Tarpy D.R., Delaney D.A., Seeley T.D. (2015). Mating frequencies of honey bee queens (*Apis mellifera* L.) in a population of feral colonies in the northeastern United States. PLoS One. 10:e0118734
Tomé H.V.V., Schmehl D.R., Wedde A.E., Godoy R.S.M., Ravaiano S.V., Guedes R.N.C., Martins G.F., Ellis J.D. (2020). Frequently encountered pesticides can cause multiple disorders in developing worker honey bees. Environmental Pollution, 256.
Uzunov A., Costa C., Panasiuk B., Meixner M., Kryger P., Hatjina F., Bouga M., Andonov S., Bienkowska M., Le Conte Y., Wilde J., Gerula D., Kiprijanovska H., Filipi J., Petrov P., Ruottinen L., Pechhacker H., Berg S., Dyrba W., Ivanova E., Büchler R. (2014). Swarming, defensive and hygienic behaviour in honey bee colonies of different genetic origin in a pan-European experiment. Journal of Apicultural Research. 53(2): 248-260.
Uzunov A., Brascamp P., Büchler R. (2017). The Basic Concept of Honey Bee Breeding Programs. Bee World. 94(3): 84-87.
Uzunov A., Büchler R., Costa C., Mondet F., Bienkowska M., Hatjina F., Meixner M., Andonov S., Kovačić M., Dall'Olio R., Basso B., Wilde J., Panasiuk B., Charistos L., Brechensbauer D. (2021). Book of methods – performance testers. EU pilot study: Restructuring of the honey bee chain and Varroa resistance breeding & selection programme. AGRI-2017-0346.
Williams G.C. (1966). Adaptation and natural selection. Princeton University Press
Withrow J.M., Tarpy D.R. (2018). Cryptic "royal" subfamilies in honey bee (*Apis mellifera*) colonies. PLoS ONE. 13(7): e0199124.
Woyke J. (1955). Multiple mating of the honeybee queen (*Apis mellifica* L.) in one nuptial flight. Bulletin of the Polish Academy of Sciences. 3: 175–180.

Nema mjesta kao što je dom - važnost uzgoja lokalno prilagođene medonosne pčele (*Apis mellifera*)

Sažetak
Medonosna pčela (*Apis mellifera*) osigurava oprašivanje mnogih biljnih vrsta. Tijekom posljednjeg desetljeća u svijetu su zabilježeni veliki gubici pčelinjih zajednica kao posljedica novih invazinvnih bolesti, klimatskih promjena, poljoprivredne i pčelarske prakse. Kako bi poboljšali stanje na svojim pčelinjacima pčelari se moraju prilagoditi primjenom novih strategija i tehnologija pčelarenja ili uvođenjenjem novog koncepta zaštite zajednica od bolesti. Jedan od koraka koji se može poduzeti je držanje i uzgoj pčela lokalnog porijekla, pčela koje su prilagođene specifičnom okruženju u kojem žive. U ovom preglednom članku prikazana je važnost lokalne adaptacije i trenutni status kranjske pčele u Hrvatskoj.

Ključne riječi: *Apis mellifera*, kranjska pčela, lokalna adaptacija, uzgoj

Maslina kao tvornica fitokemikalija

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Sažetak
Kao jedna od najvažnijih mediteranskih voćnih vrsta, maslina (*Olea europaea* L.) je poznata kao bogat izvor fenola i drugih nutritivno važnih spojeva.
Proteklih godina na Institutu za poljoprivredu i turizam provedena su brojna istraživanja vezana uz utjecaj ishrane masline na fenolne komponente lista ili maslinovog ulja.Temeljem odabranog seta podataka prikazanih u ovom radu, sorta 'Istarska bjelica' pokazala je najveću koncentraciju oleuropeina u listu i ukupnih fenola u maslinovom ulju. U hidroponskom pokusu smanjena razina magnezija u hranivoj otopini povećala je, a niska razina mangana, cinka i bakra smanjila koncentraciju ukupnih fenola u listu masline sorte 'Istarska bjelica'. Folijarna gnojidba sumporom imala je pozitivan učinak na količinu ulja po stablu u rodnoj godini neovisno o proučavanoj sorti.

Ključne riječi: oleuropein, fenoli, gnojidba, *Olea europaea* L., Istarska bjelica cv.

Uvod

Maslina (*Olea europaea* L.), kao tipična mediteranska kultura, uz badem i smokvu je jedna od prvih kultiviranih voćnih vrsta na prostoru mediteranskog bazena te se njezin uzgoj spominje i pred više od 6.000 godina. Za stanovnike Mediterana maslinovo ulje oduvijek predstavlja značajan izvor masti u prehrani i ekonomski je vrijedan izvozni proizvod (Liphschitz i sur., 1991.; Kapellakis i sur., 2008.; Pasković i sur., 2020.).
Maslina se u svijetu uzgaja na više od 11 milijuna ha s ukupnom proizvodnjom od preko 3 milijuna tona od čega Europska unija predstavlja oko 67 % svjetske proizvodnje (EC, 2022.). U Republici Hrvatskoj maslina se uzgaja na više od 20.000 ha s proizvodnjom većom od 40.000 hl maslinova ulja (DSZ, 2020.).
Zbog načina ekstrakcije, pri kojoj se svježe cijeđeni sok iz ploda dobiva jednostavnim mehaničkim postupcima bez dodataka industrijskih otapala, djevičansko maslinovo ulje (DMU) ima, uz specifičan sastav masnih kiselina, niz specifičnih fenolnih spojeva što ga svrstava u skupinu funkcionalne hrane (Calabriso i sur., 2015.; Gouvinhas i sur., 2017.).
Maslina uz svoju tradicionalnu ulogu u mediteranskoj prehrani ima i značajno mjesto u proizvodnji raznih nutraceutika ili bioloških/medicinskih agensa. Naime, nusproizvodi

masline poznati su kao bogat izvor raznih fitonutrijenata, među kojima je i list masline, često korišten kao lijek u narodnoj medicini, značajan izvor različitih fenolnih spojeva (Pasković i sur., 2020.) s poznatim antioksidantivnim, antimikrobnim, hipoglikemičnim djelovanja kao i utjecajem na snižavanje lipida u krvi ili snižavanju krvnog tlaka (Lukić i sur., 2020.). Nastavno na navedeno, upravo korištenje ekstrakta lista masline danas je uobičajeno u prehrambenoj, kozmetičkoj i farmaceutskoj industriji.

U redovnoj maslinarskoj proizvodnji, gnojidba je neizostavna agrotehnička mjera kojom se održava odgovarajući status ishranjenosti biljke što pak rezultira optimalanim rastom i razvojem masline (Erel i sur., 2018.). Pri tome, najčešće je istraživana primjena gnojiva s dušikom, kalijem ili borom (Perica, 1996.; Perica i sur., 2001.; Fernández-Escobar i sur., 2006.) dok je pregledom literature uočeno da je primjena ostalih pojedinačnih makro (kalcij, magnezij, sumpor) ili mikoelementata (Zn, Mn, Cu) znatno rijeđe u fokusu istraživača. Također, iako je općenito poznata povezanost gnojidbe i njezinog utjecaja na fenolni profil biljaka samo nekoliko istraživanja proučavalo je utjecaj minerale ishrane na fenolni profil lista ili ulja masline (Antonnen i sur., 2006.; Talhaoui i sur. 2015.; Marcelić i sur., 2022.).

Kako koncentracija hraniva u listu masline, između ostalog, ovisi o kultivaru i razini gnojidbe (Therios, 2009.) cilj naših višegodišnjih projektnih istraživanja bio je definirati utjecaj sorte i gnojidbe na kvalitetu DMU kao i utjecaj istih na modulaciju specifičnih i/ili ukupnih fenolnih spojeva u listu masline.

Materijal i metode

Pokus 1.

Pokus je proveden po shemi potpuno slučajnog rasporeda na tri sorte masline ('Leccino', 'Oblica', 'Istarska bjelica'). Uzorci su prikupljeni u maslinicima na četiri različite lokacije (Novalja, otok Pag; Poličnik, kod Zadra; Linardići, Otok Krk; Vodnjan, Istra) s dobro razvijenim i ujednačenim stablima starosti od 7 do 15 godina. Na svakoj lokaciji, na odabranim sortama, uzorkovani su listovi s tri stabla po svakoj sorti tijekom tri roka uzorkovanja i to: nakon berbe maslina (listopad, 2017.), u zimskom mirovanju (siječanj, 2018.) i u rezidbi (ožujak, 2018.).

Pokus 2.

Pokus je proveden u plasteniku Instituta za poljoprivredu i turizam, Poreč, Hrvatska, od sredine veljače do sredine srpnja 2019 godine u uvjetima prirodnog svjetla, temperature i fotoperioda. Prosječni raspon dnevne/noćne temperature varirao je između 10 i 35 °C. Jednogodišnje sadnice masline sorte 'Istarska bjelica' svedene su na jedan izbojak te uzgajane u posudama od 3,5 L koje su sadržavale supstrat sastavljen od perlita (Agroperl; Europerl d.o.o., Samobor, Hrvatska) i pijeska (Fuga Sand; Kema d.o.o., Puconci, Slovenija) pomiješanih u jednakom omjeru (w/w). Eksperiment je postavljen kao slučajni blokni raspored u četiri ponavljanja. Svaki od 10 gnojidbenih tretmana imao je pet sadnica po jednom ponavljanju, odnosno 20 sadnica po svakom gnojidbenom tretmanu. Stoga, ukupan broj sadnica u pokusu bio je 200. Za svaki gnojidbeni tretman, Hoaglandova hraniva otopina bila je modificirana s ili bez dodatka odabranog hraniva kako slijedi: Ca– (1 mmol/L Ca), Ca+ (6 mmol/L Ca), Mg– (0 mmol/L Mg), Mg+ (1 mmol/L Mg), Cu- (0 µmol/L Cu), Cu+ (4 µmol/L Cu), Zn- (0 µmol/L Zn), Zn+ (18 µmol/L Zn), Mn- (0 µmol/L Mn), Mn+ (24 µmol/L Mn).

Pokus 3.

Tijekom dvije godine (2019., 2020.) proveo se dvofaktorijalni poljski pokus (gnojidba x sorta) po shemi potpuno slučajnog rasporeda. Tretmani (Kontrola, Thiotrac 22,5 ml/L) (Thiotrac je SN gnojivo s 200 g N i 750 g SO_3) primjenjeni su na dvije sorte ('Leccino', 'Istarska bjelica') folijarno 50 dana od pune cvatnje u fenofazi okoštavanja endokarpa ploda (BBCH 75) i 80 dana od pune cvatnje u fenofazi početka sinteze ulja (BBCH 79) (Marcelić

i sur., 2022.). Ubrani listovi (pokus 1. i 2.) dostavljeni su u svježem stanju u laboratorij, u papirnatim vrećicama i oprani od vanjskih nečistoća u 1 % octenoj kiselini, a potom su dva puta oprani u destiliranoj vodi (Pasković i sur., 2020.). Tako pripremljeni listovi maslina sušeni su na 35 °C do konstantne mase te samljeveni u fini prah za daljnje analize pojedinačnih (pokus 1.) ili ukupnih (pokus 2.) fenola (Pasković i sur., 2020.).
Plodovi (pokus 3.) su uzorkovani (ubrani) sa središnjeg obodnog dijela krošnje tijekom polovice listopada 2019. i 2020. godine, ravnomjerno sa svih strana svijeta, te su nakon vaganja transportirani u laboratorij gdje su prerađeni u maslinovo ulje. Na osnovi randmana ulja prilikom prerade i mase ploda određena je ukupna količina ulja po stablu. Također, u maslinovom ulju određen je sadržaj ukupnih fenola. Svi uzorci u prije opisanim pokusima (pokus 1. i 2. lišće, pokus 3. ulje) analizirani su prema metodologiji navedenoj u našim prethodnim publikacijama (Pasković i sur. , 2020.; Lukić i sur. 2020.; Vidović i sur., 2021.; Marcelić i sur., 2022.), a prikupljeni podatci obrađeni su jednosmjernom (pokus 1. i 2.) ili dvosmjernom (pokus 3.) analizom varijance (ANOVA), te nakon signifikantnog F-testa Fisherovim LSD post-hoc testom, pomoću statističkog programa Statistica® v.12.0 software (Tibco Software Inc., Palo Alto, CA, USA).

Rezultati i rasprava

Pokus 1.

Rezultati sastava fenolnih spojeva u listu masline na odabrane tri sorte iz pokusa 1. prikazani su u tablicama 1., 2. i 3.. U dostupnoj literaturi upravo sorte 'Leccino', 'Istarska bjelica' i 'Oblica' opisane su kao sorte masline tolerantne na niske temperature (Connor i Ferreres, 2005.; Strikić i sur., 2010.; Biško i sur., 2015.) dok se razina oleuropeina u listu smatra se jednim od najvažnijih čimbenika kapaciteta za oporavak i otpornost masline na temperaturne stresove (Ortega i Peragon, 2008.). U našem istraživanju koncentracija jedostavnih fenola (hidroksitirozola, tirozola) (Tablica 1) kao i flavonoida (apigenin-7-O-glukozida) (Tablica 3) bila je značajno veća kod kod sorte 'Leccino' u odnosu na sorte 'Istarska bjelica' i 'Oblica' dok se koncentracija verbaskozida nije razlikovala između promatranih sorti. Iako se razina tirozola smatra jednim od najvažnijih fenolnih prekursora za biostinezi oleuropeina (Ryan i sur., 2002.; Obied i sur., 2008.) sorta 'Istarska bjelica' imala je značajno veću razinu oleuropeina (4547 mg/100 g suhe tvari (ST)) u odnosu na 'Leccino' (3454,97 mg/100 g ST) ili 'Oblicu' (2638,34 mg/100 g ST) (Tablica 2) te između navedenih fenolnih spojeva, u ovom istraživanju, nije pronađena značajna korealcija (podatci nisu prikazani). Također, 'Istarska bjelica' pokazala je značajno veću koncentraciju ukupnih fenola u odnosu na koncentraciju istih kod 'Oblice' (Tablica 2). Naime, sekoiridoid oleuropein je, neovisno o promatranom kultivaru, uobičajeno najzastupljeniji fenolni spoj u listu masline (Lukić i sur., 2020.). Viša koncentracija Luteolin-7-O-glukozida zabilježena je u odnosu na druge istražene flavonoide neovisno o istraženom kultivaru (Tablica 3). Luteolin-7-O-glukozid navodi se kao dominanti flavonoid kod španjolskih odnosno portugalskih sorata masline (Talhaoui i sur., 2015.; Meirinhos i sur., 2005.).

Tablica 1. Utjecaj sorte na koncentracije jednostavnih fenola i verbaskozida u listu masline

	Jednostavni fenoli		Fenolne kiseline
SORTA	Hidroksitirozol	Tirozol	Verbaskozid
I. bjelica	24,85±2,11 c	7,45±0,56 b	435,8±63,34
Leccino	58,98±3,3 a	10,22±0,52 a	411,74±75,51
Oblica	39,97±3,12 b	6,47±0,57 b	336,28±63,22
p	***	***	n.s.

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± SE, n=36). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Jednosmjerna analiza varijance (ANOVA).

Tablica 2. Utjecaj sorte na koncentracije oleuropeina i ukupnih fenola u listu masline

	Sekoiridoidi	
SORTA	Oleuropein	Ukupni fenoli
I. bjelica	4547,57±397,55 a	5489,07±436,53 a
Leccino	3454,97±332,39 b	4501,14±399,03 ab
Oblica	2638,94±344,02 c	3518,11±409,32 b
p	**	**

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± SE, n=36). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Jednosmjerna analiza varijance (ANOVA).

U ovom istraživanju 'Istarska bjelica' imala je značajno nižu koncentraciju luteolin-7-O-glukozida i značajno višu koncentraciju rutina u odnosu na ostale proučavane sorte (Tablica 3). U prethodnim istraživanjima (Pasković i sur., 2020., Lukić i sur., 2020.) 'Istarska bjelica' je pokazala višu razinu rutina u listu i od drugih autohtonih sorata poput 'Drobnice', 'Lastovke' i 'Levantinke'. Također, Cukrov i sur. (2020.) navode 'Oblicu' kao sortu s najnižom razinom rutina u odnosu na ostale proučavane hrvatske autohtone sorte.

Tablica 3. Utjecaj sorte na koncentracije flavonoida u listu masline

	Flavonoidi		
SORTA	Rutin	Apigenin-O-7-glukozid	Luteolin-O-7-glukozid
I. bjelica	93,23±7,17 a	35,53±1,36 b	313,15±13,12 b
Leccino	58,1±3,12 b	95,23±4,34 a	373,64±11,93 a
Oblica	31,72±2,07 c	27,81±0,98 c	386,7±16,76 a
p	***	***	***

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± SE, n=36). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Jednosmjerna analiza varijance (ANOVA).

Pokus 2.

Pravilna ishrana bilja preduvjet je za osiguranje kofaktora za mnoge enzime fenilpropanoidnog i flavonoidnog puta. Naime, Mg^{2+} i Mn^{2+} ioni osiguravaju funkcioniranje PAL (Fenilalanin-amonij-liaze), ključnog enzima odgovornog za biosintezu fenola (Treutter, 2010.). Ipak, u našem istraživanju Mg+ tretman (5230,33 mg/100 g ST) rezultirao je smanjenjem, a Mn+ tretman (6095.81 mg/100 g ST) povećanjem koncentracije ukupnih fenola u listu masline u odnosu na Mg- (6777,56 mg/100 g ST) odnosno Mn- (5150,56 mg/100 g ST) gnojidbene tretmane (Tablica 4). Koncentracije Mg odnosno Mn u Mg- i Mn-tretmanima bile su ispod literaturno definirane razine relativnog deficita kod masline od 0,7 g/kg ST za Mg odnosno 20 mg/kg ST za Mn (Fernandez-Escobar, 2019., Therios, 2009.). Koncentracije Mg i Mn u Mg+ odnosno Mn+ tretmanu bile su optimalne (Therios, 2009.) (Tablica 5).

U ovom istraživanju nije bilo zabilježenih razlika u koncentraciji ukupnih fenola između primjenjenih Ca+ i Ca- tretmana (Tablica 4). Teixeira i sur. (2006.) i Treutter (2010.) navode da se koncentracija fenolnih spojeva smanjuje u odnosu na koncentracije kalcija. Castaneda i Perez (1996) primjećuju da folijarna primjena $CaCl_2$ povećava koncentraciju fenola, što dovodi do povećane otpornosti na fitopatogene gljive kod citrusa.

Tablica 4. Utjecaj različitih gnojidbenih tretmana u hidroponskom pokusu na koncentracije ukupnih fenola u listu masline sorte Istarska bjelica

TRETMAN	UKUPNI FENOLI
Ca-	5758,99±119,37 b
Ca+	5871,5±69,27 b
Mg-	6777,56±114,39 a
Mg+	5230,33±288,56 c
Zn-	4717,48±24,94 d
Zn+	6101,89±167,43 b
Cu-	2525,19±120,19 e
Cu+	6023,13±179,97 b
Mn-	5150,56±162,66 c
Mn+	6095,81±194,72 b
p	***

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± standardna pogreška, n=36). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Jednosmjerna analiza varijance (ANOVA).

Tablica 5. Koncentracije hraniva u listu masline u hidroponskom pokusu

TRETMAN	KONCENTRACIJA U SUHOJ TVARI LISTA
Ca-, Ca+	5-7 g/kg, 9-10 g/kg
Mg-, Mg+	0,5-0,6 g/kg, 1,0-1,1 g/kg
Zn-, Zn+	5-6 mg/kg, 11-13 mg/kg
Cu-, Cu+	1-2 mg/kg, 4 mg/kg
Mn-, Mn+	11-16 mg/kg, 21-24 mg/kg

Razina ukupnih fenola u Zn- tretmanu (4717,48 mg/100 g ST) bila je značajno različita u odnosu na Zn+ tretman (6101,89 mg/100 g ST) (Tablica 4). Pri tome je razina ishranjenosti cinkom kod Zn- tretmana bila ispod definirane razine relativne deficijencije cinka u listu masline od 10 mg/kg ST (Therios, 2009.) dok su zabilježene vrijednosti cinka u Zn+ u literaturno definiranom optimumu >10 mg/kg ST (Tablica 5). Cink kao sastojak folijarnog gnojiva Brotomax, uz Mn, Cu i N, povećao je razinu fenola kod masline i citrusa (Del Rio i sur., 2003.; Ortuno i sur., 2006.). Naime, metaboličke funkcije cinka temelje se na njegovoj sposobnosti stvaranja tetraedarskih kompleksa s N-, O- i S-ligandima, čime utječe na funkcionalnu (katalitičku) i strukturnu ulogu u enzimatskim reakcijama (Marschner, 2012.). Koncentracija ukupnih fenola kod Cu- (2525,19 mg/100g ST) bila je značano niža od koncentracije ukupnih fenola kod tretmana Cu+ (6023,13 mg/100 ST) (tablica 4.). Kod masline optimalnom razinom ishranjenosti masline smatra se koncentracija bakra u listu od 4 mg/kg ST (Fernandez-Escobar, 2019.). U našem istraživanju ista je zabiljezena u Cu+ tretmanu dok je u Cu- tretmanu bila ispod razine defcijencije s vidljivim zaostajanjem u rastu i razvoju biljke (tablica 5.). Saha i sur. (2012.) navode da ukupan sadržaj fenola u listovima čaja (*Camellia sinensis* L. (O.) Kuntze) raste povećanjem koncentracije bakra (Cu).

Pokus 3.

Pojam 'alternativna rodnost“ definira se kao izmjena visokog (maksimalnog) priroda jedne godine te niskog ili čak izostanka priroda sljedeće godine, što je karakteristika višegodišnjih drvenastih biljaka pa tako i masline (Fichtner i Lovatt, 2018.). Opterećenje plodom u tekućoj godini, koji je usko povezan s razvojem koštice, usporava vegetativni prirast na kojem bi se trebao razviti cvijet u sljedećoj godini, što ima za posljedicu smanjen intenzitet cvatnje u idućoj godini (Dag i sur., 2010.). Ova pojava predstavlja značajno ograničenje u

maslinarskoj proizvodnji jer u 'nerodnoj' godini maslina daje velike plodove, ali premalu količinu plodova po stablu da bi se osigurao dobar urod, dok u 'rodnoj' godini daje veliki broj sitnih plodova koje kasnije dozrijevaju i nakupljaju ulje u manjoj količini (Fichtner i Lovatt, 2018.).

Rezulati pokusa 3. prikazani su u tablici 6. i tablici 7.. Količina ulja po stablu između tretmana nije se razlikovala u 'nerodnoj' 2019. godini dok je signifikantno veća količina ulja u 'rodnoj' 2020. godini zabilježana u folijarnom tretmanu sa SN gnojivom u odnosu na kontrolni tretman (Tablica 7). Naime, poznato je da primjena S gnojiva povećava količinu ulja kod raznih drugih uljarica. Tako je pozitivan utjecaj gnojidbe sumporom na količinu ulja zabilježen na uljanoj repici (Sattar i sur., 2011.), soji (Burkitbayev i sur., 2021.) ili sezamu (Moradi i sur., 2020.). Premda je zabilježen pozitivan utjecaj gnojidbe sumporom na količinu fenola u biljkama (Zhoui i sur., 2013.), u obje godine ovog istraživanja nije bilo razlike u koncentraciji fenola u ulju između gnojidbenih tretmana.

Poznat je snažan učinak sorte na prirod ploda i količinu ulja (Vossen, 2013.). Iako se ‘Istarska bjelica’ smatra sortom s visokim, a 'Leccino' sortom s niskim udjelom ulja (Barranco i sur., 2000.) rezultati našeg istraživanja pokazali su ukupno veću količinu ulja po stablu u „nerodnoj godini“ za sortu Leccino (Tablica 6). U 2020. godini nije bila vidljiva razlika između promatranih sorti u količini ulja po stablu (Tablica 7). Nadalje, 'Istarska bjelica' imala je 2 do 3 puta veću koncentraciju ukupnih fenola u ulju u odnosu na 'Leccino' u obje promatrane godine (Tablica 6, Tablica 7). Nastavno na navedeno, dosadašnja istraživanja o kvaliteti ulja provedena u Republici Hrvatskoj pokazala su dominantno više vrijednosti ukupnih fenola kod 'Istarske bjelice' u odnosu na sortu 'Leccino' (Šindrak i sur., 2007., Koprivnjak i sur., 2012.).

Tablica 6. Utjecaj folijarnog tretmana (Kontrola, Thiotrac 22,5 ml/L) i sorte ('Istarska bjelica' i 'Leccino') te njihove interakcije (sorta x tretman) na količinu ulja po stablu i koncentraciju ukupnih fenola u maslinovom ulju u 2019. godini

FAKTORI TRETMAN	Količina ulja po stablu (kg)	Ukupni fenoli u ulju (mg/kg)
Kontrola	1,05 ± 0,22	897,91 ± 122,32
Thiotrac 22,5 ml/L	1,49 ± 0,23	989,56 ± 116,15
SORTA (Cv.)		
'Istarska bjelica' (IB)	1,07 ± 0,22 b	1243,71 ±25,70a
'Leccino' (LE)	1,46 ± 0,23 a	643,76 ± 40,41b
T	n.s.	n.s.
Cv.	*	***
T x Cv.	n.s.	n.s.

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± standardna pogreška, n = 4). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Dvosmjerna analiza varijance (ANOVA).

Tablica 7. Utjecaj folijarnog tretmana (Kontrola, Thiotrac 22,5 ml/L) i sorte ('Istarska bjelica' i 'Leccino') te njihove interakcije (sorta x tretman) na količinu ulja po stablu i koncentraciju ukupnih fenola u maslinovom ulju u 2020. godini

FAKTORI TRETMAN	Količina ulja po stablu (kg)	Ukupni fenoli u ulju (mg/kg)
Kontrola	1,38±0,21b	677,20±111,12
Thiotrac 22,5 ml/L	2,52±0,34a	598,93±96,09
SORTA (Cv.)		
'Istarska bjelica' (IB)	2,02±0,46	903,79,34±39,12a
'Leccino' (LE)	1,88±0,20	372,33±17,77 b
T	*	n.s.
Cv.	n.s.	***
T x Cv.	n.s.	n.s.

Srednje vrijednosti označene različitim slovima značajno su različite uz p<0,05 prema Fisherovom LSD testu (srednje vrijednosti ± standardna pogreška, n = 4). n.s. nije signifikantno; ***p < 0.001; **p < 0.01; *p < 0.05. Dvosmjerna analiza varijance (ANOVA).

Zaključak

Sorta 'Istarska bjelica' pokazala je najveću koncentraciju oleuropeina u listu i ukupnih fenola u maslinovom ulju. U hidroponskom pokusu smanjena razina magnezija u hranivoj otopini povećala je, a niska razina mangana, cinka i bakra smanjila koncentraciju ukupnih fenola u listu masline sorte 'Istarska bjelica'. Folijarna gnojidba sumporom imala je pozitivan učinak na količinu ulja po stablu u rodnoj (2020.) godini neovisno o proučavanoj sorti.

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Literatura

Anttonen M. J., Hoppula K. I., Nestby R., Verheul M. J., & Karjalainen R. O. (2006). Influence of fertilization, mulch color, early forcing, fruit order, planting date, shading, growing environment, and genotype on the contents of selected phenolics in strawberry (Fragaria x ananassa Duch.) fruits. Journal of Agricultural and Food Chemistry. 54(7): 2614-2620.

Barranco D., Cimato A., Fiorino P., Rallo L., Touzani A., Castaneda C., Serafini F., Trujillo I. (2000). World catalogue of olive varietes, 51-216. Madrid, Spain: International Olive Oil Council.

Biško A., Prlić M., Barbarić M., & Leko M. (2019). Olive growing in Herzegovina. Pomologia Croatica. 22(3–4): 137-146.

Burkitbayev M., Bachilova N., Kurmanbayeva M., Tolenova K., Yerezhepova N., Zhumagul M., Mamurova A., Turysbek B., & Demeu G. (2021). Effect of sulfur-containing agrochemicals on growth, yield, and protein content of soybeans (*Glycine max* (L.) Merr). Saudi Journal of Biological Sciences. 28(1): 891-900.

Calabriso N., Scoditti E., Pellegrino M., Carluccio M. A. (2015). Olive oil. The Mediterranean diet, Preedy V. R., Watson R. R., 135-142. Lecce, Italy: Elsvier.

Castañeda P., & Pérez L. M. (1996). Calcium ions promote the response of Citrus limon against fungal elicitors or wounding. Phytochemistry. 42(3): 595-598.

Connor D. J., & Fereres E. (2010). The Physiology of Adaptation and Yield Expression in Olive. In Horticultural Reviews. 31: 155-229.

Cukrov M., Žurga P., Majetić Germek V., Brkljača M., Ban D., Lukić I., Goreta Ban S., & Paskovi, I. (2021). Effect of olive (Olea europaea L.) variety on leaf biophenolic profile. Agriculturae Conspectus Scientificus. 86(3): 277-282.

Dag A., Bustan A., Avni A., Tzipori I., Lavee S., & Riov J. (2010). Timing of fruit removal affects concurrent vegetative growth and subsequent return bloom and yield in olive (Olea europaea L.). Scientia Horticulturae. 123(4): 469-472.

del Río J. A., Báidez A. G., Botía J. M., & Ortuño A. (2003). Enhancement of phenolic compounds in olive plants (Olea europaea L.) and their influence on resistance against Phytophthora sp. Food Chemistry. 83(1): 75-78.

Državni zavod za statistiku DZS (2020). Dostupno na: www.dzs.hr (Pristupljeno 1.2.2022.)

Erel R., Kerem Z., Ben-Gal A., Dag A., Schwartz A., Zipori I., Basheer L., & Yermiyahu U. (2013). Olive (Olea europaea L.) tree nitrogen status is a key factor for olive oil quality. Journal of Agricultural and Food Chemistry. 61(47): 11261-11272.

Erel R., Yermiyhu Y., Ben-Gal A., & Dag A. (2018). Olive fertilization under intensive cultivation management. Acta Horticulturae. 1217: 207-224.

Fernández-Escobar R. (2019). Olive Nutritional Status and Tolerance to Biotic and Abiotic Stresses. In Frontiers in Plant Science. 10: 1151.

Fernández-Escobar R., Beltrán G., Sánchez-Zamora M. A., García-Novelo J., Aguilera M. P., & Uceda M. (2006). Olive oil quality decreases with nitrogen over-fertilization. HortScience. 41(1): 215-219.

Fichtner E. J., Lovatt C. J. (2018). Alternate bearing in olive. Acta Horticulturae. 1199: 103-108.

Gouvinhas I., Machado N., Sobreira C., Domínguez-Perles R., Gomes S., Rosa E., & Barros A. I. R. N. A. (2017). Critical review on the significance of Olive phytochemicals in plant physiology and human health. In Molecules. 22(11): 1986.

https://ec.europa.eu/info/food-farming-fisheries/plants-and-plant-products/plant-products/olive-oil_en (pristupljeno 24.02.2022)

Kapellakis I. E., Tsagarakis K. P., & Crowther J. C. (2008). Olive oil history, production and by-product management. In Reviews in Environmental Science and Biotechnology. 7(1): 1-26.

Koprivnjak O., Vrhovnik I., Hladnik T., Prgomet Ž., Hlevnjak B., Majetić Germek V. (2012). Obilježja prehrambene vrijednosti djevičanskih maslinovih ulja sorti Buža, Istarska bjelica, Leccino i Rosulja. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam. 7 (3-4): 172-178.

Liphschitz N., Gophna R., Hartman M., & Biger G. (1991). The beginning of olive (olea europaea) cultivation in the old world: A reassessment. Journal of Archaeological Science. 18(4): 441-453.

Lukić I., Pasković I., Žurga P., Germek V. M., Brkljača M., Marcelić Š., Ban D., Grozić K., Lukić M., Užila Z., & Ban S. G. (2020). Determination of the variability of biophenols and mineral nutrients in olive leaves with respect to cultivar, collection period and geographical location for their targeted and well-timed exploitation. Plants. 9(12): 1–22.

Marcelić Š., Vidović N., Pasković I., Lukić M., Špika M.J., Palčić I., Lukić I., Petek M., Pecina M., Herak Ćustić M., Ban D., Grozić K., Polić Pasković M., Cukrov M., Pongrac P., Goreta Ban S. (2022). Combined Sulfur and Nitrogen Foliar Application Increases Extra Virgin Olive Oil Quantity without Affecting Its Nutritional Quality. Horticulturae. 8: 203.

Marschner H. (2003). Mineral Nutrition of Higher Plants', 212-223.London, UK: Academic Presss.

Meirinhos J., Silva B. M., Valentão P., Seabra R. M., Pereira J. A., Dias A., Andrade P. B., & Ferreres F. (2005). Analysis and quantification of flavonoidic compounds from Portuguese olive (olea europaea L.) leaf cultivars. Natural Product Research. 19(2): 189-195.

Moradi R., Dejam M., Madandoust M., Mohajeri F. (2020). Effects of sulfur foliar application on the yield, yield components, and contents of oil and protein in different sesame cultivars in the south of Iran. International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR). 10(5): 216-225.

Obied H. K., Prenzler P. D., Ryan D., Servili M., Taticchi A., Esposto S., & Robards K. (2008). Biosynthesis and biotransformations of phenol-conjugated oleosidic secoiridoids from Olea europaea L. In Natural Product Reports. 25(6): 1167-1179.

Ortega-García F., & Peragón J. (2009). The response of phenylalanine ammonia-lyase, polyphenol oxidase and phenols to cold stress in the olive tree (*Olea europaea* L. cv. Picual). Journal of the Science of Food and Agriculture. 89(9): 1565-1573.

Ortuño A., Gómez P., Báidez A., Frías V., & del Río J. A. (2006). Citrus sp.: A source of flavonoids of pharmaceutical interest. ACS Symposium Series. 936: 175-185.

Pasković I., Lukić I., Žurga P., Germek V. M., Brkljača M., Koprivnjak O., Major N., Grozić K., Franić M., Ban D., Marcelić Š., & Ban S. G. (2020). Temporal variation of phenolic and mineral composition in olive leaves is cultivar dependent. Plants. 9(9): 1–16.

Perica S. (1996). Utjecaj folijarne gnojidbe dušikom, kalijem i borom na razinu ishranjenosti, vegetativnu i generativnu aktivnost masline. Doktorska disertacija, Agronomski fakultet Sveučilišta u Zagrebu, Zagreb, Hrvatska.

Perica S., Bellaloui N., Greve C., Hu H., & Brown P. H. (2001). Boron transport and soluble carbohydrate concentrations in olive. Journal of the American Society for Horticultural Science. 126(3): 291-296.

Ryan D., Antolovich M., Herlt T., Prenzler P. D., Lavee S., & Robards K. (2002). Identification of phenolic compounds in tissues of the novel olive cultivar Hardy's Mammoth. Journal of Agricultural and Food Chemistry. 50(23): 6716-6724.

Saha D., Mandal S., & Saha A. (2012). Copper induced oxidative stress in tea (Camellia sinensis) leaves. Journal of Environmental Biology. 33(5): 861-866.

Sattar A., Cheema M. A., Wahid M. A., Saleen M. F., Hassan M. (2011). Interactive effect of sulphur and nitrogen on growth, yield and quality of canola. Crop & Environment. 2 (1): 32–37.

Strikić F.; Klepo T.; Rošin J.; Radunić M.. (2010). Udomaćene Sorte Maslina u Republici Hrvatskoj; Institut za jadranske kulture i melioraciju krša: Split, Croatia.

Šindrak Z., Benčić Đ., Voća S., & Barberić A. (2007). Ukupne fenolne tvari u sortnim istarskim maslinovim uljima Total phenols in varietal olive oils from Istria. Pomologia Croatica. 13(1): 17-29.

Talhaoui N., Gómez-Caravaca A. M., León L., de la Rosa R., Segura-Carretero A., & Fernández-Gutiérrez A. (2014). Determination of phenolic compounds of "Sikitita" olive leaves by HPLC-DAD-TOF-MS. Comparison with its parents "Arbequina" and "Picual" olive leaves. LWT - Food Science and Technology. 58(1): 28-34.

Talhaoui N., Taamalli A., Gómez-Caravaca A. M., Fernández-Gutiérrez A., & Segura-Carretero A. (2015). Phenolic compounds in olive leaves: Analytical determination, biotic and abiotic influence, and health benefits. In Food Research International. 77: 92-108.

Teixeira A. F., de Bastos Andrade A., Ferrarese-Filho O., & de Lourdes Lucio Ferrarese M. (2006). Role of calcium on phenolic compounds and enzymes related to lignification in soybean (Glycine max L.) root growth. Plant Growth Regulation. 49(1): 69-76.

Therios I. (2009). Olives: Crop Production Science in Horticulture 18. Experimental Agriculture, Wallingford, UK: CABI Publishing.

Treutter D. (2010). Managing phenol contents in crop plants by phytochemical farming and breeding-visions and constraints. In International Journal of Molecular Sciences. 11(3): 807-857.

Vidović N., Pasković I., Lukić I., Žurga P., Germek V. M., Grozić K., Cukrov M., Marcelić Š., Ban D., Talhaoui N., Palčić I., Rubinić V., & Ban S. G. (2021). Biophenolic profile modulations in olive tissues as affected by manganese nutrition. Plants. 10(8): 1724.

Vossen P. (2013). Growing olives for oil. Handbook of Olive Oil: Analysis and Properties. Aparicio R., Harwood J., 19-56. New York: Springer Science+Business Media.

White P. J., & Broadley M. R. (2005). Biofortifying crops with essential mineral elements. In Trends in Plant Science. 10(12): 586–593.

White P. J., & Broadley M. R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets - Iron, zinc, copper, calcium, magnesium, selenium and iodine. In New Phytologist. 182(1): 49–84.

Younis M., Shah S., Inamullah Gul R., Jalal A., Khalil F., Hussain I., & Fahad M. A. (2020). Effect of phosphorus and sulphur on yield and yield components of sesame. Sarhad Journal of Agriculture, 36(2), 722-728.

Zhou C., Zhu Y., & Luo Y. (2013). Effects of sulfur fertilization on the accumulation of health-promoting phytochemicals in radish sprouts. Journal of Agricultural and Food Chemistry. 61(31): 7552-7559.

Olive Tree as Phytochemical Factory

Abstract

As one of the most important Mediterranean fruits, olives (*Olea europaea* L.) are known as a rich source of phenolic and other nutritionally important compounds.

In recent years the Institute of Agriculture and Tourism has conducted numerous studies related to influence of olive mineral nutrition on phenolic compounds in leaf or olive oil. Based on the selected data set presented in this paper, the cultivar 'Istarska bjelica' showed the highest concentration of oleuropein in the leaf as well as total phenols in olive oil. In the hydroponic experiment, the reduced level of magnesium in the nutrient solution increased, while manganese, zinc, and copper low nutrient solution level decreased the concentration of total phenols in the cv 'Istraska bjelica' olive leaves. Foliar sulfur fertilization had a positive effect on the oil quantity in 'on' year regardless of the variety studied.

Key words: oleuropein, phenolic, fertilization, *Olea europaea* L., Istarska bjelica cv.

Choice of maize maturity group and climate change: current status and predictions

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Abstract
The idea of this paper was to give insight into interconnection between maize genotypes categorized by maturity groups, environment and agronomic management for improving future grain yield in the context of climate change. Current status and predictions suggest that the right choice of maize maturity to attain higher yields in future is not straightforward and it is not made only according to prevalent climate change expectations. Globally, the effects of management might play pivotal role in maize phenology and yield formation. In future, the integrative genotype×environment×management framework should be applied along with high-throughput genotyping and phenotyping using solutions for precision agriculture and machine learning to capture more data and to provide crucial information to farmers in real-time.

Key words: maize, maturity groups, climate change, grain yield, predictions

Introduction

Choosing maize hybrids is one of the most important decisions a maize grower makes each year. Grain yield potential along with relative maturity are the decisive factors that need to be addressed. Hybrid relative maturity, i.e. plant cycle duration is becoming more important in the context of climate change to maximize yield, whereby farmers should continuously adapt maize cycle duration and planting dates to the diversity of environmental conditions (Parent et al., 2018; Abendroth et al., 2021). Several systems for rating of hybrid maize maturity are employed around the world. In North America, relative maturity in days (RM), crop heat units (CHU) and growing degree days (GDD) both calculated in °C, are used (Dwyer et al., 1999).The uniform method recommended by Food and Agriculture Organization of the United Nations (FAO) (Jugenheimer et al., 1976.) is still used in Europe where maize varieties should be officially characterized (European Commission, 2022) using the expression „Index maturitas“. Like RM, FAO maturity groups represent the length of time necessary for a hybrid to reach harvest-ready moisture determined relatively to a standard hybrid. This maturity classification is also an important component for evaluation of new maize hybrids before they can be registered in the recommended or national lists of varieties and released for commercial use. The official registration trials, i.e. trials for assessment of value for cultivation and use (VCU) are valuable source of information on general maize yield trends and across maturity groups showing genetic potential of the newly registered hybrids projecting future yields. However, there is a gap between on-farm yields and VCU trial yields (Laidig et al., 2014; Schils et al., 2020), which is particularly wide in Eastern Europe primarily due to suboptimum field management in on-farm maize production (Zorić et al., 2017). Ultimately, the increase in maize yield is equally influenced by the development of new genotypes and improved management (Duvick, 2005). The idea of this paper was to give insight into interrelationship among genotypes categorized by different FAO maturity groups, environment and agronomic management for improving future maize yield in the context of climate change.

Current status

Globally, maize yields are declining with climate change due to increasing air temperatures. It was observed that high temperatures above 30°C play a more critical role than drought due to increased air moisture deficit and increased evapotranspiration (Di Salvo et al., 2021). The increase in air temperature had a negative effect on yield even under irrigated conditions (Di Salvo et al., 2021). Also, high temperatures can limit yields by reducing life cycle duration (Bonhomme et al., 1994). Zhu et al. (2019) combined crop models, satellite observations, and field data to investigate how heat stress affects maize yield in the U.S. Midwest. When the effects of warming were decomposed into direct heat stress and indirect water stress, observational data suggest that the yield was reduced more by direct heat stress than by indirect water stress. They suggest that adaptation strategies should focus on heat stress during grain formation as it poses a marked threat to cause the decline in maize yields and that changes in management should be designed to adequately assess the effects of heat stress during different developmental stages.

Climate change had the effect on maize cropping system in Europe allowing earlier planting and/or growing early maturity cultivars/hybrids. These avoiding strategies are commonly applied in maize where stress can be circumvented by earlier planting dates or planting earlier hybrids to avoid assumed adverse weather conditions mostly during flowering. However, the global trends in temperature and precipitation indicate that extreme weather events may occur at any time throughout the growing season. Thus, the negative effect of temperature during the growing season of maize can be mitigated by selecting later hybrid maturities that require a longer thermal time period to complete development (Di Salvo et al., 2021). Generally, a full-season hybrid can take more advantage of available heat units and performs better when everything else is balanced. At the physiological level, it was shown via chlorophyll fluorescence parameters that early and medium maturity groups of FAO 300 and FAO 400 displayed more sensitive reactions to high temperatures compared to the FAO 500 group (Galić et al., 2021). However, recent climate change considerably impacts maize production causing various (a)biotic stresses (IPCC, 2012) and multiple interactions among them, both documented or new (Šimić, 2018; Malenica et al., 2021). Nevertheless, investigations in France (Caubel et al., 2018), India (Kleinknecht et al., 2013.) and China (Liu et al., 2013a; Liu et al., 2013b) showed that one of the ways to mitigate the effect of heat stress is the detection and selection of the optimal maturity group for each breeding area that can prevent a reduction in maize yield. In the US Midwest, Abendroth et al., (2021) used Pioneer® brand hybrids sales across 17 years in 650 counties to show that in northern counties hybrid maturities of GDD units are more related to the environmentally available GDD units compared to central and southern counties. Overall, hybrid maturities shortened across the majority of the Midwest contrasting with prevalent expectations of hybrid maturity aligning with scale of warming.

In Croatia, Buhiniček et al. (2021) demonstrated that heat stress significantly affected grain yield in all maturity groups, but negative associations between heat stress represented by "stress degree days" (SDD) and grain yield was weakened by later-maturity groups. However, this research was conducted only at one location in Central Croatia (Rugvica near Zagreb) in pre-registration trials. Nevertheless, it was clearly shown that in Croatia heat stress conditions were becoming more frequent and more severe in the last two decades. To our knowledge, there are no publicly available long-term results on relations between maize grain yield and environmental stresses affected by climate change based on comprehensive datasets from Croatia. In average, grain yield of maize in the last two decades increased substantially in Croatia with a growth rate of about 16% per year (Figure 1). Comparable average yield increase in the EU was 3.7% per year in the period 1993-2017 (European Commission, 2018). The total on-farm yield increased in Croatia despite three extremely low-yielding growing seasons in 2000, 2003 and 2012 primarily affected by adverse weather

conditions. At first glance, it seems that there is no reason to worry about the future maize production in Croatia. However, average yields in the first decade lagged behind the average yields in the EU (European Commission, 2018). On the other hand, results of VCU trials in the Republic of Croatia demonstrated that there was a wide gap between on-farm and official variety trials for maize grain yield (Zorić et al., 2017). It suggests that "potential yield" of hybrids candidates for registration in VCU trials are not attainable in on-farm production in the imminent future. Compared to on-farm yield, there was a small annual increase for yield in Croatian VCU trials which was also observed in VCU trials in other European countries (Laidig et al., 2014).

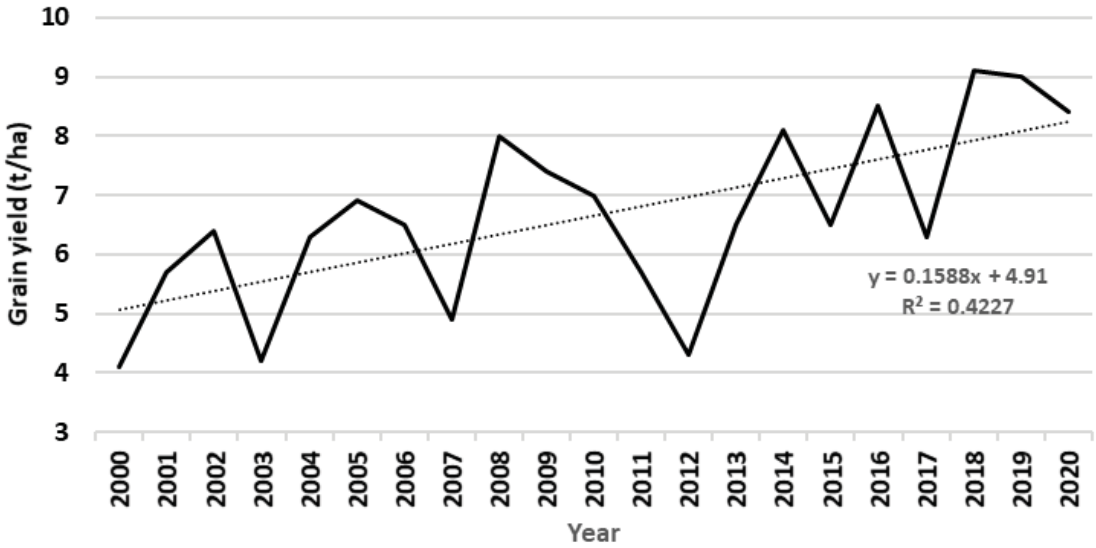

Figure 1. Trend of on-farm yield means of maize in Croatia in the period 2000-2020 (Source: Croatian Bureau of Statistics)

There is no official data on maize production in Croatia categorized by maturity groups. However, according to brand hybrid sales' share of different maturity, it is possible to analyze trends of general shortening or lengthening of maize maturity time. In the last four years, sales' share of brand hybrids of Agricultural Institute Osijek according to the FAO maturity groups was gradually changed. Hybrids of early and medium maturity groups (FAO 300 and FAO 400) increased their share, whereas later hybrids of the FAO 500 group had negative annual growth rate (Figure 2). It indicates that shortening of maize vegetation period is currently present also in Croatia. Apparently, hybrid choice by Croatian farmers is made according to other factors than recent climatic developments.

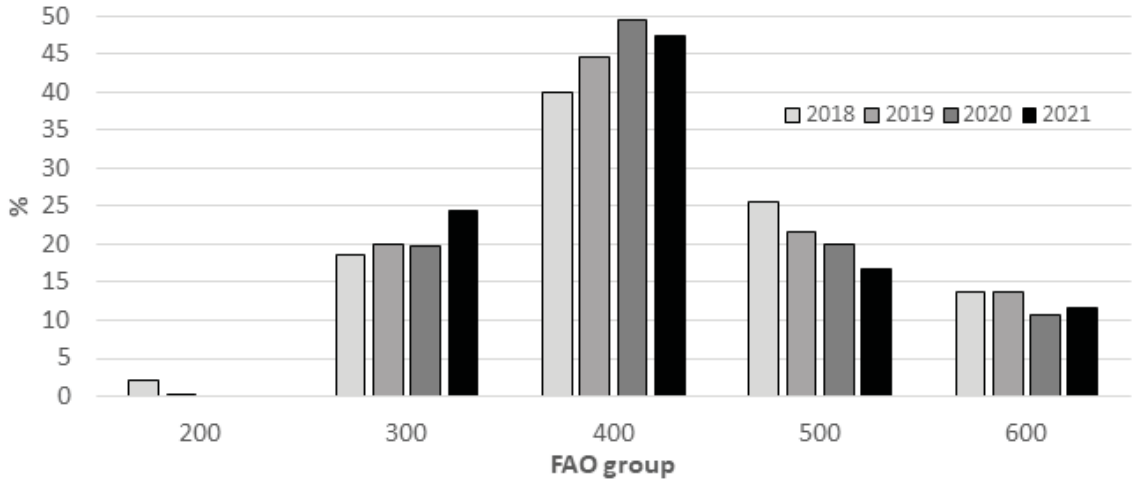

Figure 2. Sales' share of maize hybrids at Agricultural Institute Osijek according to the FAO maturity groups in the period 2018-2021

Predictions

Apart from simple linear regression analyses, there are several phenotype modeling approaches to predict maize yield including crop or statistical models under different climate change scenarios. Parent et al. (2018) simulated grain production over Europe in 2050 based on vast datasets using weather data from AGRI4CAST database (AGRI4CAST, 2022) and applying crop growth simulation model Agricultural Production Systems Simulator (APSIM) (Holzworth et al., 2014). They concluded that in spite of climate change, maize yield in Europe may increase with appropriate hybrid choice. Under the hypotheses of unchanged hybrid relative maturity, production may be stable (-1 to +1%), but if the relative maturity was optimized, grain yield may increase for 4-7%. However, the possible yield increase depends on different climate scenarios. The simulation results in the period 2020 to 2100 by Žydelis et al. (2021) using AgroC model (Huang et al., 2009) showed for Nemoral climate zone in Lithuania, that average yield may increase under RCP2.6 (low), RCP4.5 (medium) and RCP8.5 (high) emission scenarios by 69, 197 and 304 kg ha^{-1} per decade, respectively. In France, Caubel et al., (2018) demonstrated that heat and water stress incidents will be more frequent and severe between flowering and maturity irrespective of climate change scenario and planting date.

In Croatia, Buhiniček et al. (2021) used the same data and analytical resources as Parent et al., (2018) (AGRI4CAST and APSIM) to compare the simulations with observed data. Generally, the simulation followed well the observed results with the lowest (or next to the lowest) yields across all FAO groups in 2003, which was the worst year according to stress degree day accumulation in all 20 years (Figure 3). Regression analysis revealed negative observed yield trends for FAO 200 - 500, and stagnation for FAO 600 over the years. Almost the same trends were obtained by the simulation highlighting FAO 600 as nearly no yield-reducing FAO group over the two decades.

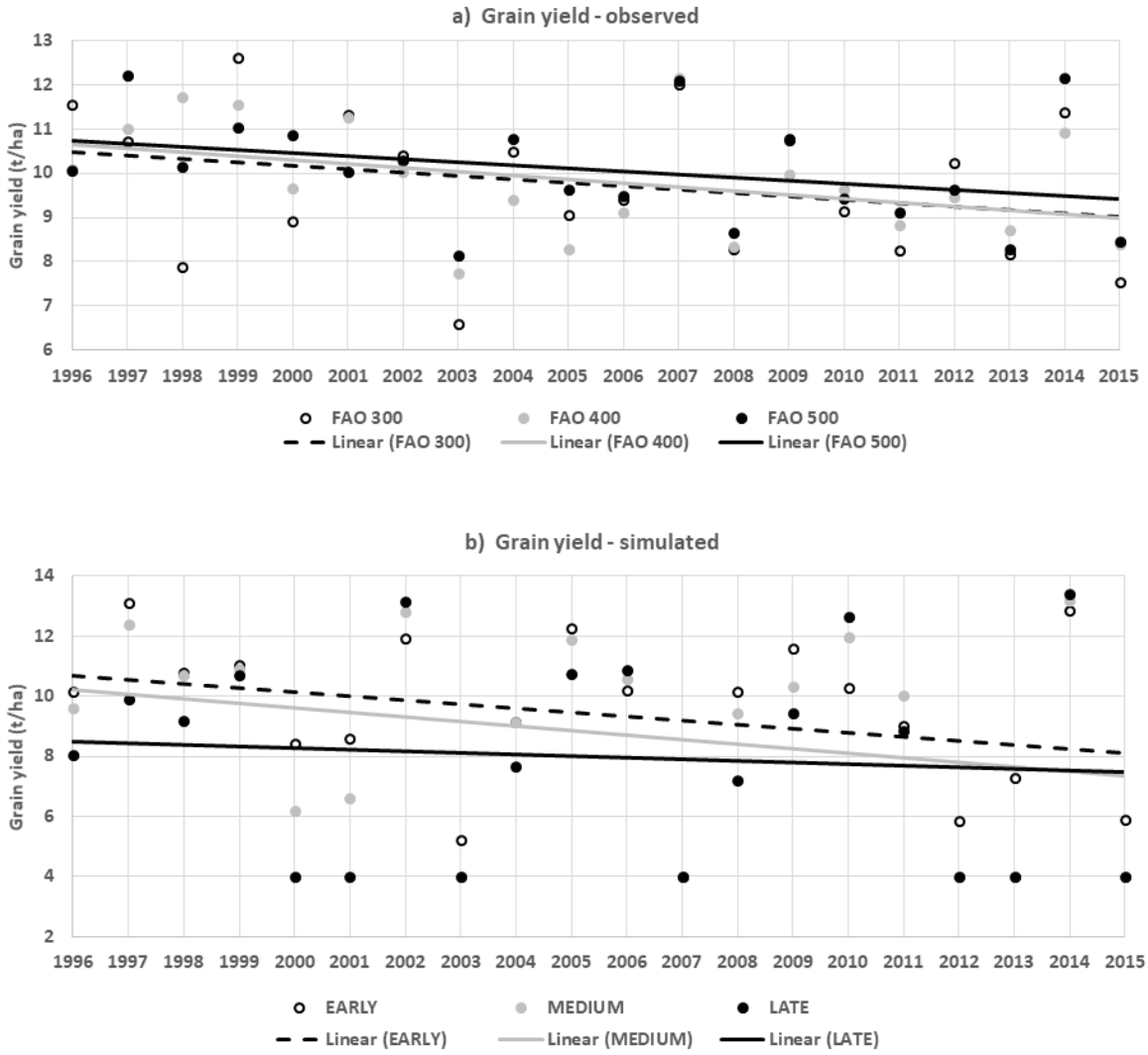

Figure 3**.** Grain yield trend during the period of 1996 to 2015 in Rugvica near Zagreb, Croatia in maize hybrids differing in maturity groups according to: (a) observed data in pre-registration trials (FAO 300 - FAO 500) and (b) APSIM simulated data (early - medium – late). Adapted from Buhiniček et al. (2021)

Grain yield estimates according to AGRI4CAST data and APSIM simulations were generated also for other two remote locations in Croatia: Kutjevo and Osijek for the period 1991-2019 (Stepinac et al., 2021). Generally, there was a slight increase of grain yield in all three locations for the three maturity groups along with considerable deviations over the years, particularly in Osijek, when heat and drought took place more often. No discernible effect of maturity groups was detected.

However, Abendroth et al., (2021) stated that factors other than thermal availability are more important in the US Midwest when choosing maize hybrids, such as drying costs, field operability, labor constraints or crop germplasm availability. Moreover, it seems that the contribution of agronomic management to maize phenology, *i.e.* life cycle is larger than climate change (Liu et al., 2021). Best linear unbiased predictions (BLUPs) (Piepho et al., 2008) revealed that the effect of location became stronger with later maturity, either positive or negative in Croatia (Zorić, unpublished) whereby no geographic pattern was detected. Thus, the effect of location is directly connected to micro-climatic and edaphic conditions:

yields at sites with higher yield potential were less prone to adverse effects than at sites with lower yield, highlighting the crucial role of agronomic management.

The negative effect of the location and its edaphic properties can be undoubtedly mitigated by applying appropriate agro-technical measures (Berzsenyi et al., 2006; Pepo et al., 2021). However, in Southeast Europe, there are inadequate (suboptimum) cultural practice and crop management due to economic reasons (Zorić et al., 2017) resulting in complex and mostly unknown multiple stress growing conditions in field environments. Altogether, investigating simultaneous genotype × environment × management (G×E×M) interactions should be employed (Messina et al., 2009; Cooper et al., 2021) to overcome traditional crop improvement approach seeking firstly genotypes adapted broadly under a standard management regime, and then manipulation of management regionally in response to average local environmental conditions. Traditional discipline-centered approach that dealt with separate components of G×E×M, usually as G×E by breeders and E×M by agronomists should be integrated in order to search the full spectrum of G×E×M combinations forming the adaptation landscape. Understanding the high dimensionality of the complex G × E × M interactions generates opportunities to predict novel Genotype–Management technology combinations suitable to attain sustainable higher yields in future. It implies integration of vast data sets including genotyping and high-throughput phenotyping data.

Outlook

The interplay among genotype, environment and management across maize maturity groups could be investigated by applying new paradigms such as precision agriculture and machine learning. Data rich approaches in agricultural research are scarce, especially considering the dissection of the full spectrum of the G×E×M. Generally, the inflation is present in the data matrices of G, while the data in E and M is often unstandardized, redundant, missing or of poor quality. However, novel approaches such as machine learning show promise in integration of the spatiotemporally auto correlated data with dense genotypic matrices (Westhues et al., 2021) and management information. Contrarily to the traditional linear genomic prediction models, methods such as deep neural networks are agnostic of the data linearity and offer solving of topology related data issues in complex hyperplanes (Krizhevsky et al., 2012). This comes at price that the higher the model complexity, the more high-quality data it needs for a robust performance due to the exponential growth in number of parameters with model complexity. Most importantly, high amounts of low quality data are unable to compensate the data quality, implying the garbage-in, garbage-out heuristics. With this in mind, the maize growing community, as well as all other stakeholders of the agricultural value chain, have come to adopt the Internet of Things (IoT) as novel prospective game-changer in the industry, aiming to simultaneously increase information resolution and quality on the plant, management and the environment, by creating a dense grid of intercommunicating devices at low cost (Spišić et al 2022, in review). Combining the dense data in real-time, but also in historical context can provide important information to farmers. Deployment of such paradigmatic shift, where the on-farm decisions would be supported by dense hyperplanes of data covering many aspects of plant-environment interactions will certainly influence the future trends in hybrid maturity optimization to drain the maximum resource utilization efficiency through improvement of hybrid-environment-management fitness.

Conclusion

The right choice of maize maturity for a given region to attain higher yields in future is not straightforward and it is not made only according to prevalent climate change expectations.

Apparently, the effects of management might play more critical role in maize phenology and yield formation compared to climate change, not only in suboptimum growing conditions often found in Southeast Europe. To facilitate robust analysis of the maize improvement, the integrative G×E×M framework should be applied along with high-throughput genotyping and phenotyping data. Further, precision agriculture including Internet of Things and machine learning could capture more relevant data providing important information to farmers in real-time.

Acknowledgement
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References

Abendroth L.J., Miguez F.E., Castellano M.J., Carter P.R., Messina C.D., Dixon P.M., Hatfield J.L. (2021). Lengthening of Maize Maturity Time Is Not a Widespread Climate Change Adaptation Strategy in the US Midwest. Global Change Biology. 27: 2426–2440.

AGRI4CAST Resources Portal. Available online: https://agri4cast.jrc.ec.europa.eu/DataPortal/Index.aspx

Berzsenyi Z.Z., Dang Q.L. (2006). Effect of Crop Production Factors on the Yield and Yield Stability of Maize (*Zea Mays L.)* Hybrids. Acta Agronomica Hungarica. 54: 413–424.

Bonhomme R., Derieux M., Edmeades G.O. (1994). Flowering of Diverse Maize Cultivars in Relation to Temperature and Photoperiod in Multilocation Field Trials. Crop Science. 34: 156-164.

Buhiniček I., Kaučić D., Kozić Z., Jukić M., Gunjača J., Šarčević H., Stepinac D., Šimić, D. (2021). Trends in Maize Grain Yields across Five Maturity Groups in a Long-Term Experiment with Changing Genotypes. Agriculture-Basel. 11(9): 887.

Caubel J., Cortazar-Atauri I.G., de Vivant A.C., Launay M., Noblet-Ducoudré, N. (2018). Assessing Future Meteorological Stresses for Grain Maize in France. Agricultural Systems. 159: 237–247.

Cooper M., Voss-Fels K.P., Messina C.D., Tang, T., Hammer G.L. (2021). Tackling G × E × M Interactions to Close On-Farm Yield-Gaps: Creating Novel Pathways for Crop Improvement by Predicting Contributions of Genetics and Management to Crop Productivity. Theoretical and Applied Genetics. 134: 1625–1644.

Di Salvo J.I., Lee C., Salmerón M. (2021). Regional Multi-Environment Analysis of Corn Productivity and Yield Stability as Impacted by Hybrid Maturity. Field Crops Research. 262: 108025.

Duvick, D.N. (2005). The Contribution of Breeding to Yield Advances in Maize (Zea Mays L.). Advances in Agronomy. 86: 83–145.

Dwyer L.M., Stewart D.W., Carrigan, L., Ma B.L., Neave P., Balchin D. (1999). Guidelines for Comparisons among Different Maize Maturity Rating Systems. Agronomy Journal. 91: 946–949.

European Commission (2022). Common Catalogue of Varieties of Agricultural Plant Species. Supplement 2022/1. Official Journal of the European Union. C58/01. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:C2022/058/01&from=EN

European Commission (2018). Production, yields and productivity. Available online: https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/production-yields-productivity_en.pdf

Franić M., Galić V., Ledenčan T., Jambrović A., Brkić I., Zdunić Z., Brkić A., Brkić J., Šimić D. (2017). Changes of chlorophyll a fluorescence parameters in water limited

maize IBM population. in *Proceedings of 52nd Croatian and 12th International Symposium on Agriculture*. Vila, S., Antunović, Z. (eds.), 208-211. Osijek, Croatia: Faculty of Agriculture.

Galić V., Ledenčan T., Jambrović A., Zdunić Z., Podnar Žarko I., Šimić D. (2021). Time-resolved chlorophyll fluorescence measurements in maize reveal different patterns of responses to increasing temperatures among maturity groups. In *Proceedings of 56th Croatian and 16th International Symposium on Agriculture*. Rozman V., Antunović Z. (eds.), 344-347. Osijek, Croatia: Faculty of Agrobiotechnical Sciences.

Huang Y., Yu Y., Zhang W., Sun W., Liu S., Jiang J., Yang, Z. (2009). Agro-C: A biogeophysical model for simulating the carbon budget of agroecosystems. Agricultural and forest meteorology. 149(1): 106-129.

Holzworth, D.P.; Huth, N.I.; Devoil, P.G.; Zurcher, E.; Herrmann, N.; McLean, G.; Chenu, K.; van Oosterom, E.J.; Snow, V.; Murphy, C.; Keating B.A. (2014). APSIM–evolution towards a new generation of agricultural systems simulation. Environmental Modelling & Software 62: 327–350.

IPCC (2012). Managing the risks of extreme events and disasters to advance climate change adaptation: special report of the intergovernmental panel on climate change. Field C.B., Barros V., Stocker T. F., and Dahe Q. (Eds.). Cambridge University Press, Cambridge, UK.

Jugenheimer R.W. (1976). Corn: Improvement, Seed Production, and Uses. New York, N.Y., USA: Wiley.

Krizhevsky A., Sutskever I., Hinton G. (2012). ImageNet classification with deep convolutional neural networks. Proceedings of the Advances in Neural Information Processing Systems 25 (NIPS'2012).

Laidig F., Piepho H.P., Drobek T., Meyer U. (2014). Genetic and Non-Genetic Long-Term Trends of 12 Different Crops in German Official Variety Performance Trials and on-Farm Yield Trends. Theoretical and Applied Genetics. 127: 2599–2617.

Liu Z., Hubbard K.G., Lin X., Yang, X. (2013a). Negative Effects of Climate Warming on Maize Yield Are Reversed by the Changing of Sowing Date and Cultivar Selection in Northeast China. Global Change Biology. 19: 3481–3492.

Liu Z., Yang X., Chen F., Wang E. (2013b). The Effects of Past Climate Change on the Northern Limits of Maize Planting in Northeast China. Climatic Change. 117: 891–902.

Liu Y., Zhang J., Pan T., Ge, Q. (2021). Assessing the adaptability of maize phenology to climate change: The role of anthropogenic-management practices. Journal of Environmental Management. 293: 112874.

Malenica N., Dunić J. A., Vukadinović L., Cesar V., Šimić D. (2021). Genetic Approaches to Enhance Multiple Stress Tolerance in Maize. Genes - Basel. 12(11): 1760.

Messina C., Hammer G., Dong Z., Podlich D., Cooper M. (2009). Modelling Crop Improvement in a G×E×M Framework via Gene–Trait–Phenotype Relationships. Crop physiology: applications for genetic improvement and agronomy. 235–265.

Parent B., Leclere M., Lacube S., Semenov M.A., Welcker C., Martre P., Tardieu, F. (2018). Maize Yields over Europe May Increase in Spite of Climate Change, with an Appropriate Use of the Genetic Variability of Flowering Time. Proceedings of the National Academy of Sciences of the USA. 115: 10642–10647.

Pepo P. (2021). Effect of Agrotechnical Factors on Soil Chemical Traits and Maize Yield on Chernozem in the Long-Term Experiment. Plant, Soil and Environment. 67: 453–459.

Piepho H.P., Möhring J., Melchinger A.E., and Büchse, A. (2008). BLUP for Phenotypic Selection in Plant Breeding and Variety Testing. Euphytica. 161: 209–228.

Schils R.L.M., Van den Berg W., Van der Schoot J.R., Groten, J.A.M., Rijk B., Van de Ven G.W.J., Van Middelkoop J.C., Holshof G., van Ittersum M.K. (2020). Disentangling

Genetic and Non-Genetic Components of Yield Trends of Dutch Forage Crops in the Netherlands. Field Crops Research. 249: 107755.

Spišić J., Balen J., Šimić D., Galić V. (2022, *in review*). Assessment of machine learning algorithms for analysis of multispectral proximal remote sensing node reads in maize leaf rolling detection, Remote Sensing 14(X).

Stepinac D., Pejić I., Šimić D. (2021). Modeling the long-term response of yield to heat stress for maize genotypes of different maturity. In *Proceedings of 56th Croatian and 16th International Symposium on Agriculture*. Rozman V., Antunović Z. (eds.), 368-372. Osijek, Croatia: Faculty of Agrobiotechnical Sciences.

Šimić D. (2018). Maize and climate change–coping with multiple stress. In *Proceedings of 56th Croatian and 16th International Symposium on Agriculture*. Rozman V., Antunović Z. (eds.), 7-16. Osijek, Croatia: Faculty of Agriculture.

Westhues C. C., Mahone G. S., da Silva S., Thorwarth P., Schmidt M., Richter J. C., Simianer H., and Beissinger T. M. (2021). Prediction of Maize Phenotypic Traits With Genomic and Environmental Predictors Using Gradient Boosting Frameworks. Frontiers in plant science. 12: 699589.

Zhu P., Zhuang Q., Archontoulis S.V., Bernacchi, C., Müller, C. (2019). Dissecting the Nonlinear Response of Maize Yield to High Temperature Stress with Model-Data Integration. Global Change Biology. 25: 2470–2484.

Zorić M., Gunjača J., Šimić D. (2017). Genotypic and environmental variability of yield from seven different crops in Croatian official variety trials and comparison with on-farm trends. The Journal of Agricultural Science - Cambridge. 155(5): 804-811.

Žydelis R., Weihermüller L., Herbst M. (2021). Future climate change will accelerate maize phenological development and increase yield in the Nemoral climate. Science of the Total Environment. 784: 147175.

Odabir skupine zriobe kukuruza i klimatske promjene: stanje i predviđanja

Sažetak

Zamisao ovoga rada je pokazati međupovezanost genotipova kukuruza kategoriziranih prema skupinama zriobe, okolinskih uvjeta i agronomskog upravljanja za povećanje budućih prinosa zrna u uvjetima klimatskih promjena. Sadašnje stanje i predviđanja ukazuju da optimalan odabir skupine zriobe za postizanje viših prinosa nije jednostavan i ne ovisi samo o prevladavajućim očekivanjima vezanih za klimatske promjene. Agronomsko upravljanje u proizvodnji kukuruza moglo bi imati važniji učinak na fenologiju kukuruza i formiranje prinosa nego klimatske promjene. U budućnosti bi se trebao koristiti integrativni okvir analize interakcije genotip×okolina×upravljanje zajedno s visokopropusnom genotipizacijom i fenotipizacijom. Uporabom metoda precizne poljoprivrede i strojnoga učenja obuhvatilo bi se više relevantnih podataka i pružile poljoprivrednim proizvođačima presudne informacije u realnom vremenu.

Ključne riječi: kukuruz, skupine zrobe, klimatske promjene, prinos zrna, predviđanja

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01

Agroecology, Ecological Agriculture and Environmental Protection

ORIGINAL SCIENTIFIC PAPER

Effects of intercropping system of maize and walnut on agronomical properties of maize

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Abstract

Aim of this study was to determine the influence of intercropping system of walnut and maize on agronomic properties of maize (yields and 1000 grain mass). Study was conducted in Eastern Croatia – near the town of Đakovo, where the site consisted of three plots: 1. sole maize production plot – control, 2. walnut orchard and 3. intercropping system of maize and walnut. Although maize yields did not significantly differ in intercropping (8 t ha^{-1}) and control (sole maize production) (8,4 t ha^{-1}), other parameters did, like plant height, which was more pronounced in the intercropping than in control as well as thousand-grain mass which was 367,1 g in intercropping and 322 g in control. Mass of leaves beneath cobs were higher in intercropping as well as leaf area index (LAI) when compared to that in control. Results of this research suggest that intercropping systems could have positive effects on the agronomical properties of maize.

Key words: intercropping, maize, walnut, yields, productivity

Introduction

Rapid expansion of global population mandated new, innovative and sustainable systems of agricultural production which would maximise yields while reducing monetary investments. Agroforestry could be excellent tool in efforts to combat negative impacts of conventional agricultural production (Jose, 2009). Agroforestry is a system of agricultural production that combines agricultural and forestry production which has numerous advantages and benefits like increase in yields because of, among other reasons, more favourable microclimate conditions, better usage of resources etc. (Kuyah et al., 2019). Intercropping system in this research is silvoarable agroforestry in which rows of maize were planted between rows of walnuts, but the combinations of agricultural and forestry species are endless. The aim of this research was to determine and quantify the effects and impacts of intercropping system of maize and walnut on maize cultivation as well as its agronomical properties.

Material and methods

The field trial was set in Eastern Croatia – Đakovo. Walnut orchards were 13 years old in which the distance between rows was 8 meters. The orchard was divided in two parts – first part in which maize was planted between rows of walnuts and the second part without maize. In the vicinity of the orchard, control plot without trees was set up. Insolation was measured multiple times during vegetation, in the middle of the row and in the shade of walnut trees. Measurements were carried out by luxmeter – manufacturer Sauter, model SO 200K. Leaf area index (LAI) of maize was calculated according to Yi et al. (2010) by calculating the area of every individual leaf. Obtained values were summed for each individual leaf on every plant and by that the leaf area of every plant was calculated and multiplied by number of plants per m^2. In addition to LAI, tree row volume (TRV) was measured too. The TRV was

calculated by multiplying tree height with tree width which was further multiplied by 10.000. Obtained number was then divided by distance between rows. Walnut canopy width was determined by measuring longest and shortest branches which were perpendicular to each other and from that data the mean values were extracted. The height of walnut trees was measured by mobile application. Soil pH values were determined in water and KCl solution (ISO 10390: 1994) and humus was determined by ISO 14235 (1998). Content of P and K in soil was determined with AL method (Egner et al., 1960). Sowing was done on April 26th 2021. Samples of plant materials were collected during 3-5 leaves stage (DAS 31), blister phase (DAS 86), dough phase (DAS 114) and during harvest (DAS 171). A total of 8 samples of plant material was collected, 4 from control and 4 from intercropping system, in each of the phases, where each sample contained 4 plants. Once in all of the phases maize plant height, mass of dry plant, LAI and the mass of leaves beneath the cob were measured and the yield and 1000 grain mass were determined during harvest. Maize yields were determined by harvesting 14.3 m of length. Yield was converted into kg ha^{-1} on basis of 14% moisture. Grain mass of 5 cobs was weighted and the mass of one cob was multiplied by the number of cobs in aforementioned set space. Statistical analysis (statistically significant differences in maize parameters and yield between two location) was carried out in ANOVA with Minitab® Statistical Software version 15 (2007), followed by Tukey's test.

Results and discussion

Levels of phosphorus were highest in orchard (11.2 kg ha^{-1}), then in control (9.8 kg ha^{-1}) and finally lowest levels of phosphorus were in intercropping (8.7 kg ha^{-1}). Potassium levels ranged from 14.1 kg ha^{-1} in control, 15.4 kg ha^{-1} in intercropping to 18.7 kg ha^{-1} in the orchard. Hygroscopicity was significantly higher in control than in intercropping. Insolation values differ from location to location and from plot to plot and there were statistical differences in insolation measured beneath the walnut canopy and in the direct sunlight. During March insolation ranged from 52.000 lux in shade to 60.500 lux in direct sunlight, in April values ranged from 80.000 lux in shade to 94.000 lux in direct sunlight. During May insolation values ranged from 10.000 lux in shade to 85.000 lux in direct sunlight. During June, July and October range of values of insolation were higher in direct sunlight than in the shade of walnut canopy. Maize can potentially be very sensitive to shading and to the conditions of reduced light intensity (Kovačević and Rastija, 2014). As shown before minimal values of lux in intercropping were 10.000 lux and when compared with minimal values of lux needed for maize of 1.400 – 1.800 it can be concluded that intercropping or walnut canopy, to be more precise, didn't negatively impact maize production, but did positively impacted soil moisture in intercropping. Average height of walnut tree was 5.2 m, average width was 5.6 m and interrow distance was 8 m, and with this data TRV was calculated. In comparison to standard walnut tree volume, TRV in this research amounted to 102%. Maize height was measured 3 times: during phase of 5-6 leaves, blister phase and dough phase. Height values ranged from 22.9 cm to 228 cm, depending on a phase. During all 3 aforementioned phases plants were significantly higher in intercropping than in control. Mass of whole (dry) plant was measured in the same 3 phases as the measurements of plant height, and there were statistical differences in plants mass from control and intercropping. Plants mass from phase of 5-6 leaves was 0.39 g for plant from control and 0.66 g for plant from intercropping. In blister and dough phases plants mass was higher in plants from intercropping than plants from control. Mass of leaf beneath the cob was measured two times: during blister and dough phase. Leaf mass in intercropping during blister phase was twice as big as the leaf mass of plant in control (4.1 g - 8.2 g). Leaf mass of intercropped plant in dough phase was 8.6 g while the leaf mass of plant from control was 5.6 g. The LAI calculated during phase of 5-6 leaves was 0.06 plants m^{-2} in control and 0.13 plants m^{-2} in

intercropping. During blister and dough ripening LAI was higher in intercropping than in control. During all three phases statistical differences were determined (p<0,05). From 5 measurements of agronomic properties of maize, in 4 of them statistical differences were determined. Only agronomic property in which statistical differences weren't determined was the yield. Maize yield was 8t/ha in intercropping and 8.4 t ha^{-1} in control. Ivezić et al. (2019) in their research during season 2017/2018 determined that the yield of wheat on control plot was 6.7 t ha^{-1} while the wheat yield in intercropping was 6.0 t ha^{-1} (4.5 t ha^{-1} because only in 75% of plot was wheat planted) while the walnut yield was same in the intercropping and in orchard. Similar to Ivezić et al. (2019), Pardon et al. (2019) and Ivezić et al. (2021) had similar findings, i.e. every culture covered by their research had reduced yield due to the presence of trees, especially maize and sugar beet grown in the vicinity of trees (closer than 10 m). Ivezić et al. (2021) stated in their research that relative yields of cereals grown in silvoarable system were 96% of that in monocultural production, and that in average yields decrease by yearly rate of 2.6% for the first 21 years so the older trees will be more competitive. Mantzanas et al. (2021) stated in their research that the grain yield of barley in intercropping system amounted to 80% of average yields in monocultural production of barley.

There were 54.000 plants per ha in intercropping and 67.750 plants per ha in control. Plant height in this study was 2.2 m in intercropping and 1.8 m in control. Number of cobs was higher in intercropping (11.813) than in control (9.515). 1000 grain mass was too bigger in intercropping (367.1 g) than in control (322 g).

Table 1 Temperatures and moisture in three months throughout different growing systems

	Maximal temperatures (°C) in May	Difference	Maximal temperatures (°C) in June	Difference	Maximal temperatures (°C) in July	Difference
Control	17.7	B	28.1	A	29.3	A
Intercropping	18	B	27.8	B	29.2	A
Orchard	19.4	A	28.5	C	29.2	A
	Minimal temperatures (°C) in May	**Difference**	**Minimal temperatures (°C) in June**	**Difference**	**Minimal temperatures (°C) in July**	**Difference**
Control	14.5	A	22.9	A	24.4	A
Intercropping	14.1	A	22.9	A	25.1	A
Orchard	14.5	A	22.5	A	23.9	A
	Maximal moisture (%) in May	**Difference**	**Maximal moisture (%) in June**	**Difference**	**Maximal moisture (%) in July**	**Difference**
Control	72.5	C	76.40	A	68.6	A
Intercropping	66	A	80.273	A	65.6	A
Orchard	75.5	B	55.69	B	29.1	B
	Minimal moisture (%) in May	**Difference**	**Minimal moisture (%) in June**	**Difference**	**Minimal moisture (%) in July**	**Difference**
Control	34	C	33,42	B	41.7	B
Intercropping	57.5	A	49.85	A	53.2	A
Orchard	46.8	B	32.36	B	23.6	C

*Values denoted by different letters indicate significant differences between systems (p<0,05, Tukey test)

All of the aforementioned microclimate conditions and changes are most likely one of the reasons for statistically higher values regarding plant height, number of cobs per hectare, 1.000 grain mass, mass of whole plant, mass of leaf beneath the cob and LAI. Puškarić et al. (2021) in their research had similar results to these, which they explained by positive impacts of agroforestry systems on nematodes, bacteria and fungi in soil, as well as positive impacts on their biodiversity. Humus content was highest in intercropping system (1.9%), then in

orchard (1.8%) and finally, the lowest values were measured in control (1.6%) which coincides with the results from Puškarić et al. (2021). Highest measured values of moisture, in the month of May, were in orchard, 75.5%, then 72.5% in control and finally 66% in intercropping (Table *1*). Lowest measured values of moisture were in control, 34%, then 46.8% in orchard and 57.5% in intercropping. Temperatures in shown months (Table *1*) are an important microclimate parameter for the growth of maize. During June highest values of moisture were in orchard, 28%, and lowest values were in control, 14.3%. On the contrary to the month of June, in July and August situation was different, so the highest values were in control, 68.6% and 77.7%, and lowest values were in orchard, 23.6% and 30%. Maize has the ability to compete with the forest species for soil moisture and the resulting microclimate changes suit it (Simpson, 1999). Intercropping positively impacted soil moisture in summer months, so moisture in intercropping was statistically higher than moisture in control, as shown in the aforementioned data. During the month of May highest recorded temperatures ranged from 19.4 °C in orchard, 18 °C in intercropping and 17.7 °C in control. Highest temperature in orchard was significantly higher than in intercropping and control. Lowest temperatures ranged from 14.1 °C in intercropping to 14.5 °C both in control and orchard. Similar to the month of May, highest temperatures in June were in orchard, 28.5 °C, then in control, 28.1 °C and finally 27.8 °C in intercropping. Lowest temperatures were measured in orchard, 22.5 °C and 22.9 °C in control and intercropping. During the month of July highest temperature was measured in control, 29.3 °C and then in intercropping and orchard 29 °C. Lowest temperatures were measured in orchard, 23.9 °C, then in control 24.4 °C and finally in intercropping 25.1 °C. Unlike previous month the highest temperature in August was measured in intercropping, 29.1 °C while the lowest temperature was measured in orchard, 23.1 °C. Months shown in the Table 1 are important microclimatic parameter for maize production. Intercropping of walnut trees and maize had numerous positive effects on maize production, so intercropping had lesser oscillations between minimal and maximal values of moisture.

Conclusions

Data presented in this research clearly shows the differences between different production systems. Insolation was statistically higher in direct sunlight (60.500 lux in March and 85.000 lux in May) than beneath the walnut canopy (52.000 lux in March and 10.000 lux in May) which shows great potential of intercropping on the reduction of negative effects of solar radiation on crops. Plants from intercropping were statistically higher throughout all three phases (5-6 leaves, blister and dough phase) in comparison to plants from control. Similarly, the weight of plants had statistical differences as well. Plant weight in 5-6 leaves phase was statistically higher in intercropping (0,66 g) than those from control (0,39 g). Statistical differences were determined on different properties of maize like LAI, 1.000 grain mass, number of cobs per hectare and mass of leaf beneath the cob. Such data suggests a positive effect of intercropping systems on maize production. These positive impacts can be explained by more favourable microclimate conditions in intercropping systems and by positive effects of intercropping systems on soil moisture and on soil organic content. From 5 measured agronomical properties in this research, 4 had statistical differences between control and intercropping and the only property which didn't had aforementioned differences was the yield – 8,0 t ha^{-1} in intercropping and 8,4 t ha^{-1} in control. Intercropping systems are not perfect and they don't have positive impact on all of the properties and parameters of agricultural production. Some properties were impacted in negative manner, such as number of plants per hectare, or in neutral manner like maize yields. Despite these negative and/or neutral impacts on maize production, intercropping systems show potential compared to monocultural systems in regards to other agronomical properties.

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References

Egner H., Riehm H., and Domingo, W.R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nahrstoffzustandes der Boden, II: Chemische Extractionsmetoden zu Phosphor- und Kaliumbestimmung. Kungliga Lantbrukshügskolans Annaler, 26: 199-215.

International Standard Organisation, [ISO 10390: 1994 (E)] (1994c): Soil quality – determination of pH.

International Standard Organisation, [ISO 14235: 1998 (E)] (1998): Soil quality – determination of organic carbon by sulfochromic oxidation.

Ivezić V., Yu Y., van der Werf W. (2021). Crop Yields in European Agroforestry Systems: A Meta-Analysis. Frontiers in Sustainable Food Systems. 5: 1-13.

Ivezić V., Stošić M., Zebec V., Popović B., Puškarić J., Ilić J., Jović J. (2019). Konsocijacija drvenastih vrsta i poljoprivrednih kultura kao inovativnih pristup u agroekosustavima. In the *Book of Abstracts* 54th Croatian & 14th International Symposium on Agriculture. Mioč B., Širić I. (ed.), 51-52. Zagreb, Croatia, Univeristy of Zagreb, Faculty of Agriculture.

Jose S. (2009). Agroforestry for ecosystem services and environmental benefits: an overview. Agroforestry Systems 76: 1–10

Kovačević V., Rastija M. (2014). ŽITARICE. Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet. Osijek, Croatia

Kuyah S., Whitney C.W., Jonsson M., Sileshi W.G., Öborn I., Muthuri C.W., Luedeling E. (2019). Agroforestry delivers a win-win solution for ecosystem services in sub-Saharan Africa. A meta-analysis. Agronomy for Sustainable Development 39.

Mantzanas K., Pantera A., Koutsoulis D., Papadopoulos A., Kapsalis D., Ispikoudis S., Fotiadis G., Sidiropoulou A., Papanastasis V.P. (2021). Intercrop of olive trees with cereals and legumes in Chalkidiki, Northern Greece. Agroforestry Systems 95 (2): 895-905.

Pardon P., Reubens B., Mertens J., Reheul D. (2019). Juglans regia (walnut) in temperate arable agroforestry systems: Effects on soil characteristics, arthropod diversity and crop yield. Renewable Agriculture and Food Systems. 35 (5): 1-17.

Puškarić J., Jović J., Ivezić V., Popović B., Paponja I., Brmež M. (2021.). The communities of the Nematodes, Bacteria and Fungi and the Soil's Organic Matter in an Agroforestry Ecosystem in Croatia. Poljoprivreda, 27 (1): 66-74.

Simpson J. A. (1999). Effects of shade on maize and soybean productivity in a tree based intercrop system. The University of Guelph. National Library of Canada. 106.

Yi L., Shenjiao Y., Shiqing L., Xinping C., Fang C. (2010). Growth and development of maize (Zea mays L.) in response to different field water management practices: Resource capture and use efficiency. Agricultural and Forest Meteorology 150: 606–613

IZVORNI ZNANSTVENI RAD

Utjecaj temperature i eteričnih ulja na *Rhizoctonia solani*

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Sažetak

Cilj je ovog istraživanja u *in vitro* uvjetima ispitati antifungalni utjecaj dvanaest eteričnih ulja na rast micelija *Rhizoctonia solani*. Navedena eterična ulja primijenjena su u količini 25 µL pri temperaturama 15, 20 i 30 °C. Mjerenje zone inhibicije provedeno je 96 h i 192 h nakon inokulacije. Najjače antifungalno djelovanje na gljivu *R. solani* pokazala su eterična ulja anisa, cimet kore, citronele, čajevca, lavande, ružmarina i timijana. Na jačinu antifungalnog djelovanja ulja anisa, čajevca i timijana nisu utjecale različite temperature i vrijeme inkubacije. Najslabije antifungalno djelovanje imalo je ulje naranče slatke.

Ključne riječi: antifungalno djelovanje, eterična ulja, *Rhizoctoni solani*

Uvod

Jedna od najznačajnih i ekonomskih važnih vrsta roda *Rhizoctonia* je *Rhizoctonia solani* Kühn (teleomorf *Thanatephorus cucumeris* (Frank) Donk). Ovaj zemljišni polifag široko je rasprostranjen u cijelom svijetu i destruktivni je patogen na mnogim biljnim vrstama (Tsror, 2010.). Različite vrste roda *Rhizoctonia* uzrokuju bolesti na većini svjetski važnih usjeva kao što su žitarice, šećerna repa, pamuk, travnjaci, ukrasno bilje, voćke i šumsko drveće (Sneh i sur., 2013.). *R. solani* parazitira na više od 200 biljnih vrsta u svim agroklimatskim područjima. Optimalna temperatura za rast gljive je između 23 i 28 °C, iako je kod nekih izolata najbolji porast micelija utvrđen kod nešto nižih ili viših temperatura (Goswami i sur., 2011.). Suzbijanje biljnih bolesti često se provodi kemijskim fungicidima koji su vrlo toksični i nebiorazgradivi te uzrokuju veliko onečišćenje okoliša, a kod sve većeg broj patogena razvija se rezistentnost na iste (Bajpai i sur., 2010.) Međutim, zabranom uporabe sve većeg broja aktivnih tvari te ograničavanjem primjene kemijskih fungicida promiče se smanjenje uporabe ekološki neprihvatljivih pripravaka (Diánez i sur., 2018.), a priorit se daje traženju alternativa kemijskoj kontroli (Valadares i sur., 2018.). Zbog svoga inhibitornog djelovanja na rast patogena, eterična ulja predstavljaju alternativne biološke spojeve i to kao glavne komponente ili kao antimikrobna pomoćna sredstva (Abdel-Kader i sur., 2011.; Nazzaro i sur., 2017.). Eterična ulja predstavljaju složene prirodne mješavine od 20 do 60 kemijskih spojeva zastupljenih u različitim koncentracijama koje su dobivene iz biljnog materijala. Ona su biološki aktivna te mnoga od njih ima antimikrobna, alelopatska, antioksidativna i bioregulacijska svojstva (Bakkali i sur., 2008.; Abbaszadeh i sur., 2014.; Bajpai i sur., 2010.). Od oko 3000 različitih eteričnih ulja komercijalnu važnost ima njih oko 300 (Bakkali i sur., 2008.). Smatraju se nefitotoksičnim spojevima i predstavljaju potencijalno učinkovita prirodna sredstva za zaštitu biljaka (Isman, 2000.; Bajpai i sur. 2008.). Cilj ovog rada je u *in vitro* uvjetima ispitati antifugalno djelovanje 12 eteričnih ulja pri različitim temperaturama na porast micelija fitopatogene gljive *Rhizoctonia solani*.

Materijal i metode

Ispitivanje antifungalnog djelovanja eteričnih ulja na fitopatogenu gljivu *R. solani* provedeno je u Centralnom laboratoriju za fitomedicinu Fakulteta agrobiotehničkih znanosti

Osijek. Ispitivano je antifungalno djelovanje 12 eteričnih ulja u količini 25 µL, pri različitim temperaturama (15, 20 i 30 °C). Korištena su sljedeća ulja: anis (*Pimpinella anisum* L.), bor (*Pinus sylvestris* L.), cimet kora (*Cinnamomum verum* Bercht & Presl), citronela (*Cymbopogon nardus* (L.) Rendle), čajevac (*Melaleuca alternifolia* Cheel.), čempres (*Cupressus sempervirens* L.), eukaliptus (*Eucalyptus globulus* Labill.), klinčić (*Syzygium aromaticum* (L.) Merr. & L. M. Perry), lavanda (*Lavandula angustifolia* L.), naranča slatka (*Citrus sinensis* (L.) Osbeck.), ružmarin (*Rosmarinus officinalis* L.) i timijan (*Thymus vulgaris* L). Navedena eterična ulja su komercijalni pripravci tvrtke Fargon Hrvatska d.o.o. (Kemig). Pokus je postavljen u tri ponavljanja za svaku temperaturu i svako eterično ulje prema metodi Saikia i sur. (2001.). U središte Petrijeve zdjelice ispunjene s krumpir dekstroznim agrom (KDA) postavljen je kružni isječak sterilnog filter papira na koji je aplicirano 25 µL eteričnog ulja. Kružni isječci rastuće kulture *R. solani* stare osam dana sterilnom iglom su stavljeni nasuprotno na četiri mjesta na način da je svaki isječak udaljen 5 mm od ruba zdjelice. Kao kontrola umjesto eteričnog ulja korištena je sterilna destilirana voda. Petrijeve zdjelice su inkubirane u klima komorama pri temperaturama 15, 20 i 30 °C, relativnoj vlažnosti zraka 70 % i svjetlosnom režimu 12 h svjetlo / 12 h tama. Mjerenje zone inhibicije (mm) je obavljeno 96 h i 192 h nakon inokulacije.
Statistička analiza rezultata iz pokusa analizirana je i obrađena u statističkom programu SAS 9. 2 Statistički paket (SAS Institute Inc., Cary, NC, SAD) faktorskom analizom varijance ANOVA i Fisher LSD testom (P= 0,05 i 0,01).

Rezultati i rasprava

Rezultati istraživanja antifungalnog djelovanja eteričnih ulja primjenjenih u količini 25 µL pri različitim temperaturama na porast micelija *R. solani* prikazani su u tablicama 1 i 2.

Tablica 1. Utjecaj eteričnih ulja i temperature na porast micelija *R. solani* 96 sati nakon inokulacije (zona inhibicije mm)

	Temperatura			LSD	
Ulje	15 °C	20 °C	30 °C	0,05	0,01
Anis	**30,00 ± 0,00**	**30,00±0,00**	**30,00±0,00**	0	0
Bor	16,17±5,39	9,92±1,70	20,67±8,10	11,39	17,26
Cimet kora	**30,00±0,00**	**28,83±1,81**	**30,00±0,00**	2,09	3,16
Citronela	**30,00±0,00**	**27,92±3,61**	**30,00±0,00**	4,16	6,31
Čajevac	**30,00±0,00**	**30,00±0,00**	**30,00±0,00**	0	0
Čempres	21,50±5,20	19,58±1,46	28,33±2,89	7,06	10,70
Eukaliptus	24,67±9,24	9,17±0,88	27,08±3,47	11,43	17,31
Klinčić	19,33±2,08	19,25±0,50	21,25±0,75	2,62	3,96
Lavanda	**30,00±0,00**	**21,75±7,61**	**30,00±0,00**	8,78	13,30
Naranča slatka	11,33±2,16	5,00±1,09	17,08±1,46	3,26	4,94
Ružmarin	**30,00±0,00**	**13,92±3,47**	**30,00±0,00**	4,00	6,06
Timijan	**30,00±0,00**	**30,00±0,00**	**30,00±0,00**	0	0
Kontrola	0,00±0,00	0,00±0,00	0,00±0,00	0	0
LSD 0,05	5,71	4,50	4,38		
LSD 0,01	7,72	6,08	5,92		

Kako je već potvrđeno velikim brojem istraživanja, antimikrobno djelovanje eteričnih ulja ovisi o različitim čimbenicima i potrebna su daljnja istraživanja antifungalnog djelovanja eteričnih ulja na rast fitopatogenih gljiva (Ćosić i sur., 2010.; Palfi i sur., 2019.). Eterična ulja anisa, čajevca i timijana u potpunosti 96 sati nakon inokulacije su inhibirala porast gljive

R. solani na svim ispitivanim temperaturama. Kod primjene ulja cimeta kore i citronele na 20 °C utvrđen je vrlo slabi porast gljive no statistički značajne razlike između veličine zone inhibicije u odnosu na zonu inhibicije pri 15 i 30 °C nisu utvrđene. Pri svim temperaturama najslabije antifungalno djelovanje imalo je ulje naranče slatke. Ipak, na temperaturama 15 i 30 °C zona inhibicije je bila statistički značajno veća u odnosu na kontrolnu varijantu. Potpuna inhibicija rasta micelija na temperaturama 15 i 30 °C utvrđena je kod primjene eteričnih ulja anisa, kore cimeta, cintronele, čajevca, lavande, ružmarina i timijana. Kotba i sur. (2020.) su utvrdili da se niti jedan izolat *R. solani* ne razvija na temperaturama ispod 5 i iznad 35 °C. Također su utvrdili da rast gljive na temperaturama između 10 i 33 °C značajno ovisi o izolatu. Isti autori su ispitivali i djelovanje sedam eteričnih ulja na rast micelija gljive te navode da je ulje timijana najjače inhibiralo rast (75,9 %).

Tablica 2. Utjecaj eteričnih ulja i temperature na porast micelija *R. solani* 192 sata nakon inokulacije (zona inhibicije mm)

	Temperatura			LSD	
Ulje	15 °C	20 °C	30 °C	0,05	0,01
Anis	**30,00±0,00**	**30,00±0,00**	**30,00±0,00**	0	0
Bor	0,00±0,00	0,00±0,00	3,33±5,77	6,66	10,09
Cimet kora	30,00±0,00	18,33±16,04	30,00±0,00	18,50	28,03
Citronela	**30,00±0,00**	**20,58±8,20**	**30,00±0,00**	9,46	14,33
Čajevac	**30,00±0,00**	**30,00±0,00**	**29,25±0,09**	1,04	1,58
Čempres	0,00±0,00	0,00±0,00	13,33±7,43	8,57	12,98
Eukaliptus	17,92±15,52	0,00±0,00	2,08±3,61	18,38	27,84
Klinčić	0,00±0,00	0,00±0,00	0,00±0,00	0	0
Lavanda	30,00±0,00	0,00±0,00	27,00±2,88	3,33	5,04
Naranča slatka	0,00±0,00	0,00±0,00	0,00±0,00	0	0
Ružmarin	4,83±8,37	0,00±0,00	30,00±0,00	9,66	14,63
Timijan	**30,00±0,00**	**30,00±0,00**	**30,00±0,00**	0	0
Kontrola	0,00±0,00	0,00±0,00	0,00±0,00	0	0
LSD 0,05	8,21	8,38	4,90		
LSD 0,01	11,09	11,33	6,62		

Nakon 192 sata od inokulacije samo su ulja anisa i čajevca potpuno inhibirala rast micelija na sve tri temperature. Najslabije djelovanje eteričnih ulja utvrđeno je pri 20 °C što se može povezati s optimalnom temperaturom rast micelija koja je prema Grosch i Kofoet (2003.) oko 25 °C.

Zaključak

Na temelju provedenog istraživanja i prikazanih rezultata može se zaključiti da eterična ulja anisa, citronele, čajevca, i timijana imaju najjače antifungalno djelovanje na gljivu *R. solani*. Različite temperature i vrijeme inkubacije nisu imali utjecaj na jačinu antifungalnog djelovanja eteričnih ulja anisa, čajevca i timijana. Najslabije antifungalno djelovanje pri svim temperaturama i dužini trajanja inkubacije imalo je ulje naranče slatke.

Literatura

Abbaszadeh S., Sharifzadeh A., Shokri H., Khosravi A. R., Abbaszadeh A. (2014). Antifungal efficacy of thymol, carvacrol, eugenol and menthol as alternative agents to control the growth of food-relevant fungi. Journal de mycologie medicale. 24(2), e51-e56.

Abdel-Kader M., El-Mougy N., Lashin, S. (2011). Essential oils and *Trichoderma harzianum* as an integrated control measure against faba bean root rot pathogens. Journal of Plant Protection Research. 51(3): 306-313.

Bajpai V. K., Kang S. C. (2010). Antifungal activity of leaf essential oil and extracts of Metasequoia glyptostroboides Miki ex Hu. Journal of the American Oil Chemists' Society. 87(3): 327-336.

Bajpai V.K., Shukla S., Kang, S. C. (2008). Chemical composition and antifungal activity of essential oil and various extract of *Silene armeria* L. Bioresource technology. 99(18): 8903-8908.

Bakkali F., Averbeck S., Averbeck D., Idaomar, M. (2008). Biological effects of essential oils–a review. Food and chemical toxicology. 46(2): 446-475.

Ćosić J., Vrandečić K., Postić J., Jurković D., Ravlić, M. (2010). In vitro antifungal activity of essential oils on growth of phytopathogenic fungi. Poljoprivreda. 16(2): 25-28.

Diánez F., Santos M., Parra C., Navarro M. J., Blanco R., Gea, F. J. (2018). Screening of antifungal activity of 12 essential oils against eight pathogenic fungi of vegetables and mushroom. Letters in applied microbiology. 67(4): 400-410.

Goswami B. K., Rahaman M. M., Hoque A. K. M. A., Bhuiyan K., Mian, I. H. (2011). Variations in different isolates of *Rhizoctonia solani* based on temperature and pH. Bangladesh Journal of Agricultural Research. 36(3): 389-396.

Grosch R., Kofoet, A. (2003). Influence of temperature, pH and inoculum density on bottom rot on lettuce caused by Rhizoctonia solani/Einfluss von Temperatur, pH und Inokulumdichte auf die Salatfäule verursacht durch Rhizoctonia solani. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection. 366-378.

Isman M. B. (2000). Plant essential oils for pest and disease management. Crop protection. 19(8-10): 603-608.

Kotba I., Bouaichi A., Lougraimzi H., Habbadi K., Errifi A., Touhami A. O., Douira A. (2020). Effect of temperature, pH and essential oils on the mycelial growth of *Rhizoctonia solani* Kühn (Cantharellales: Ceratobasidiaceae) isolates. Journal of microbiology, biotechnology and food sciences. 10(3): 461-466.

Nazzaro F., Fratianni F., Coppola R., Feo V. D. (2017). Essential oils and antifungal activity. Pharmaceuticals. 10(4): 86.

Palfi M., Vrandečić K., Popijač V., Ćosić J. (2019). Utjecaj eteričnih ulja na fitopatogene gljive. Poljoprivreda. 25(1): 32-40.

Saikia D., Khanuja S. P., Kahol A. P., Gupta S. C., Kumar S. (2001). Comparative antifungal activity of essential oils and constituents from three distinct genotypes of Cymbopogon spp. Current Science. 80(10): 1264-1266.

Sneh B., Jabaji-Hare S., Neate S. M., Dijst G. (Eds.). (2013). Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Springer science & business media.

Tsror L. (2010). Biology, epidemiology and management of *Rhizoctonia solani* on potato. Journal of Phytopathology. 158(10): 649-658.

Valadares A. C. F., Alves C. C. F., Alves J. M., De Deus I. P., DE Oliveira J. G., Dos Santos T. C. L., Miranda M. L. (2018). Essential oils from *Piper aduncum* inflorescences and leaves: chemical composition and antifungal activity against *Sclerotinia sclerotiorum*. Anais da Academia Brasileira de Ciências. 90: 2691-2699.

Influence of temperature and essential oils on *Rhizoctonia solani*

Abstract
The aim of this study was to examine the *in vitro* antifungal activity effect of twelve essential oils on the growth of *Rhizoctonia solani*. Essential oils were applied in an amount of 25 µL, at temperatures of 15 ° C, 20 ° C and 30 ° C. The inhibition zones were measured 96 h and 192 h after inoculation. Essential oils of anise, cinnamon bark, citronella, tea tree, lavender, rosemary, and thyme showed the strongest antifungal activity on the growth of *R. solani*. The strength of the antifungal activity of anise, tea tree, and thyme oils was not affected by different temperatures and incubation periods. Sweet orange oil had the weakest antifungal activity.

Key words: antifungal activity, essential oils, *Rhizoctonia solani*

STRUČNI RAD

Intenzitet zaraze plodova masline s najznačajnijim štetnicima na području Dalmacije tijekom 2021. godine

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Sažetak
Tijekom 2021. godine utvrđen je intenzitet zaraze ploda masline s najvažnijim štetnicima: maslinina muha, maslinin moljac i maslinin svrdlaš. Ocjena zaraze s štetnicima utvrđena je na uzrocima plodova masline prikupljenih s 63 lokaliteta. Plodovi masline s 58,7 % lokaliteta nisu bili zaraženi maslininom muhom, a najviši intenzitet zaraze muhom utvrđen je na jednom lokalitetu i iznosio je 24 %. Utvrđen je visok intenzitet zaraze karpofagnom generacijom maslininog moljca do čak 100 % (Vrana 2). Najviši intenzitet zaraze (61 %) sa simptomima napada maslininog svrdlaša utvrđen je na lokalitetu Supetar, otok Brač. U 2021. značajne štete uzrokovao je maslinin moljac, a u većini maslinika je izostao jak napad maslinine muhe i svrdlaša.

Ključne riječi: maslinina muha, maslinin moljac, maslinin svrdlaš, ocjena zaraze

Uvod

Štetnici plodova masline su ograničavajući čimbenik koji utječu na kvantitativnu i kvalitativnu proizvodnju stolnih maslina i maslina za uljnu proizvodnju. Maslinu (*Olea europaea* Linnaeus, 1753.) napada preko 250 štetnih organizama. Maslinina muha (*Bactrocera oleae* Rossi) je ekonomski najznačajniji štetnik ploda masline, javlja se svake godine, a intenzitet napada može varirati (Mustafa i Al-Zaghal, 1987.). Najrašireniji je štetnik plodova masline (Haniotakis, 2005.). Maslinina muha kod nas ima 3-5 generacija godišnje (Bjeliš, 2009.). Maslinin moljac (*Prays oleae* Bern.) uz maslininu muhu čini ozbiljne štete na maslinama (Kovanci i Kumral, 2008.). Ličinke prve, druge i treće generacije rade štete na cvjetovima, plodovima i lišću, a razvoj ovisi o fenofazi masline. Ima tri generacije godišnje (Nave i sur., 2017.). Tijekom srpnja je moguće prvo otpadanje plodova uslijed ubušivanja gusjenice u plod, a drugo otpadanje tijekom kolovoza i rujna nakon što gusjenica napusti plod čime direktno utječe na smanjenje prinosa. Maslinin svrdlaš (*Rhynchites cribripennis* Desbr.) je pipa koja može biti prisutna u maslinicima od travnja do kraja kolovoza. Štete od svrdlaša uzrokuju sušenje i otpadanje plodova (Bjeliš, 2005.). Štete od maslinine muhe, maslininog moljca i maslininog svrdlaša se očituju u smanjenom prinosu uslijed otpadanja plodova. Maslinina muha utječe i na smanjenu kvalitetu i organoleptička svojstva dobivenog ulja (Gucci i sur., 2012.). Mjere suzbijanja predlažu se temeljem kontinuiranog praćenja pojave, intenziteta zaraze i širenja svrdlaša te analize biljnog materijala s ciljem smanjenja šteta. Cilj rada je utvrditi intenzitet zaraze ploda masline s najznačajnijim štetnicima.

Materijal i metode

Tijekom kolovoza i rujna 2021. prikupljena su 63 uzorka plodova maslina s različitih lokaliteta iz četiri županije (Zadarska, Šibensko – kninska, Splitsko – dalmatinska i

Dubrovačko – neretvanska). Svaki uzorak je sadržavao 100 plodova maslina. Uzorci su sakupljani nasumično sa stabala iz 63 odabrana maslinika. Na osam lokaliteta uzeti su uzorci iz dva ili više maslinika, radi usporedbe dobivenih rezultata iz različitih maslinika istog lokaliteta. Plodovi su pohranjeni u plastične vrećice s oznakom datuma uzimanja uzorka i lokaliteta te prijenosnim hladnjakom dostavljeni u laboratorij Centra za zaštitu bilja u Solinu. Plodovi masline su analizirani i utvrđena je prisutnost razvojnih stadija maslinine muhe (Genc, 2014.) i oštećenja uzrokovana djelovanjem maslininog moljca i maslininog svrdlaša. Vizualnim pregledom pomoću binokularne lupe Olympus SZX7 utvrđena je prisutnost različitih stadija razvoja maslinine muhe: jaja, tri različita stadija ličinki, kukuljice i otvora kroz koji je izašla odrasla maslinina muha (Zalom i sur., 2009.). Prisutnost jednog stadija maslinine muhe smatrana je oštećenim plodom. Ocjena zaraze karpofagnom generacijom maslininog moljca provedena je vizualnim pregledom plodova masline na oštećenja koju čini ličinka ove generacije moljca. Oštećenja nastala djelovanjem maslininog svrdlaša utvrđena su u vidu okruglih uboda na plodovima koja nastaju ubadanjem rila maslininog svrdlaša koji odlaže po jedno jaje i čija ličinka kasnije uzrokuje sušenje i otpadanje plodova (Bjeliš, 2005.). Intenzitet zaraze izražen je kao učestalost (%) pojave štetnika i simptoma oštećenja. Relativna učestalost zaraze ploda masline s štetnicima po županijama je izračunata kao udjel frekvencije zaraze štetnikom u odnosu prema ukupnom broju lokaliteta.

Rezultati i rasprava

U Tablica 1. je prikazan intenzitet zaraze ploda masline s najznačajnijim štetnicima po lokalitetima. Plodovi masline s 58,7 % lokaliteta nisu bili zaraženi maslininom muhom, dok se na preostalih 41,3% lokaliteta zaraza ploda masline kretala između 1 i 24 %. Najveći postotak zaraze od 24 % utvrđen je na lokalitetu Dolčić, otok Hvar (Splitsko – dalmatinska županija). –Zaraza ploda masline karpofagnom generacijom maslininog moljca utvrđena je od 10 do 40 % kod 60 % uzoraka, a kod 27 % uzorka je utvrđeno više od 40 % zaraženih plodova masline s ovim štetnikom. Kod ostalih uzoraka je bilo manje od 10 % zaraženih uzoraka. Najveći postotak zaraze (100 %) maslininim moljcem u Zadarskoj županiji utvrđen je na lokalitetu Vrana 2. Najmanji postotak zaraze utvrđen je na lokalitetu Ražanac (15 %). U Šibensko – kninskoj županiji, uzorak s lokaliteta Jezera na otoku Murteru je imao najveći postotak zaraze od 48 %, dok je na lokalitetu Grebaštica utvrđen najmanji postotak zaraze od 10 %. U Splitsko – dalmatinskoj županiji najveći postotak zaraze utvrđen je pregledom plodova masline s lokaliteta Krvavica (92 %), a uzorci bez zaraze utvrđeni su na lokalitetima Mravince, Split, Kaštel Stari i Kaštel Štafilić. U Dubrovačkoj županiji najveći postotak zaraze utvrđen je na uzorku s lokaliteta Slano (45 %), a najmanji s lokaliteta Gruda (6 %). Pregledom maslina utvrđeno je otpadanje plodova ukoliko bi se uhvatila grana s plodovima, kao i prisutnim, već otpalim plodovima na tlu, ispod maslina. Na 11 lokaliteta je utvrđena zaraza maslininim svrdlašem od 2 do 8 %, četiri uzorka su imali postotke zaraze do 27 %, a tri uzorka postotke zaraze od 48, 60 i 61 %. Tijekom 2021. godine zaraza maslininim svrdlašem nije učinila značajnije štete u većini maslinika.

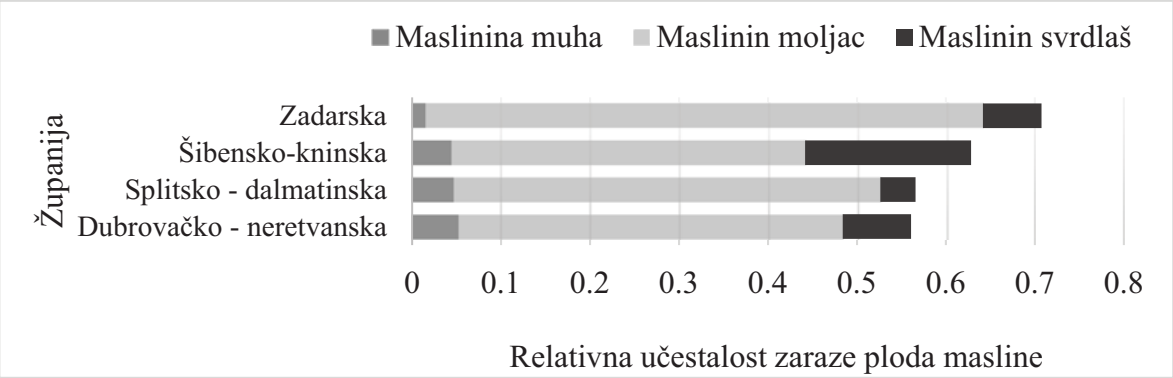
Tablica 1. Intenzitet zaraze ploda masline s maslininom muhom, maslininim moljcem i maslininim svrdlašem po lokalitetima i županijama

Županija / Lokalitet	**Intenzitet zaraze (%)**		
	Maslinina muha	**Maslinin moljac**	**Maslinin svrdlaš**
Dubrovačko - neretvanska			
Raba	6	32	10
Gruda	0	6	2
Gruda 2	0	40	0

Slano	2	38	0
Slano 2	4	45	0
Orašac	8	30	2
Ston	0	22	20
Ston 2	10	18	24
Baćina	0	21	0
Rogotin	0	30	0
Komarna 2	6	20	0
Zaton Doli	4	24	0
Splitsko - dalmatinska			
Mravince	1	0	0
Mravince 2	0	8	0
Mravince 3	0	12	0
Mravince 4	0	64	0
Kaštel Stari	3	0	0
Split, Poljud	2	0	0
Split, Poljud 2	2	30	0
Split, Poljud 3	0	30	0
Stari Grad, otok Hvar	0	2	0
Sveta Nedjelja, otok Hvar	6	14	8
Vrbanj, otok Hvar	2	26	2
Dolčić, otok Hvar	24	46	0
Kaštel Štafilić	0	0	0
Kaštel Štafilić	0	18	0
Kaštel Štafilić 2	0	74	0
Gornje Selo, otok Šolta	0	26	0
Stomorska, otok Šolta	0	24	4
Grohote, otok Šolta	0	30	2
Klis	0	26	0
Sućuraj, otok Hvar	0	40	0
Bogomolje, otok Hvar	0	17	0
Hvar (Kopito), otok Hvar	0	8	0
Dražinica, otok Brač	6	30	0
Supetar, otok Brač	4	46	61
Splitska, otok Brač	0	32	0
Drvenik	16	20	0
Brela	6	48	3
Krvavica	14	92	0
Živogošće	0	56	0
Baška Voda	0	68	0
Tučepi	0	28	0
Kaštel Sućurac	10	50	0
Šibensko - kninska			
Primošten	8	22	6
Primošten Burnji	0	12	48
Grebaštica	0	10	0
Grebaštica 2	0	42	8
Pirovac	0	18	60

Vodice	2	22	16
Ražanac	0	36	3
Dvornica	0	21	0
Šibenik	14	18	0
Jezera, otok Murter	0	48	0
Betina, otok Murter	10	18	0
Vrsine	0	33	0
Zadarska			
Biograd n/m	3	18	27
Vrana 2	0	100	0
Vrana 3	0	51	0
Mirovanje	0	44	0
Komarna	4	30	2
Ražanac	0	15	0
Turanj	0	18	0

Tijekom 2021. godine najveće štete na plodovima masline uzrokovao je napad karpofagne generacije maslininog moljca. Relativna učestalost zaraze ploda masline s štetnicima najveća je bila u Zadarskoj županiji (Grafikon 1). U većini maslinika nije utvrđena zaraza maslininom muhom, iako drugi autori navode da se intenzitet zaraze kreće i do 100 % (Gucci i sur., 2012.).



Grafikon 1. Relativna učestalost zaraze ploda masline s maslininom muhom, maslininim plodom i maslininim svrdlašem u 2021. godini po županijama

U pojedinim godinama napad maslininog moljca može utjecati na smanjenje prinosa od 50 – 90 % (Kumral i sur., 2005.). Karpofagna generacija maslinina moljca uzrokuje štete na plodovima masline od 49 do 63 % (Ramos i sur., 1998.). Štete na plodovima nastale djelovanjem maslininog svrdlaša uglavnom su se odnosile na maslinike sa sortimentom sitnijeg ploda, uz rubne položaje šuma, makije, te u maslinicima gdje se ne provodi agrotehnička mjera frezanja, te redovita i pravovremena zaštita. Ovim istraživanjem ustanovili smo da su se na određenim lokalitetima postotci zaraze maslininim svrdlašem kretali u okvirima ranije utvrđenih postotaka zaraze od 30 do 80 % (Lykouressis i sur., 2005.).

Zaključak

Istraživanjem je potvrđena prisutnost i oštećenja od najznačajnijih štetnika masline u 2021. godini u četiri županije srednje i južne Dalmacije. Intenzitet zaraze plodova masline je bila najveća u Zadarskoj i Šibensko-kninskoj županiji, dok su ostale dvije županije imale podjednaku zarazu plodova masline s najznačajnijim štetnicima. Najvišu relativnu učestalost

zaraze plodova masline je uzrokovala karpofagna generacija maslininog moljca. Ostali štetnici i njihova oštećenja na plodu masline zabilježeni su u maloj brojnosti. Rezultati ukazuju na varijabilnost u pojavnosti najznačajnijih štetnika masline u odnosu na već objavljene podatke, te nužnost kontinuiranog praćenja njihovih populacija radi donošenja odluka o potrebi primjene metoda zaštite masline.

Literatura

Bjeliš M. (2009). Zaštita masline u ekološkoj proizvodnji. II prošireno izdanje, Solin: Vlastita naklada. 7-154.

Genc H. (2014) Embryonic development of the olive fruit fly Bactrocera oleae Rossi (Diptera: Tephritidae), in vivo. Turkish Journal of Zoology. 38: 598-602.

Gucci R., Caruso G., Canale A., Loni A., Raspi A., Urbani S., Taticchi A., Esposto S., Servili M. (2012). Qualitative changes of olive oils obtained from fruit damaged by *Bactrocera oleae* (Rossi). American Society for Horticultural Science vol.47 (2)

Haniotakis G. H. (2005). Olive pest control. Present status and prospects. Integrated protection of Olive crops. 28 (9), 1-9.

Kovanci B., Kumral N. (2008). Insect pests in olive groves of Bursa (Turkey).Acta Horticulturae. 791: 569 – 576.

Kumral N., Kovanci B., Akbudak B. (2005.).Pheromone trap catches of the olive moth, Prays oleae (Bern.)(Lepidopera, Plutellidae) in relation to olive phenology and degree - day models. Journal of Applied Entomology. 129 (7): 375 - 381.

Lykouressis D., Kapsaskis A., Perdikis D., Vatos A., Fanitou A. (2004.). Fruit damage by *Rhynchites cribripennis* (Desbr.), (Coleoptera: Attelabidae) and its 35 population in an olive grove. Integrated Protection of Olive Crops. IOBC/WPRS Bulletin 28(9)

MustafaT.M., Al-Zaghal K. (1987). Frequency of *Dacus oleae* (Gmelin) immature stages and their parasites in seven olive varieties in Jordan. Insect Science and its Application. 8: 165–169.

Nave A., Goncalves F., Teixeira R., Amaro Costa C., Campos M., Torres L. (2017). Hymenoptera parasitoid complex of Prays oleae (Bernard) (Lepidoptera: Parydidae) in Portugal. Turkish Journal of Zoology. 41: 502-512.

Ramos P., Campos M., Ramos J.M. (1998). Long - term study on the evaluation of yield and economic losses caused by *Prays oleae* Bern. in the olive crop of Granada (southern Spain). Crop Protection. 17 (8): 645 - 647.

Zalom, F.G., Van Steenwyk, R. A., Burrack,H. J., Entomology, Johnson, M. W. (2009). Pest Notes: Olive Fruit Fly, UC ANR Publication 74112.

Intensity of infection of olive fruits with the most significant pests in Dalmatia in 2021

Abstract

During August and September 2021, the occurrence and intensity of infection of the most important pests of olives were monitored: the olive fly, the olive moth and the olive weevil. Infection assessment was carried out by sampling fruits at 63 sites. Of all the samples examined, 37 were found without olive fly infection and one with the highest percentage of 24% (Dolčić, island of Hvar). High percentages of infection with the fertile generation of the olive moth have been found to be as high as 100% (Vrana 2). The highest percentage of infection (61%) with symptoms of olive weevil attack was found in the locality of Supetar, island of Brač. In 2021, significant damage was caused by the olive moth, and in most olive groves there was no strong attack of olive flies and weevils.

Key words: olive, olive fruit fly, olive moth, olive weevil, infection rate

IZVORNI ZNANSTVENI RAD

Mogućnost primjene izmeta šturka (*Gryllus assimilis* Fab.) kao poboljšivača tla

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Sažetak
Održivi uzgoj proteina može se ostvariti uzgojem insekata. U uzgoju nastaju nusproizvodi među kojima su ekskrementi insekata koji se mogu koristiti kao poboljšivač tla. Njihova kvaliteta i utjecaj na tlo znanstveno su malo ispitani. Cilj rada bio je utvrditi kemijski sastav izmeta šturka *(Gryllus assimilis* Fab.*)* te efikasnost njegove primjene kao poboljšivača tla u poljskim uvjetima uzgoja salate (*Lactuca sativa* L.*)*. Primjena izmeta šturka je pozitivno utjecala na tlo i kemijski sastav biljke ali nisu ustanovljeni statistički značajni rezultati u prinosima. Utvrđen NPK omjer šturkovog izmeta iznosio je 5:1:4.

Ključne riječi: gnojivo, salata, nusproizvod, industrija insekata, održivo gospodarenje hranivima

Uvod

Upotreba nusproizvoda industrije insekata važna je kako bi se povećala ekonomska održivost industrije i kruženje hraniva na kopnenim ekosustavima (Kronberg i sur., 2021.). Mikro i makro hraniva potrebna za poljoprivrednu proizvodnju često se gube iz kopnenih ekosustava i javlja se potreba za njihovim recikliranjem. Razvojem legislativnog okvira EU potiče razvoj ove industrije (Regulacija 893/2017, 2015/2283 i 2021/1372) te se očekuje i porast količine njenog nusproizvoda, u ovom slučaju izmeta šturka.U Hrvatskoj je prva certificirana farma insekata osnovana 2015. godine, te je u Registar farmi Hrvatske poljoprivredne agencije upisana 2018. godine (Insektarij, 2021.). Na farmi se uzgajaju vrste *Gryllus assimilis Fab. (*Jamajčanski poljski šturak) i *Hermetia illucens* L. (Crna vojnička muha) (Insektarij, 2021.).Više istraživanja utvrdilo je pozitivno djelovanje ovog nusproizvoda na rast i razvoj biljaka (Laland i sur., 2014.; van Huis i sur., 2013.; Čičkova i sur., 2015. prema Zahn, 2017.). Izmet insekata može imati pozitivan utjecaj na rast biljaka zbog potencijalno visokog sadržaja mikrobiološke biomase, hitina, hormona rasta i enzima (Zahn, 2017.). Ovim radom ispituje se mogućnost korištenja nusproizvoda uzgoja šturka, njegovog izmeta miješanog s drugim ostacima poput hitinske ovojnice mrtvih insekata i otrusina proizvodne podloge, u poljoprivrednom kontekstu. Cilj rada bio je utvrditi kemijski i mineralni sastav (makro i mikroelemenata) izmeta šturka (*Gryllus assimilis* Fab.) te efikasnost njegove primjene kao poboljšivača tla u poljskim uvjetima uzgoja salate (*Lactuca sativa* L.).

Materijali i metode

Pokus je proveden na pokušalištu Maksimir Zavoda za povrćarstvo u uvjetima kontinentalne klime na praškasto ilovastom tlu. Na pokusnoj površini od 9 m^2, raspodijeljenu na devet dijelova (tri tretmana s tri ponavljanja), posađene su presadnice salate 29.3.2021 godine. U svakom kvadratu posađeno je 14 jedinki salate sorte „Melina“. Dva tretmana su se razlikovala u sterilizaciji sirovine sat vremena na 80 °C i bez sterilizacije, a jedan tretman je

bio kontrolni i u njemu nije primijenjen šturkov izmet kao poboljšivač tla. Primijenjena je ista doza izmeta sukladno potrebama uzgojne kulture (u ekvivalentu od 100 kg N ha^{-1}) i iznosila je 50 g m^{-2}. Izmet se rasporedio na površini tla, oko posađene biljke, u korijenovoj zoni 01.04.2021. Berba salate je bila 01.6.2021. godine. Uzorci tla prikupljeni su iz oraničnog sloja. Agrometeorološki uvjeti su analizirani prema podacima iz Državnog hidrometeorološkog zavoda te je utvrđeno da su bili nepovoljni za optimalni rast i razvoj salate (Jantol, 2021.).Prije postavljanja pokusa provedene su laboratorijske analize šturkovog izmeta i tla (0-30 cm), u tri ponavljanja. Po završetku pokusa analizirani su uzorci biljnog materijala i tla na svim tretmanima. Uzorci su pripremljeni prema protokolima (HRN ISO 11464:2006), određen je ukupan sadržaj ugljika, vodika, dušika i sumpora CHNS analizom (HRN ISO 10694:2004), provedena je elementarna analiza (37 elemenata) pXRF metodom (ISO 13196) i utvrđen je udio suhe tvari gravimetrijskom metodom (HRN ISO 11465:2004). Na uzorcima tla utvrđen je pH u KCl-u (HRN ISO 10390:2004), ukupni sadržaj karbonata metodom po Scheibleru (HRN ISO 10693:2004), mehanički sastav (HRN ISO 11277:2004), specifična električna vodljivost (HRN ISO 11265:2004), sadržaj biljkama dostupnog fosfora i kalija AL-metodom (Škorić, 1982.), Napravljena je mikrobiološka analiza MALDI-TOF masenom spektrometrijom (HRN EN ISO 16140-6:2020). Prinosi salate bili su izmjereni gravimetrijskom metodom odmah nakon berbe (HRN ISO 11465:2004).
Mikrobiološka analiza provedena je na Institutu Ruđer Bošković u suradnji s Veterinarskim fakultetom Sveučilišta u Zagrebu. Ostale analize napravljene su u laboratoriju Zavoda za opću proizvodnju bilja na Agronomskom fakultetu Sveučilišta u Zagrebu. Prikupljeni podaci analiza tla i biljaka su analizirani u statističkom program SAS 9.1. (SAS Inst.Inc.). Korištena je analiza varijance (ANOVA) i post-hoc t-test (Fisher LSD test). Razina statističke značajnosti u svim testovima potvrđivala se na razini $p < 0.05$.

Rezultati i rasprava

Analiza inicijalnog stanja tla ukazala je na dobru opskrbljenost ukupnim dušikom i umjerenu organskom tvari, za koje nije bila potrebna osnovna gnojidba (Tablica 1). Alkalna reakcija tla, ne zaslanjenost, slabo karbonatno tlo, dobra opskrbljenost biljci pristupačnim fosforom, te slaba kalijem, također su bili pogodni za uzgoj kulture *Lactuca sativa* L.(HAPIH, 2020.).

Tablica 1. Fizikalna i kemijska svojstva tla prije postavljanja pokusa

inicijalno stanje tla	**pH** (u KCl)	**EC** (µS cm^{-1})	**P_2O_5** (mg 100 g^{-1})	**K_2O** (mg 100 g^{-1})	**Humus** (%)	**$CaCO_3$** (%)
	7,34	201	15,7	10,6	2,93	3,71
N (%)	**C** (%)	**S** (%)	**H,** (%)	**Pijesak** (%)	**Prah** (%)	**Glina** (%)
0,15	1,94	0,07	0,9	6	79	15

Napravljene su preliminarne analize šturkovog izmeta 2020. godine (PT). Druga šarža (2021. godine) se razlikovala u postupku sterilizacije i ne sterilizacije (SŠ, NŠ) te je jedan uzorak dolazio od šturka kojem je dio prehrane bio nadomješten kukuruznim brašnom (KŠ). Rezultati se mogu usporediti u tablici 2. Utvrđen je udio vlage ove sirovine koji je iznosio 2,4 % (Tablica 2). To je mnogo niže od klasičnih organskih gnojiva. Na primjer, kokošji izmet koji ima najmanji udio vlage od klasično korištenih organskih gnojiva, sadrži čak 40 % vlage (Lorimor i sur., 2004.). Niži udio vlage znači lakše rukovanje i veću koncentriranost hraniva dajući prednost šturkovom izmetu pred drugim organskim gnojivima.

Tablica 2. Rezultati elementarne analize šturkovog izmeta

	H (%)	N (%)	C (%)	S (%)	P (%)	K (%)	Ca (%)	Mg (%)
PT	-	5,72	40,7	0,63	1,78	3,73	1,91	0,91
NŠ	7,64	5,49	36,67	0,37	0,54	4,53	1,65	<LOD
SŠ	7,57	5,51	37,61	0,38	-	-	-	-
KŠ	8,13	4,83	40,16	0,91	0,46	4,39	1,82	<LOD

	Si (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Mo (mg kg^{-1})	H_2O (%)
PT	0,19	966	613	383	33	3	2,4
NŠ	0,77	864	522	361	48	0,08	-
KŠ	0,85	831	230	464	21	0,03	-

PT preliminarno testiranje
NŠ nesterilizirani izmet
SŠ sterilizirani izmet
KŠ izmet šturka hranjenog kukuruzom

Iz rezultata se može vidjeti da su udio ugljika i NPK omjeri povoljni i dovoljni kako bi se ova sirovina smatrala upotrebljivom za gnojivo. Razlog zbog kojeg se ne naziva gnojivom je Hrvatski zakon o gnojivima u kojima se navodi kako su organska gnojiva podrijetlom domaćih životinja, što šturak nije (NN 163/2003.). NPK omjer prosjeka triju uzoraka (PT, NŠ, KŠ) ove sirovine je iznosio 5:1:4 (Tablica 3), te je u usporedbi s drugim organskim gnojivima sadržavao veći udio dušika (Paudel i sur., 2004.; Houben i sur., 2020.) (Tablica 3.).

Tablica 3. Usporedba sastava šturkovog izmeta s organskim gnojivima životinjskog podrijetla

	N (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
1.	5,35	39,18	0,93	4,22	1,79	0,91	887	455	402	34
2.	5,00	39,30	0,20	0,17		-	-	94	-	10
3.	1,79		2,05	0,75	0,93	0,45	3400	72	384	19
4.	1,72		1,11	1,51	1,09	0,49	2900	48	416	142

1. - prosjek vrijednosti uzoraka izmeta šturka (N=3)
2. - izmet vrste Tenebrio molitor L. (podaci prema Houben i sur., 2020)
3.- goveđi izmet (podaci prema Paudel i sur., 2004)
4.- kokošji gnoj (podaci prema Paudel i sur., 2004)

Elementarnom analizom utvrđene su statistički značajne razlike u količini fosfora i biljkama pristupačnog fosfora u uzorcima tla koji su tretirani sa šturkovim izmetom. Rasponi vrijednosti za fosfor je bila 266 - 415 mg kg^{-1} i za biljkama pristupačan fosfor 141- 570 mg kg^{-1} (Jantol, 2021.).Rezultatima mikrobiološke analize identificirane su sljedeće vrste: *Bacillus cereus, Bacillus megaterium, Enterobacter asburiae, Enterobacter cloacae, Enterobacter bugandensis, Enterobacter kobei, Pseudomonas monteili* i *Paeni bacillus* sp. Nisu utvrđene razlike između steriliziranog i nesteriliziranog tretmana te se nije radila daljnja analiza. Slična istraživanja su utvrdila kako postoji povezanost između primjene izmeta insekata i poželjne mikrobiološke aktivnosti u tlu (Houben i sur., 2020.; Schmitt i Vries, 2020.), što u ovom istraživanju nije zabilježeno. Navedena kategorija bi trebala biti bolje istražena u pokusu u kontroliranim uvjetima rasta biljaka npr. u pokusu s posudama uz promatranje kroz duži period. Elementarnom analizom biljnog materijala u ovom

istraživanju utvrđeni su sljedeći rasponi elemenata (%): fosfor 0,32 - 0,35, kalij 5,4 - 5,6, kalcij 1,98 - 2,04, sumpor 0,13 - 0,17. Prinosi salate u pokusu su bili izrazito varijabilni: 5,65- 31,84 t ha^{-1},što je rezultiralo velikim LSD vrijednostima i ne signifikantnim rezultatima u prinosu. Najbolji rezultat u prinosima biljnog materijala (31,84 t ha^{-1}) je postignut u tretmanu s nesteriliziranim šturkovim izmetom u drugom ponavljanju pri čemu je prinos usporediv s onim u komercijalnoj proizvodnji (Lešić i sur., 2004.) što ukazuje na mogućnost postizanja ciljanih rezultata prinosa korištenjem ove sirovine u komercijalnoj proizvodnji salate.Razlozi za velike varijabilnosti u prinosu bili su i nepovoljni agroklimatski uvjeti ali i štetočine (puževi) koje su utvrđene na pokusnom polju.

Zaključak

Provedeno istraživanje ostvarilo je ciljeve utvrđivanja elementarnog sastava izmeta šturka. Nije dobivena statistički značajna razlika u prinosima među tretmanima, djelom i zbog nepovoljnih agroklimatskih uvjeta i pojave štetnika tijekom istraživanja. Razlog otežanog isticanja pozitivnog djelovanja sirovine moguć je i zbog inicijalno povoljnih karakteristika korištenog supstrata za uzgoj salate. Usporedbom ove sirovine s drugima na tržištu koje se koriste kao poboljšivači tla, uvidio se povoljni elementarni sastav i potvrdila se legitimnost korištenja izmeta šturka u tom kontekstu. Ta činjenica se očituje ponajviše u visokom udjelu organske tvari i dušika u sirovini. Povoljni utjecaj očitava se u vidu povećanja fosfora i biljkama pristupačnog fosfora u tlu i povećanim relativnim prinosima biljnog materijala u tretmanima u kojima se koristila ova sirovina. Najviši prinos i udio suhe tvari utvrđen je u tretmanu s nesteriliziranim šturkovim izmetom. Takvi trendovi ukazuju na potencijal primjene ove sirovine u poljoprivredi te su potrebna daljnja, obimnija istraživanja, posebno u tlima siromašnim hranjivima i organskom tvari. Za nastavak istraživanja preporučuju se kontrolirani uvjeti u pokusu.

Literatura

EU law - EUR-Lex. (n.d.). Retrieved December 2, 2021, from https://eur-lex.europa.eu/homepage.html

HAPIH (Hrvatska Agencija za Poljoprivredu i Hranu, 2020,:Tehnološke upute za tumačenje analiza tla za pračenje stanja poljoprivrednog zemljišta,Osijek, veljača 2020.https://www.hapih.hr/wpcontent/uploads/2020/03/Tehnolo%C5%A1ke-upute-14022020.pdf. Acessed 12.9.2021.

Houben D., Daoulas G., Faucon MP., Dulaurent AM. (2020) Potential use of mealworm frass as a fertilizer: Impact on crop growth and soil properties. Sci Rep 10:1–9. https://doi.org/10.1038/s41598-020-61765-x

HRN EN ISO 13196:2015- Kvaliteta tla -- Provjera tla za odabrane elemente rendgenskom fluorescentnom spektrometrijom uporabom ručnog ili prijenosnog instrumenta (ISO 13196:2013; EN ISO 13196:2015), Hrvatski zavod za norme

HRN EN ISO 16140-6:2020- Mikrobiologija u lancu hrane -- Validacija metode -- 6. dio: Protokol za validaciju alternativnih (zaštićenih) metoda za postupke mikrobiološke potvrde i tipizacije (ISO 16140-6:2019; EN ISO 16140-6:2019) , Hrvatski zavod za norme

HRN ISO 10390, 2004: Kakvoća tla – Određivanje pH vrijednosti, Hrvatski zavod za norme

HRN ISO 10693:2004: Kakvoća tla -- Određivanje sadržaja karbonata -- Volumetrijska metoda (ISO 10693:1995) , Hrvatski zavod za norme

HRN ISO 11261:2004 Kakvoća tla – Određivanje ukupnog dušika – Prilagođena Kjeldahlova metoda, Hrvatski zavod za norme

HRN ISO 11265:2004 Kakvoća tla – Određivanje specifične električne vodljivost, Hrvatski zavod za norme

HRN ISO 11277:2004 Kakvoća tla – Određivanje raspodjele veličine čestica u mineralnom dijelu tla – Metoda prosijavanja i sedimentacije, Hrvatski zavod za norme
HRN ISO 11464:2009 Priprema uzorka za fizikalno-kemijske analize (ISO 11464:2006) , Hrvatski zavod za norme
HRN ISO 11465:2004 Kakvoća tla – Određivanje suhe tvari i sadržaja vode na osnovi mase – Gravimetrijska metoda, Hrvatski zavod za norme
HRN ISO 14235:2004 Kakvoća tla – Određivanje organskog ugljika sulfokromnom oksidacijom, Hrvatski zavod za norme
Insektarij (2021). O nama : insektarij.com. https://www.insektarij.com/o-nama/. Accessed 10 Aug 2021
Jantol A. (2021). Mogućnost primjene izmeta šturka (Gryllus assimilis Fab.) kao poboljšivača/gnojiva u uzgoju salate. Diplomski rad. Agronomski fakultet u Zagrebu
Kronberg SL, Provenza FD, van Vliet S, Young SN. (2021). Review: Closing nutrient cycles for animal production – Current and future agroecological and socio-economic issues. Animal 100285. https://doi.org/10.1016/j.animal.2021.100285
Lešić, R., Borošić, J., Butorac, I., Herak – Ćustić, M., Poljak, M., Romić, D. (2004). Povrćarstvo. II dopunjeno izdanje. Udžbenici Sveučilišta u Zagrebu. Manualia Universitatis studiorum Zagrabiensis. Agronomski fakultet – Zrinski d.d., Čakovec
Lorimor J., Powers W, i Sutton A. (2004.), Manure Characteristics, Manure Management Systems Series, MWPS-18, 2004, MidWest Plan Service, Iowa State University, Ames, Iowa 50011-3080 (515-294-4337)
Narodne Novine, (2003). Zakon o gnojivima i poboljšivačima tla. https://narodne-novine.nn.hr/clanci/sluzbeni/2003_10_163_2346.html. Accessed 13 Sep 2021
Paudel KP, Sukprakarn S, Sidathani K, Osotsapar Y. (2004). Effects of Organic Manures on Production of Lettuce (Lactuca sativa L.) in Reference to Chemical Fertilizer. Natural Sciences. 38:31–37
SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-2004.
Schmitt E, de Vries W. (2020). Potential benefits of using Hermetia illucens frass as a soil amendment on food production and for environmental impact reduction. Current Opinion in Green and Sustainable Chemistry 25:100335. https://doi.org/10.1016/j.cogsc.2020.03.005
Zahn NH. (2017). The effects of insect frass created by Hermetia illucens on Spring onion growth and soil fertility. University of Stirling 1–65.

Possibility of using cricket frass (*Gryllus assimilis* Fab.) as a soil amendment

Abstract
Sustainability of protein cultivation can be achieved with insect breeding so this industry is increasingly encouraged. The quality and efficiency of products from insect frass that are used as soil amendments have been scientifically little tested. The aim of this study was to determine the chemical composition of cricket frass (*Gryllus assimilis* Fab.) and the effectiveness of its application as a soil amendment in field-grown lettuce (*Lactuca sativa* L.). The application of cricket frass had a positive effect on the plant and soil but no statistically significant differences in yield were determined. The NPK ratio of frass was determined to be 5: 1: 4.

Key words: fertilizer, lettuce, by-product, insect industry, sustainable nutrient management

ORIGINAL SCIENTIFIC PAPER

Antifungal activity of *Lactobacillus casei* and *Lactobacillus brevis*

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Abstract
Lactic acid bacteria (LAB) are indigenous and present in various substrates: soil, vegetables, meat, fermented products, etc. They are irreplaceable in dairy production and the feed industry. Antimicrobial metabolites are formed by LAB, and some of them have antifungal properties. Fungi are the main causes of plant diseases and contaminants of stored products in agricultural production, so the aim of this study was to determine the antifungal activity of the *Lactobacillus* casei and *Lactobacillus brevis* on wheat grain contaminants: *Alternaria alternata* and *Fusarium graminearum*. The results showed 94% growth inhibition of *F. graminearum* by both investigated species of *Lactobacillus* with a statistically significant difference ($p < 0.001$) compared to the control. Weaker growth inhibition of 72% was found by the action of both *Lactobacillus* species on *A. alternata* with a statistical difference ($p < 0.001$) compared to the control. This study confirmed the antifungal activity of investigated *Lactobacillus* species on the growth of investigated fungi on wheat grains.

Key words: *Lactobacillus* spp., *Fusarium, Alternaria*, wheat, grains

Introduction

Lactic acid bacteria (LAB) are present and isolated from soil, water, manure, plants, milk, and fermented foods (Holzapfel et al., 2001). They occur naturally on the skin, in the oral cavity, intestines, and urogenital tracts of humans and animals (Harzallah and Belhadj, 2013). Some genera taken orally, have a beneficial effect on the intestinal microbiota and are used as probiotics. Their use in the food and feed industry is irreplaceable today. Indigenously present bacteria are the cause of spontaneous fermentations used by our ancestors in the past. Today, selected strains of desirable properties are used as starter cultures in the fermentation processes of milk, meat, vegetables, and cereals (Bintsis, 2018). By their action, carbohydrates are converted into lactic acid, which has a conservative effect, and fermentation improves the organoleptic, nutritional, and technological properties of the product (Chan and Hang, 2019). Changes in fermented products affect the extension of shelf life compared to the initial product and have a beneficial effect on human health (Chan and Hang, 2019). Strategy for microorganisms introduced into the food chain or used as a producer for food and feed additives, enzymes, and amino acids is developed by The European Food Safety Authority (EFSA, 2019) based on the Qualified Presumption of Safety (QPS) concept. Among others, *Lactobacillus* spp. has QPS status. In addition, LABs produce a number of metabolites, some of which have antifungal activity (Magnusson and Schnürer, 2001; Salas et al., 2018). From the aspect of agricultural production, this activity is very interesting considering that fungi are the most common pathogens in field conditions and they are contaminants of stored products. In addition to contamination, the undesirable effect of fungi growth is the biosynthesis of mycotoxins that may cause toxic effects in human and animal populations. Studies have established the inhibitory effect of lactic acid bacteria on fungal growth and mycotoxin synthesis *in vitro* and *in vivo* in agricultural

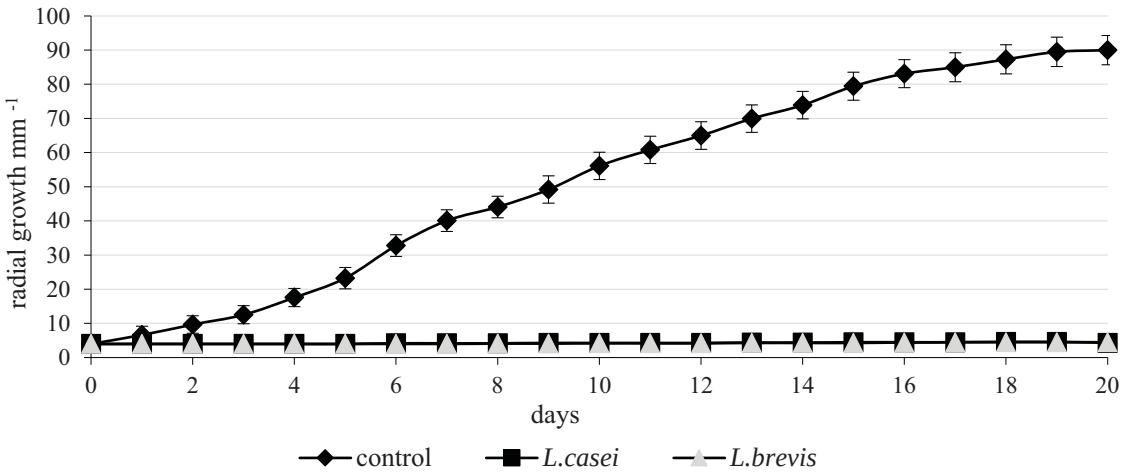
products (Zhao et al., 2013; Kivanc et al., 2014; Sellamani et al., 2016; Lipinska et al., 2016; Shehata et al., 2019). According to the frequency of isolated types of fungi on wheat grain, *Fusarium graminearum* and *Alternaria alternata* were included in this study. *F. graminearum* is the dominant species isolated from wheat and barley grain samples in the Republic of Croatia (Ćosić et al., 2007). Cereal grain is also frequently contaminated with *A. alternata* (Logrieco et al., 1990; Vrandečić et al., 2013). Different species of the genus *Alternaria* were isolated from wheat samples in the Republic of Croatia depending on the year, fertilization, and tillage, *Alternaria* infection ranges from 21 to 36% (Vrandečić et al., 2013). The aim of this study was to determine the antifungal activity of *Lactobacillus casei* and *Lactobacillus brevis* on the growth of these frequently identified types of fungi on wheat grain.

Material and methods

Lactobacillus casei and *Lactobacillus brevis* from the collection of the Chair of Microbiology of the Faculty of Agrobiotechnical Sciences Osijek were used in the research. De Man, Rogosa, Sharpe (Merck, Germany) liquid medium was used to cultivate lactic acid bacteria at 37 °C for 48 h. The phytopathogenic fungi used include *Fusarium graminearum* Schwabe R-05796 (Fusarium Research Center Culture Collection, Department of Plant Pathology, Pennsylvania U.S.A) and *Alternaria alternata* from the collection of the Chair of Microbiology of the Faculty of Agrobiotechnical Sciences Osijek. Pure cultures of fungi were inoculated on potato dextrose agar (Biolife, Italy) at 25 °C ± 1 °C for seven days. Antifungal activity was tested on the wheat grain of the Xenos variety, produced on the organic farm "OPG Nedjeljko Bošnjak", which was weighed in the amount of 10 g in Petri dishes and autoclaved at 121 °C for 15 minutes. The seeds were treated with $3x10^9$ LAB cells per ml (Juodeikiene et al., 2018), according to Grant Instruments^{TM} liquid McFarland standards (Fisher Scientific Ltd., UK). Control samples were treated with sterile water. Inoculation was performed with a mycelial disc taken from the edges of a fungi colony cultures using a cork borer with a diameter of 4 mm. Incubation was performed at 30 ºC ± 0.2 °C in a laboratory thermostat (Inko, Croatia). Over a period of 20 and 30 days, respectively, until the fungi reached the edge of the petri dish, fungi growth was monitored by measuring radial growth. The experiment was set up in two repeats with four replicates. Statistical analysis included determining the difference between fungi growth rate and control by Student's t-test. Microsoft Excel (2013) and Statistica 13.5 (Tibco Software Inc.) were used for statistical data analysis.

Results and discussion

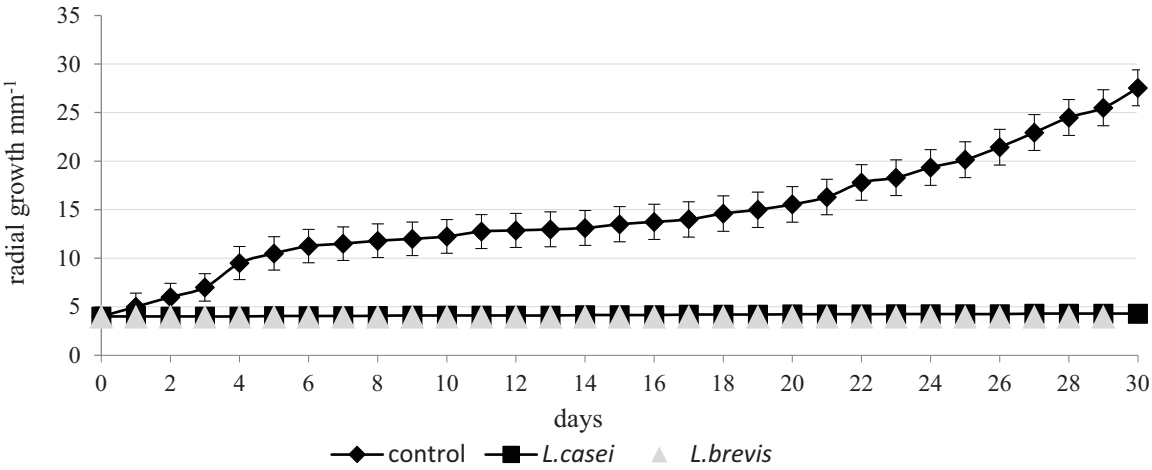
In this study, a strong inhibitory activity of *L. casei* was recorded, and reduced mycelial growth of *F. graminearum* by 94.5% was noted compared to the control (Graph 1). Similar results were obtained with *L. brevis* where growth inhibition of the same fungi of 94.2% was observed compared to the control, with a statistical difference ($p < 0.001$) for both tested species of LAB.



Graph 1. *F. graminearum* growth on wheat grains treated with *L. casei* and *L. brevis*

Studies by other researchers have confirmed the antifungal effects of LAB on fungi. Shehata et al. (2019) applied cell-free supernatants (CFS) from *Lactobacillus* sp. RM1 to wheat grains and *Aspergillus parasiticus* mycelia was completely inhibited after 2 weeks of incubation. Complete inhibition of aflatoxins B1 and ochratoxin A was achieved at a CFS concentration of 15 mg mL^{-1} in broth culture. The chemical composition of CFS determined by GC–MS showed antimicrobial metabolites with antifungal and antibacterial properties. The authors concluded that fungal growth and mycotoxin synthesis with tested *Lactobacillus* RM1 are reduced and it's suitable as a biological preservative in agricultural and food products. Cell-free supernatants of *Pediococcus pentosaceus* isolated from traditional fermented dairy products from India showed mycelial inhibitory activity of *F. graminearum* and zearalenone production *in vitro* (Sellamani et al., 2016). Zhao (2013) found that 10% CFS of *Lactobacillus rhamnosus* VT1 successfully inhibited the growth of 83.7% *F. graminearum in vitro*. Also in the same study CFS of the same strain showed strong suppression of deoxynivalenol, zearalenone, and 3-acetyl-deoxynivalenol on naturally infected barley.

In this study inhibitory activity of *L. casei* and *L. brevis* was also determined in the reduction of *A. alternata* mycelial growth of 71.6% and 72.02% (Graph 2) compared to the control with a statistical difference for both examined species of the genus *Lactobacillus* ($p < 0.001$). It should also be noted that the growth of *A. alternata* was weaker than *Fusarium* mycelial growth on wheat grains.



Graph 2. *A. alternata* growth on wheat grains treated with *L. casei* and *L. brevis*

Similar results were noted by other researchers as follows. *In vitro* study by Lipinska et al. (2016) revealed that among 60 tested *Lactobacillus* sp, *L. brevis* 0980 and *L. casei* 1020 cultivated with xylitol or galactosyl-xylitol, compounds that represent a modern prebiotic and can improve the condition of the gastrointestinal tract, showed total inhibition of *A. alternata* on agar medium. Kivanc et al. (2014) in their screening assay tested 22 LAB isolates against pathogenic fungi. *A. alternata* in the dual culture agar overlay method was the most sensitive strain. In the same study of LAB isolates, F2.1 was the most efficient and was identified as *Enterococcus durans*. This strain completely inhibited the growth of mycelia and conidia germination of *Penicillium chrysogenum* after 48 hours, *Penicillium griseofulvum* and *Aspergillus parasiticus* after 6 days in MRS broth. The authors consider the potential use of investigated LAB strains as food biopreservation. Antifungal efficacy of different genera and species of LAB has been established in this but also in several other studies. Research on LAB is well documented in the food industry, but the effect of LAB on substrates from agricultural production is not sufficient. Further investigations of interactions between the substrate and the LAB and LAB metabolite are desirable.

Conclusion

The antifungal effect of LAB is well documented in dairy products but this activity has the potential for use on grain stored products. This study confirmed the antifungal effect of *L. casei* and *L. brevis* on the growth of storage fungi *A. alternata* and *F. graminearum* on wheat grains. It is necessary to investigate the effects of other abiotic and biotic interactions that would clarify the nature of the LAB actions.

References

Bintsis T. (2018). Lactic acid bacteria: their applications in foods. Journal of Bacteriology and Mycology. 6, (2): 89-94.

Chan W., Hang F. (2019). Lactic acid Bacteria Starter. In: Lactic acid Bacteria: Bioengineering and Industrial Applications. Chen (ed), Bejing, Science Press Bejing, Springer.

Ćosić J., Jurković D., Vrandečić K., Šimić B. (2007). Pathogenicity of *Fusarium* species to wheat and barley ear. Cereal Research Communications. 35: 529-532.

EFSA Journal (2019). Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 10: Suitability of taxonomic units notified to EFSA until March 2019. 17 (7), 5753.

Holzapfel W. H., Haberer P., Geisen R., Björkroth J., Schillinger U. (2001). Taxonomy and important features of probiotic microorganisms in food nutrition. American Journal of Clinical Nutrition. 73: 365-373.

Harzallah D., Belhadj H. (2013). Lactic Acid Bacteria as Probiotics: Characteristics, Selection Criteria and Role in Immunomodulation of Human GI Mucosal Barrier in Lactic Acid Bacteria – R & D for Food, Health and Livestock Purposes. Marcelino Kongo (ed.), IntechOpen.

Juodeikiene G., Bartkiene E., Cernauskas D., Cizeikiene D., Zadeike D., Lele V., Bartkevics V. (2018). Antifungal activity of lactic acid bacteria and their application for *Fusarium* mycotoxin reduction in malting wheat grains. Food Science and Technology. 89: 307-314.

Kıvanc M., Kıvanc S.A., Pektas S. (2014). Screening of Lactic Acid Bacteria for Antifungal Activity against Fungi. Journal of Food Processing and Technology. 5: 310.

Lipinska Zubrycka L., Klewicki R., Klewicka E., Kolodziejczyk K., Sójka M., Nowak A. (2016). Antifungal Activity of Lactobacillus sp. Bacteria in the Presence of Xylitol and Galactosyl-Xylitol. Journal of Biomedicine and Biotechnology. 1: 1-8.

Logrieco A., Bottalico A., Solfrizzo M., Mule G. (1990). Incidence of *Alternaria* Species in Grains from Mediterranean Countries and Their Ability to Produce Mycotoxins. Mycologia. 82 (4): 501-505.

Magnusson J. Schnürer J. (2001). *Lactobacillus coryniformis* subsp. *coryniformis* strain Si3 produces a broad-spectrum proteinaceous antifungal compound. Applied and Environmental Microbiology. 67: 1–5.

Salas M.L., Thierry A., Lemaître M., Garric G., Harel-Oger,M., Chatel M. Lê S., Mounier J., Valence F., Coton E. (2018). Antifungal Activity of Lactic Acid Bacteria Combinations in Dairy Mimicking Models and Their Potential as Bioprotective Cultures in Pilot Scale Applications. Frontiers in Microbiology. 9: 1-18.

Sellamani M., Kalagatur N. K., Siddaiah C., Mudili V., Krishna K., Natarajan G., Rao Putcha V.L. (2016). Antifungal and Zearalenone Inhibitory Activity of Pediococcus pentosaceus Isolated from Dairy Products on *Fusarium graminearum*. Frontiers in microbiology. 7:1-12.

Shehata M.G., Badr A.N., El Sohaimy S.A., Asker D., Awad T.S. (2019). Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. Annals of Agricultural Sciences. 64: 71-78.

Vrandečić K., Jug D., Ćosić J., Stošić M., Ilić J. (2013). The impact of tillage and fertilization on wheat grain infection. Proceedings and Abstracts 2nd International Scientific Conference Soil and crop management: adaptation and mitigation of climate change, Vukadinović Vesna and Đurđević Boris.(eds.). 296-301, Osijek, Croatia.

Zhao H. (2013). Inhibition of *Fusarium* growth and trichothecene accumulation in grain by antifungal compounds from lactic acid bacteria. A Dissertation, the North Dakota State University of Agriculture and Applied Science.

ORIGINAL SCIENTIFIC PAPER

Estimation of forage value of dry grasslands on the Dinara mountain based on the analysis of the botanical composition

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Abstract
As a part of the project „Dinara back to LIFE“ (LIFE 18 NAT/HR/000847), vegetation was surveyed on 30 grassland habitats on Dinara mountain. A total of 158 taxa was recorded during the survey. The quality and total value of grasslands were estimated using the „Complex Method“. The basic quality index (BQI) was calculated for plants in the green mass and the hay. BQI varied between -6.8 and 16.6 in the green mass and -11.2 and 8.2 in the hay. Generally, it was observed that the grasslands of lower quality (especially in the green mass) are located in the southern central parts of the project area, whereas those of higher quality are situated in the northern and central parts of the area.

Key words: grassland, forage value, complex method, pastures, Dinara

Introduction

It is important to evaluate the quality and the total value of grasslands to determine which grasslands are appropriate for pasture and forage production, as well as to determine what agrotechnical and amelioration methods to use for boosting their quality (Šoštarić-Pisačić and Kovačević, 1974). Numerous authors have proposed methods for the evaluation of grassland quality, ex. Ellenberg (1952), Klapp et al. (1953), Šoštarić-Pisačić and Kovačević (1974), more recently Briemle et al. (2002), Novák (2004), and others. The „Complex Method“ (Šoštarić-Pisačić and Kovačević, 1974) for evaluation of the quality and total value of grasslands and leys is one of the strictest grassland quality evaluation methods, as well as one of the only ones that make a distinction between green mass and hay (considering that the feeding value of many plants changes through drying) and takes into account the weight share of plants. While developing the quality classification, the authors took into consideration fifteen different factors, such as morphology and anatomy of plants, developmental phase, palatability, chemical composition, digestibility, poisonousness, resistance to diseases and parasites, deterioration by haymaking, etc (Šoštarić-Pisačić and Kovačević, 1974). Another reason why „Complex Method“ was particularly suitable for our research is the fact that it contains an extensive list of classified plants belonging to Croatian flora, including Mediterranean plants that grow in our project area but are not present in Central Europe, hence are not listed in the classifications of the other mentioned authors. Dinara mountain is situated in Dalmatian Hinterland and is predominantly covered by grassland habitats – they comprise almost 50% of its' area. Most of those grasslands are submediterranean and epimediterranian dry grasslands (Basrek et al., 2020; OIKON d.o.o., 2004). Historically, the inhabitants of Dinara were cattle breeders and transhumance occurred every season when shepherds took their livestock from the lower parts of Dalmatian Hinterland and Dalmatia where it was too dry and hot during summer months to the pastures on Dinara which were rich in diverse plant species, fresh and often near water. This practice was lost towards the end of the 20th century and now only occurs sporadically (Basrek et al., 2020; Marković, 2003). The project „Dinara back to LIFE“ was launched in 2020. Among

other goals, it is set to develop management plans for the Dinara grasslands and restore them to the condition where it will be possible to use them again as pastures.

Material and methods

Thirty grassland habitats in total were surveyed during the field research, mostly belonging to the submediterranean dry grasslands (*Scorzoneretalia villosae*) (Natura2000 code: 62A0) (Interpretation manual of European Union habitats, 2007). The grasslands were surveyed in groups of ten on three locations within the project area – near the settlement Validžići (Kijevo municipality) in the northern part of the Dinara mountain, near the settlement Ježević in the central part of the project area, and near Vrdovo (settlement Donji Bitelić) in the southern central part of the project area. Thirteen grasslands were in various degrees of succession, mostly by *Juniperus oxycedrus, Fraxinus ornus, Rhamnus intermedia, Quercus pubescens, Carpinus orientalis,* etc. The vegetation survey was conducted using the Barkman scale where the cover of each taxon is marked with a symbol ranging from r (less than 1% of the plot cover, 1-2 individuals) to 5 (more than 75% of the plot cover).

The „Complex Method" was used to estimate the quality of surveyed grassland habitats. Each taxon was sorted into a quality class according to the Complex Method, ranging from Vn (very noxious) to Exc (Excellent). Quality classes and corresponding coefficients can be found in Šoštarić-Pisačić and Kovačević (1974). If a taxon wasn't available in Šoštarić-Pisačić and Kovačević (1974), it was sorted into the quality class of another taxon from its' genus, that was the most similar in morphological characteristics. The symbols from the Barkman scale were transformed into percentages of cover. The basic quality index (BQI) was calculated following this formula:

$$BQI = (1.0^* Exc + 0.8^* Vg + 0.6 * G + 0.4^* M + 0.2^* P) – (0.2^* dp + 1.0\text{-}2.0^*n + 2.5\text{-}4.0^* Vn)$$

The statistical analysis was conducted using a t-test (α= 0.05) and one-way ANOVA (α= 0.05) followed by the Bonferroni correction and post hoc test.

Results and discussion

During the vegetation survey, 158 taxa were recorded, out of which 134 species, 8 subspecies, and 16 taxa only determined to the level of genus.

Table 2. Basic quality indices of surveyed grasslands in the green mass (BQI^{gm}) and hay (BQI^{h}); (SD – standard deviation; St. error – standard error; * - grassland in succession)

Validžići			Ježević			Vrdovo		
Location	BQI^{gm}	BQI^{h}	Location	BQI^{gm}	BQI^{h}	Location	BQI^{gm}	BQI^{h}
AK1	4.8	-0.6	AJ1*	6	8.2	AV1*	3	-1.4
AK2*	0.6	-0.2	AJ2*	16.6	-0.2	AV2*	1.5	-1.1
AK3	1.6	-5.6	AJ3	5	0.2	AV3	0.4	-4.2
AK4	2.6	-4.7	AJ4	-0.8	-3.3	AV4*	-6.8	-10
AK5*	2.2	-1.9	AJ5	3.8	-2.8	AV5*	-1.4	-3
KK1*	2.4	-3	KJ1	1.6	-0.8	KV1	-4.4	-7.1
KK2	1.6	-1.2	KJ2*	3.2	1.5	KV2	3.8	-0.3
KK3	3.4	-3	KJ3	2.4	1	KV3	1.6	-0.8
KK4	1.1	-3.2	KJ4*	8.2	0.3	KV4*	-3	-10
KK5	4	-3.4	KJ5	2.4	-4.9	KV5*	-4.4	-11.2
Mean	2.43	-2.68		4.84	-0.08		-0.97	-4.91
SD	1.32	1.64		4.82	3.38		3.57	4.07
St. error	0.42	0.52		1.52	1.07		1.13	1.29

Calculated BQIs (Table 2) varied between -6.8 (location AV4) and 16.6 (location AJ2) in the green mass and -11.2 (KV5) and 8.2 (AJ1) in the hay.
At the confidence level of 0.95, it was observed that BQIs in the green mass were significantly higher than those in the hay (Table 3).

Table 3. Results of a Paired Two-Sample t-Test show a significant difference in BQI values between green mass and hay (α=0.05).

	Green mass	Hay
Mean	2.1	-2.55667
Variance	17.582069	14.58737
Observations	30	30
df	29	
P(T<=t) two-tail	**2.05×10^{-8}**	

Most of the surveyed grasslands (24) had a positive BQI value in the green mass, whereas only five had a positive BQI value in the hay. This could be explained by the negative impacts of drying, such as the crumbling of leaves, chemical changes (reduction of protein and vitamin contents, decomposition of poisonous substances), etc. (Stjepanović et al., 2008; Šoštarić-Pisačić and Kovačević, 1974).
No significant difference was found between open grasslands and those in succession, either in the green mass or in the hay. It was not expected (Čarni et al., 2021), but can be explained by the fact that most of the grasslands were in the initial stages of succession and/or woody species were only present on the margins.
Results of one-way ANOVA showed significant difference (P< 0.05) in grassland forage quality between three locations,Validžići, Ježević, and Vrdovo both in the green mass and hay (Tables 4 & 5).

Table 4. Results of one-way ANOVA comparing mean BQI values for green mass between Validžići, Ježević, and Vrdovo grasslands (α=0.05).

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	170.414	2	85.207	6.777082	**0.00412**	3.354131
Within Groups	339.466	27	12.57281			
Total	509.88	29				

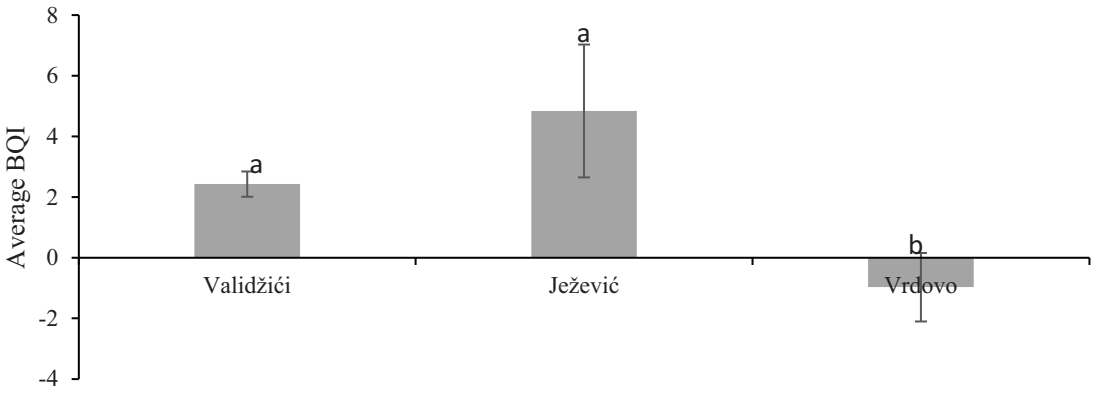
Table 5. Results of one-way ANOVA comparing mean BQI values for hay between Validžići, Ježević, and Vrdovo grasslands (α=0.05).

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	116.8727	2	58.43633	5.153435611	**0.0127134**	3.3541308
Within Groups	306.161	27	11.3393			
Total	423.0337	29				

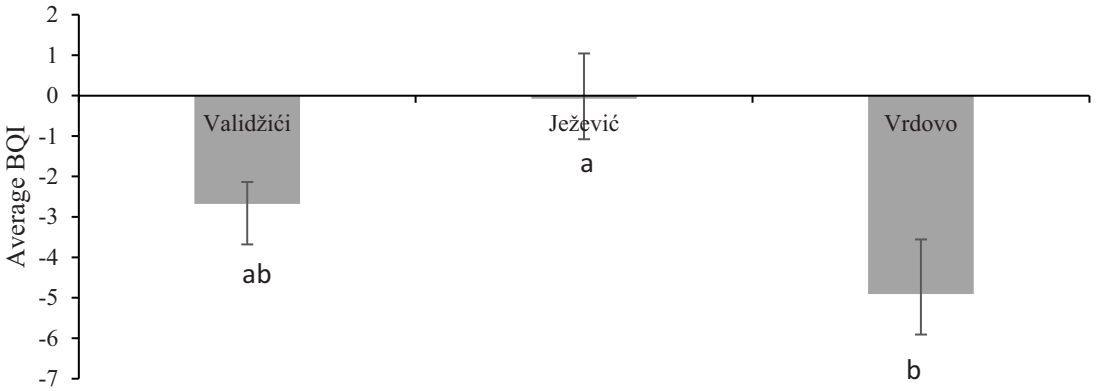
Post hoc analysis showed that, in the green mass, the grasslands from around Vrdovo had significantly lower BQI compared to the grasslands around Ježević and Validžići. In the hay, it was revealed that the grasslands from Ježević had significantly higher BQI than those from Vrdovo (Table 6) (Graph 1 & 2). We presume that the grasslands around Vrdovo show low BQIs because of the high percentage of the plot covered by *Sesleria tenuifolia*, which had in previous studies been identified as contributing to lower pastoral values of grasslands (D' Ottavio et al., 2005) or by *Sesleria autumnalis*, another species of the same genus (they covered 25% or more of the plot cover in eight of ten grasslands surveyed around Vrdovo).

Table 6. Post hoc analysis of differences between locations. * - significant difference (Bonferroni corrected p≤ 0.016666667)

	P value (green mass)	P value (hay)
Validžić vs. Ježević	0.14444846	0.052259042
Ježević vs. Vrdovo	**0.00670294***	**0.013403146***
Validžić vs. Vrdovo	**0.0112595***	0.144379016



Graph 1. Average BQIs for green mass of grasslands at Validžići, Ježević, and Vrdovo. Vertical range bars represent standard error.



Graph 2. Average BQIs for hay of grasslands at Validžići, Ježević, and Vrdovo. Vertical range bars represent standard error.

Conclusions

The results of our research confirm a better nutritional value of green forage than the potential value of the hay. It was found that there was no significant difference in nutritional value between grasslands that were not affected and those that were obviously affected by succession. Differences were found between individual localities with decreasing quality in the direction from north to south, where the average nutritional value of Vrdovo grasslands was the lowest and which was mainly due to the high proportion of *S. tenuifolia*.

Acknowledgement

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References

Basrek L., Bedek J., Boršić I., Dumbović Mazal V., Duplić A., Đud L., Hamidović D., Ilijaš I., Jeremić J., Katušić L., Kovač Konrad P., Krivanek G., Leko K., Gambiroža P., Opačić B., Partl A., Pavlinić M., Pintar V., Plavac I., Posavec Vukelić V., Starčević I., Šestani G., Zadravec M., Zwicker Kompar G., Zupan I. Žeger Pleše I., Župan D. (2020). Park prirode Dinara – stručna podloga za zaštitu. Zagreb. Hrvatska: Zavod za zaštitu okoliša i prirode. Available from: http://www.haop.hr/

Briemle G., Nitsche S., Nitsche L. (2002). Nutzungswertzahlen für Gefäßpflanzen des Grünlandes. Schriftenreihe für Vegetationskunde. 38: 203-225. Available from: https://www.ufz.de/

Čarni A., Ćuk M., Krstonošić D., Škvorc Ž. (2021). Study of Forage Quality of Grasslands on the Southern Margin of the Pannonian Basin. Agronomy 11: 2132. Available from: https://www.mdpi.com/

D'Ottavio P., Scotton M., Ziliotto U. (2005). Pastoral value of mountain pastures of Monti Sibillini (Central Apennines. Italy) grazed by sheep. In Sustainable grazing. nutritional utilization and quality of sheep and goat products. Molina Alcaide E., Ben Salem H., Biala K., Morand-Fehr P. (ed.). 93-98. Zaragoza. Spain: CIHEAM-IAMZ.

Ellenberg H. (1952). Wiesen und Weiden und ihre standörtliche Bewertung. Stuttgart. Germany: Ulmer.

Interpretation Manual of European Union Habitats (2013). Brussels. Belgium: European Commission. DG Environment. Available from: https://ec.europa.eu/

Klapp E., Boeker P., König F., Stählin A. (1953). Wertzahlen der Grünlandpflanzen. Das Grünland. Hannover. Germany: Schaper.

Marković M. (2003). Stočarska kretanja na Dinarskim planinama. Zagreb. Hrvatska: Naklada Jesenski i Turk.

Novák J. (2004). Evaluation of grassland quality. Ekológia (Bratislava). 23(2): 127-143.

OIKON d.o.o.(2004): Karta staništa RH. za Ministarstvo kulture. Zagreb. Available from: http://www.haop.hr/

Stjepanović M., Štafa Z., Bukvić G. (2008). Trave za proizvodnju krme i sjemena. Zagreb. Hrvatska: Hrvatska mljekarska udruga.

Šoštarić-Pisačić K., and Kovačević J. (1974). Kompleksna metoda za utvrđivanje kvalitete i sumarne vrijednosti travnjaka i djetelišta. Zagreb. Hrvatska: Poljoprivredni fakultet Zagreb.

PRETHODNO PRIOPĆENJE

Prvi uvid u status travnjaka u sustavu potpora na području Park prirode Dinara

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Sažetak
Na prostoru Dinare u posljednjih desetak godina intenzivirane su stočarske aktivnosti potaknute provedbom Zajedničke poljoprivredne politike kroz sustav potpora. Prostorna analiza krških pašnjaka je provedena korištenjem podataka preuzetih iz sustava ARKOD s ciljem dobivanja prvih uvida u distribuciju travnjaka koji su u sustavu poticaja na području Parka prirode Dinara. Rezultati pokazuju da ARKOD parcele krških pašnjaka i livada zauzimaju 50,1 km^2 što je 8 % površine PP Dinara, a većina parcela se nalazi unutar visinskog pojasa 300 - 500 m. Ustanovljena je umjereno jaka negativna korelacija (R^2=0,47) između nagiba terena pojedinih visinskih klasa i zastupljenosti prijavljenih travnjaka.

Ključne riječi: krški pašnjaci, Park prirode Dinara, Zajednička poljoprivredna politika, stočarstvo

Uvod

Park prirode Dinara proglašen je u veljači 2021. godine zbog svojih geomorfoloških i prirodnih osobitosti te predstavlja najmlađi park prirode u Republici Hrvatskoj (Basrek i sur., 2020.). Nalazi se u dalmatinskom zaleđu uz granicu s Bosnom i Hercegovinom obuhvaćajući tri masiva, Dinaru, Debelo brdo i Kamešnicu, koji se pružaju u smjeru sjeverozapad-jugoistok. Poznato je da se na tom području milenijima ljudi bave stočarstvom u obliku sezonske seobe (transhumanca). U zadnje vrijeme, a naročito od druge polovice 20. st., uslijed depopulacije došlo je do djelomičnog obustavljanja stočarske proizvodnje i zaraštanja travnjaka što je pokrenulo niz procesa koji su povećali opasnosti od požara i ugroze bioraznolikosti (Marković, 2003.). Ulaskom Republike Hrvatske u Europsku uniju 2013. godine i provođenjem Zajedničke poljoprivredne politike ponovo se povećava broj stočara koji prijavljuju travnjačke površine u sustav poticaja. Cilj ovog rada je po prvi puta dati uvid u stanje travnjaka koji se nalaze na području Parka prirode Dinara i koji su u sustavu poticaja kao krški pašnjaci ili kao livade te dati uvid u brojčano i površinsko stanje prijavljenih površina kao i njihovu visinsku distribuciju.

Materijal i metode

U ovom radu korišteni su podaci iz evidencije uporabe poljoprivrednog zemljišta (ARKOD) na području Republike Hrvatske koju u digitalnom grafičkom obliku vodi Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (APPRRR) u skladu s člankom 70. Uredbe (EU) br. 1306/2013 Europskog parlamenta i Vijeća. Dobivena baza se odnosi na površine prijavljene 2020. godine. Za analizu digitalnog elevacijskog modela korišten je set podataka EU-DEM v1.1 preuzet s Copericus Land servisa (EU, 2021.), prostorne rezolucije 25 m. Prostorne analize i vizualizacije izvedene su u programskim paketima ArcMap (ESRI, 2021.) i QGIS 3.16 (QGIS, 2021.).

Rezultati i rasprava

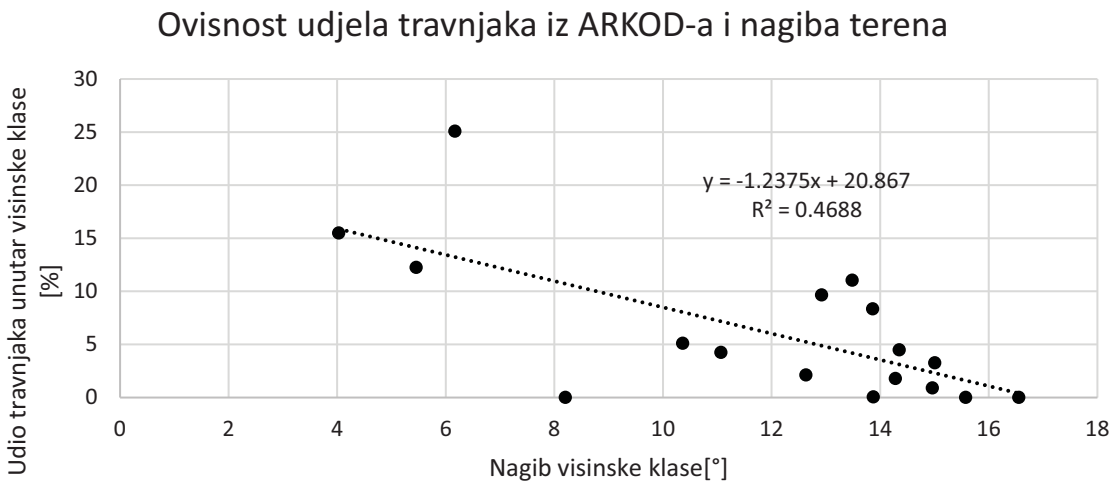
Park prirode Dinara zauzima ukupnu površinu od 629,8 km^2. Pregledom površina koje su 2020. godine bile prijavljene u ARKOD-u pri APPRRR izračunato je da krški pašnjaci (321) i livade (310) zauzimaju 50,1 km^2 što iznosi 7,95 % površine PP Dinara. Promatrajući visinsku distribuciju travnjaka vidljivo je da su većinom (31,3 % površine) zastupljeni u području između 400-500 m te s 23,4 % površine u područjima 301-400 m nadmorske visine (n.v.). To je za očekivati jer su ove dvije visinske klase površinom najzastupljenije na području Parka prirode. Nasuprot tome, i na neki način očekivano, najmanja zastupljenost travnjaka u sustavu poticaja nalazi se u području između 500-1000 m n.v. te iznad 1300 m n.v. što je u prvom slučaju vjerojatno posljedica konfiguracije terena i većeg nagiba odnosno manje pristupnosti što je očekivano jer se radi o ograničavajućim faktorima (Cook, 1966.).

Tablica 1. Pregled površine zemljišta, prijavljenih travnjaka, nagiba po visinskim klasama i prosječne veličine prijavljene parcele travnjaka u ARKOD-u

Visinska klasa	Nadmorska visina (m)	Površina zemljišta (km^2)	Površina travnjaka u ARKOD-u (km^2)	Prosječni nagib zemljišta	Prosječna površina parcele (ha)	Broj prijavljenih parcela
1	< 300	5,3	0,8	4,0 °	0,3	286
2	300-400	46,8	11,7	6,2 °	0,9	1353
3	400-500	128,0	15,7	5,5 °	1,8	864
4	500-600	57,4	2,9	10,4 °	3,5	84
5	600-700	42,6	0,8	14,3 °	2,8	27
6	700-800	40,5	0,4	15,0 °	1,1	32
7	800-900	50,9	1,1	12,6 °	1,2	93
8	900-1000	66,0	2,8	11,1 °	1,2	231
9	1000-1100	50,2	4,8	12,9 °	8,1	60
10	1100-1200	42,7	4,7	13,5 °	8,1	44
11	1200-1300	31,5	2,6	13,9 °	12,5	21
12	1300-1400	25,6	1,1	14,3 °	7,2	16
13	1400-1500	19,1	0,6	15,0 °	6,9	9
14	1500-1600	15,8	0,0	13,9 °	0,4	2
15	1600-1700	6,1	0,0	16,6 °	-	0
16	1700-1800	1,3	0,0	15,6 °	-	0
17	>1800	0,1	0,0	8,2 °	-	0
UKUPNO		**629,8**	**50,1**			**3122**

Prosječna površina prijavljenih parcela s travnjacima varirala je između visinskih klasa. Najveće su na područjima od 1000 m do 1300 m gdje im je prosječna površina između 8,1 i 12,5 ha (Tablica 1).

Analizom digitalnog elevacijskog modela izračunat je nagib za cijelo područje parka te je izračunata zonalna statistika čime je dobiven uvid u odnos udjela prijavljenih travnjaka u visinskoj klasi i nagiba ukupnog zemljiša unutar visinske klase te je ustanovljena negativna korelacija (Grafikon 1).



Grafikon 1. Ovisnost udjela travnjaka unutar visinske klase i nagiba visinske klase

Zaključak

Ovim istraživanjem je po prvi puta dan uvid u prostorni raspored travnjaka prijavljenih u sustavu poticaja na području Parka prirode Dinara. Ustanovljeno je da je 2020. godine u sustav poticaja ušlo 50,1 km^2 travnjaka. Najveće površine travnjaka zastupljene su u visinskoj klasi od 400-500 m n.v.. Najveće prijavljene parcele se nalaze u visinskoj klasi 1200-1300 m n.v i iznose u prosjeku 12,5 ha, a najviše ih je između 300 i 400 m n.v..

Ustanovljena je umjereno jaka negativna korelacija (R^2=0,47) između udjela prijavljenih travnjaka u visinskoj klasi i nagiba ukupnog zemljiša unutar visinske klase.

Napomena

Ovaj rad je napravljen sredstvima EU u sklopu projekta Dinara Back to LIFE (LIFE18 NAT/HR/00084), sufinanciranjem Fonda za zaštitu okoliša i energetsku učinkovitost (FZOU) i podacima koje je ustupila Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (APPRR).

Literatura

ARKOD (2021). Zagreb, Hrvatska: Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju. Raspoloživo: https://www.apprrr.hr/prostorni-podaci-servisi/

Basrek L., Bedek J., Boršić I., Dumbović Mazal V., Duplić A., Đud L., Hamidović D., Ilijaš I., Jeremić J., Katušić L., Kovač Konrad P., Krivanek G., Leko K., Gambiroža P., Opačić B., Partl A., Pavlinić M., Pintar V., Plavac I., Posavec Vukelić V., Starčević I., Šestani G., Zadravec M., Zwicker Kompar G., Zupan I. Žeger Pleše I., Župan D. (2020). Park prirode Dinara – stručna podloga za zaštitu. Zagreb. Hrvatska: Zavod za zaštitu okoliša i prirode. Raspoloživo: http://www.haop.hr/

Cook C.W. (1966). Factors affecting utilization of mountain slopes by cattle. Journal of Range Management 19(4): 200-204. Raspoloživo: https://repository.arizona.edu/handle/10150/647947

European Union, Copernicus Land Monitoring Service (2021). European Environment Agency (EEA). https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1

ESRI (2021). ArcGIS Desktop: Release 10.8.1. Redlands, CA: Environmental Systems Research Institute.

Marković M. (2003). Stočarska kretanja na Dinarskim planinama. Zagreb, Hrvatska: Jesenski i Turk.

QGIS Development Team (2021). QGIS Geographic Information System. Open Source Geospatial Foundation Project. Raspoloživo: http://qgis.osgeo.org

Uredbe (EU) br. 1306/2013 Europskog parlamenta i Vijeća o financiranju, upravljanju i nadzoru zajedničke poljoprivredne politike i o stavljanju izvan snage uredaba Vijeća (EEZ) br. 352/78, (EZ) br. 165/94, (EZ) br. 2799/98, (EZ) br. 814/2000, (EZ) br. 1290/2005 i (EZ) 485/2008 (2013). Raspoloživo: https://eur-lex.europa.eu/legal-content/HR/ALL/?uri=uriserv:OJ.L_.2013.347.01.0549.01.HRV

The first insight into the status of grasslands in the payments support system in the area of Nature park Dinara

Abstract

In the area of Dinara in the last ten years, livestock activities have intensified, partly due to the implementation of the Common Agricultural Policy through the support system. In this paper, a spatial analysis of karst pastures was made using plots taken from the ARKOD system in order to obtain the first data on the distribution of grassland in the incentive system in the Dinara Nature Park. The results show that ARKOD plots of karst pastures and meadows with their 50.1 km^2 make up about 8% of the area of the Dinara Nature Park, as well as that most of the plots are located in the altitude range from 300 to 500 m. A moderately strong negative correlation ($R^2 = 0.47$) was found between the share of reported grasslands in the altitude class and the slope of the total land within the altitude class.

Key words: karst pastures, Nature Park Dinara, Common Agricultural Policy, animal husbandry

IZVORNI ZNANSTVENI RAD

Primjena glifosata na području Brodsko-posavske županije u 2021. godini

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Sažetak

Cilj istraživanja bio je anketnim ispitivanjem utvrditi primjenu sredstava za zaštitu bilja na osnovi djelatne tvari glifosat na području Brodsko-posavske županije u 2021. godini. Anketirano je ukupno 107 ispitanika. Rezultati ankete pokazali su da svi ispitanici koriste sredstva na osnovi djelatne tvari glifosat, i to najviše u ratarskoj proizvodnji (46,7 %) i trajnim nasadima (22,4 %), ali i za druge namjene. Poljoprivredni proizvođači glifosat najviše koriste za suzbijanje korova na strništima te tijekom vegetacije u trajnim nasadima. Više od 65 % ispitanika glifosat primjenjuje barem jednom ili više puta godišnje, a više od 90 % ispitanika se pridržava preporučene doze primjene.

Ključne riječi: anketa, totalni herbicidi, glifosat, Brodsko-posavska županija

Uvod

Sredstva za zaštitu bilja imaju velik značaj u poljoprivrednoj proizvodnji, a primjena herbicida omogućuje brzo, učinkovito i ekonomski isplativo suzbijanje korova uz postizanje visokih prinosa usjeva. U 2021. godini u Republici Hrvatskoj za upotrebu je odobreno 68 herbicidnih djelatnih tvari, te 234 registrirana herbicidna pripravka (Bažok i sur., 2021.). Jedna od najprodavanijih i najčešće korištenih djelatnih tvari kako u svijetu tako i u Republici Hrvatskoj je herbicid glifosat (Antier i sur., 2020.a; Barić i sur., 2019.). Glifosat je djelatna tvar neselektivnih izrazito sistemičnih folijarnih herbicida, namijenjen suzbijanju jednogodišnjih uskolisnih i širokolisnih korova, te višegodišnjih korova s dubokim korijenom, rizomima i gomoljima (Bažok i sur., 2021.). Pripravci na osnovi glifosata primjenjuju se nakon nicanja korova, te kroz list i ostale zelene dijelove biljke prodiru u provodne snopove i translociraju se u sve nadzemne i podzemne organe. Biljke nakon tretiranja postupno prestaju s rastom, a potpuno odumiranje vidljivo je nakon 7 do 20 dana (Piešova, 2005.; Ostojić, 2018.). Glifosat ne iskazuje rezidualno djelovanje jer se čvrsto veže na adsorpcijski kompleks tla te ga biljke putem korijena u pravilu ne mogu usvojiti (Senseman, 2007.). U Hrvatskoj je na osnovi glifosata za različite namjene registrirano 20 pripravaka (FIS, 2021.). Područje primjene glifosata vrlo je široko pa se između ostaloga glifosat primjenjuje u poljoprivrednoj proizvodnji za suzbijanje korova na strništima, pripremu tla prije sjetve, suzbijanje korova nakon sjetve, a prije nicanja, u trajnim nasadima, za desikaciju usjeva, za predžetveno suzbijanje korova i obnovu travnjaka, livada i pašnjaka. Također se primjenjuje i na neobrađenim površinama, u ribnjacima i stajaćim vodama, na željezničkim prugama i za održavanje pojaseva uz ceste te u šumarstvu (Antier i sur., 2020.a; Bažok i sur., 2021.).

U Europskoj uniji glifosat je trenutno odobren do 15. prosinca 2022. godine i može se koristiti kao djelatna tvar u sredstvima za zaštitu bilja (EK, 2017.). S obzirom na njegovu široku primjenu, a moguću zabranu po isteku registracije, cilj rada bio je anketnim ispitivanjem na području Brodsko-posavske županije utvrditi u kojoj mjeri i za koje namjene poljoprivredni proizvođači koriste sredstva za zaštitu bilja na osnovi glifosata.

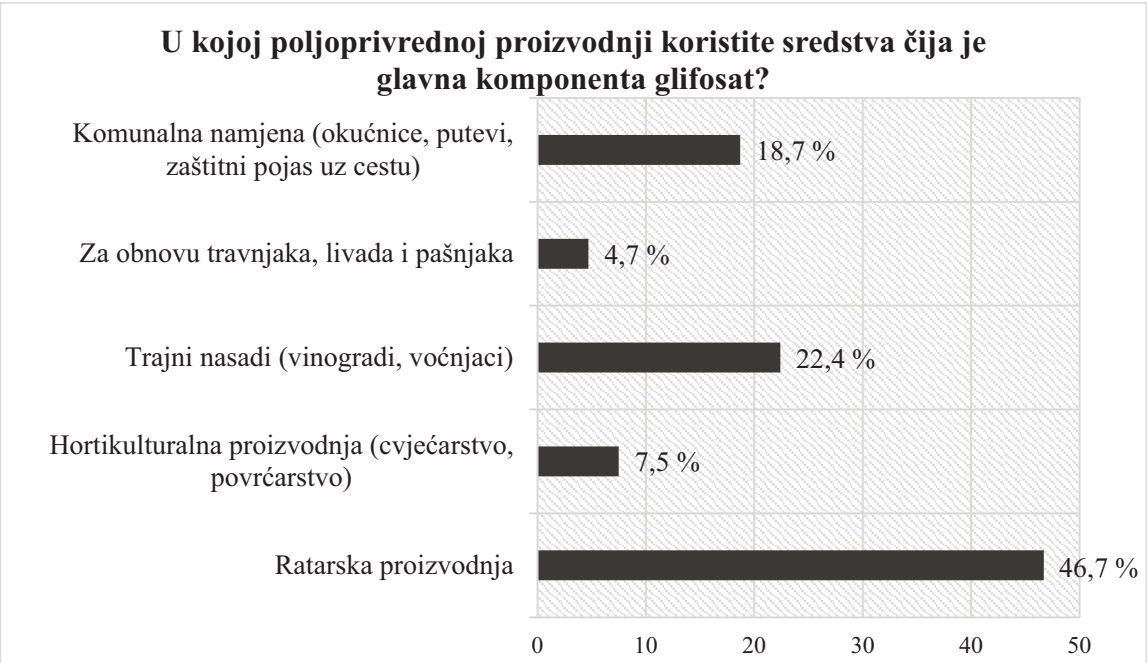
Materijal i metode

Anketno ispitivanje o primjeni herbicida na osnovu djelatne tvari glifosat provedeno je na području Brodsko-posavske županije u 2021. godini. Anketiranje je provedeno kombinirano, online i u poljoprivrednim ljekarnama u direktnom kontaktu s poljoprivrednim proizvođačima i krajnjim korisnicima sredstava za zaštitu bilja, u razdoblju od 1. veljače do 1. srpnja 2021. godine. Anketni upitnik sastojao se od pitanja zatvorenog tipa, i to seta općih pitanja (spol, dob, veličina poljoprivrednog gospodarstva), te seta pitanja vezanih za primjenu sredstava za zaštitu bilja na osnovi djelatne tvari glifosat (namjena, vrijeme primjene, učestalost primjene, poštivanje preporučenih doza, primjena ostalih totalnih herbicida). Anketa je obuhvatila ukupno 107 ispitanika.

Rezultati i rasprava

Od 107 ispitanika koji su sudjelovali u istraživanju, 83 ispitanika (77,6 %) je muškoga spola, a 24 ispitanika (22,4 %) ženskoga spola. Najveći udio ispitivane populacije pripada skupini 40-49 godina koju čini 30,8 % ispitanika, dok najmanji udio čine ispitanici koji pripadaju dobnoj skupini 60 i više godina (9,3 %). Podjednak broj ispitanika pripada dobnim skupinama 18-29 godina (20,6 %), 30-39 godina (18,7 %) i 50-59 godina (20,6 %). Na postavljeno pitanje o veličini poljoprivrednog gospodarstva kojim upravljaju, nešto manje od polovice ispitanika odnosno njih 44,9 % odgovorilo je da posjeduje poljoprivredno gospodarstvo veličine 30 i više hektara, dok poljoprivredno gospodarstvo veličine od 10 do 30 hektara posjeduje 22,4 % ispitanika. Manji broj ispitanika (15 %) posjeduje gospodarstvo veličine od 5 do 10 hektara, dok se 17,8 % ispitanika izjasnilo da posjeduje poljoprivredno gospodarstvo do 5 hektara.

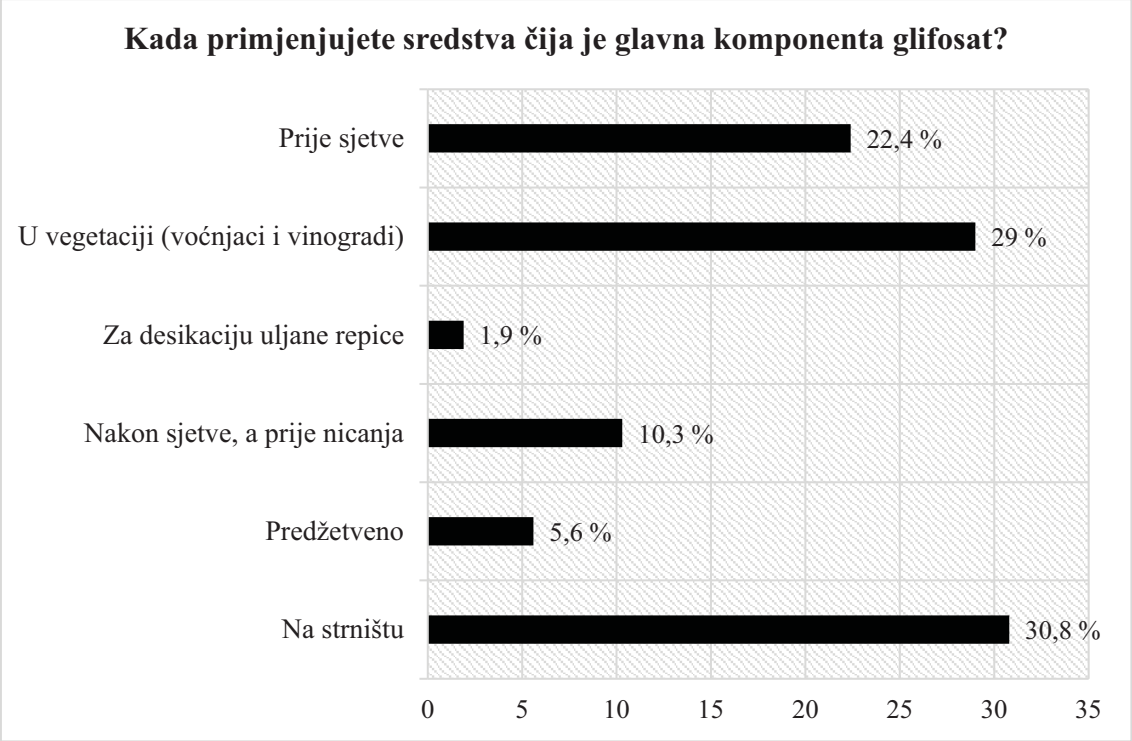
Svi ispitanici koristili su barem jedno od ponuđenih 20 registriranih pripravaka na osnovi djelatne tvari glifosat. Najveći broj ispitanika na ovo pitanje označio je pripravak Total TF (17,8 %), zatim pripravke Boom Efekt (14 %) i Satelite (11,2 %). Ostali pripravci korišteni su u manjoj mjeri, a njihov udio kretao se od 0,9 do 7,5 %.



Grafikon 1. Namjena herbicida na osnovi djelatne tvari glifosat

Ispitanici sredstva na osnovi glifosata primjenjuju u različitim poljoprivrednim proizvodnjama (Grafikon 1). Skoro polovica ispitanika, odnosno njih 46,7 % na pitanje je odgovorilo kako glifosat koriste u ratarskoj proizvodnji. Glifosat u trajnim nasadima (voćnjacima i vinogradima) primjenjuje 22,4 % ispitanika, dok je u proizvodnji cvijeća i povrća sredstva na osnovi glifosata navelo da koristi 7,5 % ispitanika. Najmanji udio ispitanika (4,7 %) koristi glifosat u obnovi travnjaka, livada i pašnjaka. Osim u poljoprivrednoj proizvodnji, ispitanici glifosat primjenjuju i u komunalne namjene, te je 18,7 % ispitanika odgovorilo da sredstvo primjenjuje za održavanje okućnica i puteva.

Poljoprivredni proizvođači upitani su i kada primjenjuju sredstva na osnovi glifosata (Grafikon 2). Najveći broj ispitanika, njih 30,8 %, glifosat koristi za tretiranje strništa. Isto tako, velik broj ispitanika (29,0 %) glifosat koristi u vegetaciji za suzbijanje korova u trajnim nasadima. Za suzbijanje korova prije sjetve glifosat koristi 22,4 % ispitanika, a njih 10,3 % glifosat koristi nakon sjetve, a prije nicanja usjeva. Najmanji broj ispitanika glifosat primjenjuje predžetveno (5,6 %) te za desikaciju uljane repice (1,9 %).

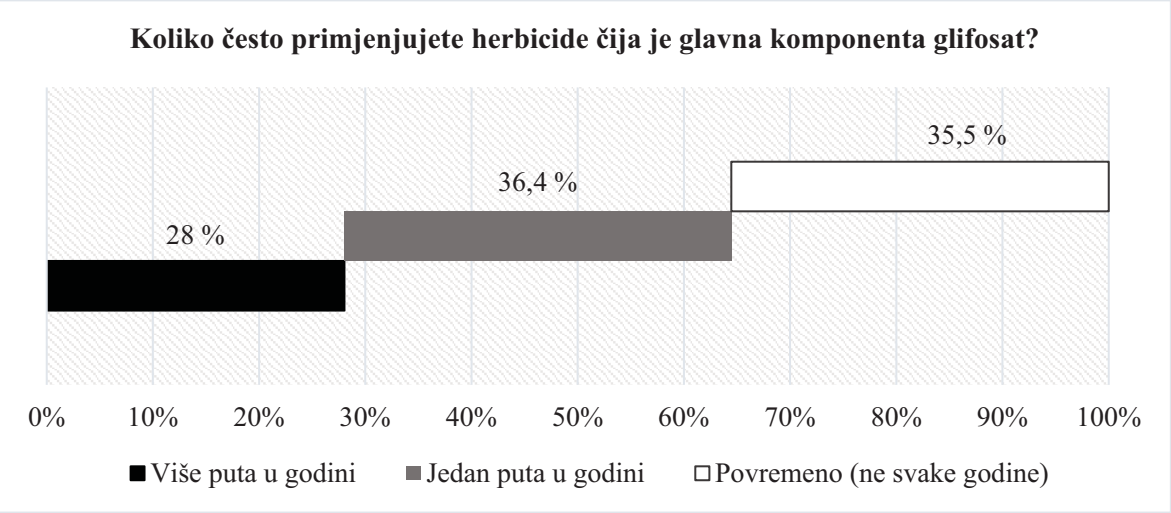


Grafikon 2. Vrijeme primjene herbicida na osnovi djelatne tvari glifosat

Učestalost primjene herbicida na osnovi djelatne tvari glifosat među ispitanicima prikazana je Grafikonom 3. Njih 36,4 % primjenjuje glifosat jedan puta u godini, a 35,5 % ispitanika sredstvo koristi povremeno, odnosno ne svake godine, dok 28 % ispitanika sredstva na bazi glifosata koristi više puta u godini. Glifosat je jedan od najčešće korištenih herbicida u poljoprivrednoj proizvodnji (Antier i sur., 2020.b). Na temelju podataka o prodaji i uporabi, glifosat čini 33 % količine prodanih herbicida u Europi u 2017. godini (Antier i sur., 2020.a). U Hrvatskoj je glifosat najprodavaniji herbicid, a udio aminofosfonata (glifosat i glufosinat) u ukupnoj potrošnji herbicida u 2017. godini iznosio je 32,5 % (Barić i sur., 2019.).

Osim primjene sredstava na osnovi djelatne tvari glifosat, poljoprivredni proizvođači upitani su jesu li ikada koristili i druge totalne herbicide. Herbicide na osnovi djelatnih tvari glufosinat i dikvat koristila su 72 ispitanika (67,3 %). Pripravke na osnovi djelatne tvari glufosinat koristilo je 59,7 %, a na osnovi djelatne tvari dikvat koristilo je 40,3 % ispitanika.

Navedenim djelatnim tvarima registracija je istekla te primjena više nije dozvoljena (Bažok i sur., 2021.; FIS, 2021.) stoga ne čude rezultati široke primjene glifosata s obzirom na suženi izbor totalnih herbicida.



Grafikon 3. Učestalost primjene herbicida na osnovi djelatne tvari glifosat

Na pitanje poštuju li preporučene doze primjene sredstava čija je glavna komponenta glifosat, 90,7 % ispitanika odgovorilo je da se pridržava propisane doze. Međutim, 9,3 % ispitanika je izjavilo da povremeno poštuje preporučene doze primjene sredstava. Baličević i sur. (2015.) navode da se visok postotak (98 %) proizvođača pridržava uputa za primjenu sredstava za zaštitu bilja, no dio ih se samo djelomično pridržava karence (13 %), dok samo 46 % proizvođača uvijek koristi zaštitnu odjeću pri rukovanju s pesticidima.

Zaključak

Provedenim istraživanjem na uzorku od 107 ispitanika utvrđena je primjena herbicida glifosata na području Brodsko-posavske županije. Veliki broj poljoprivrednih proizvođača primjenjuje pripravke na osnovi ove djelatne tvari, i to u najvećoj mjeri u ratarskoj proizvodnji i trajnim nasadima, ali i za druge namjene, a više od 65 % ispitanika ga primjenjuje barem jednom ili više puta godišnje. Potvrđena je učestala primjena djelatne tvari glifosat čija potencijalna zabrana može polučiti velike izazove i zahtjeve pred cjelokupnom poljoprivrednom proizvodnjom.

Literatura

Antier C., Kudsk P., Reboud X., Ulber L., Baret P.V., Messéan, A. (2020a). Glyphosate Use in the European Agricultural Sector and a Framework for Its Further Monitoring. Sustainability, 12(14): 5682.

Antier C., Andersson R., Auskalnienė O., Barić K., Baret P. i sur. (2020b). A survey on the uses of glyphosate in European countries. INRAE, endure diversifying crop protection, UC Louvain. 60p. https://doi.org/10.15454/A30K-D531

Baličević R., Rozman V., Raspudić E., Brmež M., Lončarić Z., Bursić V., Ravlić M., Sarajlić A., Lucić P. (2015). Uporaba sredstava za zaštitu bilja na obiteljskim poljoprivrednim gospodarstvima na području istočne Hrvatske. Proceedings of 50th Croatian and 10th International Symposium on Agriculture, Pospišil, Milan (ur.), 49-53. Opatija, 16.-20. veljače 2015., Sveučilište u Zagrebu, Agronomski fakultet, Zagreb.

Barić K., Bažok R., Pintar A. (2019). Potrošnja pesticida u Hrvatskoj poljoprivredi u razdoblju od 2012. do 2017. godine. Glasilo biljne zaštite, 19(5): 537-548.

Bažok R., Ivić D., Cvjetković B., Barić K., Ostojić Z. (2021). Pregled sredstava za zaštitu bilja u Hrvatskoj za 2021. godinu. Glasilo biljne zaštite, 21(1-2): 1-320.
EK (2017). Provedbena Uredba Komisije (EU) 2017/2324, dostupno na: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R2324
FIS (2021). Fitosanitarni informacijski sustav, dostupno na: https://fis.mps.hr/trazilicaszb/
Ostojić Z., Brzoja D., Barić K. (2018). Status, namjena i potrošnja glifosata u Hrvatskoj i svijetu. Glasilo biljne zaštite. 18(6): 531-541.
Piešova E. (2005). The effect of glyphosate on the frequency of micronuclei in bovine lymphocytes in vitro. Acta Veterinaria. 55: 101–109.
Senseman S.A. (2007). Herbicide Handbook. 9th Edition. Weed Science Society of America. Champaign. 458 p.

Use of glyphosate in Brod-Posavina County in 2021

Abstract
The aim of the research was to determine the use of plant protection products based on the active substance glyphosate in the Brod-Posavina County in 2021. A total of 107 respondents were interviewed. The results of the survey showed that all respondents use glyphosate based preparations, mostly in crop production (46.7%) and permanent crops (22.4%), but also for other purposes. Glyphosate is mostly used by farmers to control weeds in stubble and during the growing season in permanent crops. More than 65% of respondents use glyphosate at least once or several times a year, and more than 90% of respondents adhere to the recommended application dose.

Key words: survey, total herbicides, glyphosate, Brod-Posavina County

ORIGINAL SCIENTIFIC PAPER

Advanced monitoring system for agroecosystems threatened by salinization and pollution

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Abstract
Although the problem of sea intrusion and salinization of surface and groundwater, and thus the risk of land degradation, occurs throughout the coastal area of Croatia. The objective of this paper was to investigate and compare two different soil monitoring approaches, classical long-term monitoring and monitoring using automatic soil sensors at two locations in Neretva River Valley. For SMS1 mean values for EC were similar both in long-term and automatic sensor monitoring. At SMS2, mean EC value obtained by the sensors was higher compared to long-term monitoring. Variation coefficient on SMS1 vas low in both monitoring approaches indicating low soil EC varibality in the profile. On the other hand, variation coefficient was high at SMS2 especially in the long-term monitoring indicating high soil EC variability in the profile.

Key words: soil salinity, sea water intrusion, climate change

Introduction

The Strategy for Adaptation to Climate Change of the Republic of Croatia until 2040 with a view to 2070 (OG 46/2020) proposes a wide range of climate change adaptation measures for the sectors of fisheries and hydrology, as well as water and marine resource management, agriculture, biodiversity, forestry and health. It also highlights that climate change will have significant direct and indirect impacts on agriculture due to the trend of rising sea levels and salinization of karst aquifers. Although the problem of seawater intrusion and salinization of surface and groundwater occurs in all Croatian coastal areas, increasing the risk of land degradation, these processes are most evident in the Neretva River Delta area, which is considered one of the most vulnerable areas to climate change in Croatia. Monitoring the state of the environment is extremely important for the delta Neretva River due to the diversity of ecological zones, which alternate spatially, have different degrees and intensities of use, and are generally very sensitive to the effects of climate change. Moreover, the establishment of a good monitoring system is necessary because it allows for high quality and timely decision making. In this sense, two types (i.e. levels) of water quality monitoring systems are established at the national level by Croatian waters (OG 66/2019), surveillance and operational monitoring. There is also research monitoring that can be established at individual sites, such as the scientific research monitoring set in the Neretva River valley. The water and soil salinity monitoring system was established in 2009 to monitor surface and groundwater salinity and soil salinity at multiple sites in the valley, and monitoring is therefore still in operation (Romić et al., 2017). Although there are several types of monitoring systems that collect valuable data on the status of this specific agroecosystem in the area, it is important to develop and implement automatic, continuous monitoring that can provide data in real time and includes a range of sensors to monitor various parameters.The

goal of this paper is to investigate the dinamics of soil electrical conductivity in two different monitoring approaches, classical long-term monitoring and monitoring using soil sensors.

Materials and methods

Study area

The study area is located in the Neretva River Valley (NRV) (43°00 N, 17°30 E) in the Mediterranean part of Croatia. The area is semi-arid with Mediterranean climate with hot, dry summers and wet winters. Most of the precipitation falls in the period from October to April, with an annual mean of 1203 mm (1980-2010) (Romić et al., 2012). The average annual temperature is 15.7 ºC with the highest value of 25.2 ºC occurring in July (Romić et al., 2012).

As mentioned above, the long-term monitoring system, established in 2009, is organized as a comprehensive monitoring of surface water, groundwater and soil (Romić et al., 2014). It covers the entire valley, divided into 5 reclamation areas or subsystems (Vrbovci, Luke, Vidrice, Opuzen ušće and Komin). Two melioration areas, Luke and Vidrice, were selected as part of the Advanced agroecosystem monitoring system at risk of salinization and contamination project (acronym DELTASAL), funded by the European Union through European Regional Development Fund, to be upgraded with advanced automated monitoring equipment in late 2020. The equipment installed includes the installation of a shallow piezometer (4 m deep) at each site and an automatic agrometeorological station at the Vidrice site. In addition, 4 automatic radar systems were installed at the Vidrice subsystem, which are designed to provide continuous real-time measurements of water levels, water velocity, and water flow in open channels. Soil and water quality sensors were installed at both sites. The soil monitoring system includes sensors that measure soil water content, electrical conductivity, temperature, and water potential, installed at four depths, each ranging from 25 cm to 1 m. In addition to the soil sensors, multi-parameter water quality probes were installed at both sites in both shallow piezometers to monitor shallow groundwater and open channels to monitor surface water quality with a focus on electrical conductivity, i.e. salinity. Data from each station and sensor is collected at 10-minute intervals and sent to the newly developed platform. This platform contains real-time data from each installed sensor as well as historical data on soil and water quality mentioned above, historical climate data from three stations (Ploče, Opuzen and Metković) and hydrological data (water levels) from six stations in the valley (Picture 1).

Picture 1. Map of long-term and automatic continuous monitoring locations (meteorological, hydrological and soil and water quality data)

Soil sampling in the long-term monitoring

Soil sampling at 7 different sites is carried out twice a year, after the wet winter season (end of March/beginning of April) and after the dry summer season (end of September/beginning of October). Soil samples are taken at 25 cm intervals up to 1 m and the physical and chemical parameters are analyzed in the laboratory.

Soil sensors

At the end of 2020 frequency domain sensors (FDR) were installed at two representative soil monitoring sites (SMS), site Vidrice (SMS1) and Luke (SMS2) collecting data in 10 minute intervals. The TEROS 12 (Meter Group) sensors measure soil temperature, bulk electrical conductivity (EC_b), and volumetric water content using frequency-domain technology. The sensors are installed every 25 cm up to 1 m. SMS1 and SMS2 were selected due to the pronounced risk of soil salinity and the two sites differ in soil texture, organic matter content, depth of the Gleyc horizon, salinity and land use, with SMS1 mainly used as a fruit growing area and SMS2 mainly used for vegetable production. An automatic weather station (Pinova Meteo agriculture weather station) was installed at SMS1 site to record rainfall, air temperature and moisture, wind speed and global radiation. For data vizualization and analysis Microsofte Excel and XLSTAT (Addinsoft, 2021) statistical software plug-in were used.

Results and discussion

In the long-term seasonal monitoring (2009-2021) soil in the entire profile (up to 100 cm) at SMS1 was slightly saline both in wet and in dry season with mean values of electrical conductivity (EC) of 3.28 dS m^{-1} and 3.78 dS m^{-1} respectively (Table 1). At SMS2 soil was non saline since mean value of EC in wet season was 1.30 dS m^{-1} and 1.48 dS m^{-1} in dry season (Table 1). Although mean value of EC was higher in both wet and dry season at SMS1 it showed lower variability with coefficient of variation ranging from 0.35 in wet and 0.21 in dry season. Coefficient of variation was considerably higher at SMS2 both in wet (0.76) and dry season (0.60).

Table 1. Soil electrical conductivity at monitoring stations SMS1 and SMS2 obtained by long-term monitoring within NRV

	Electrical conductivity at SML1 and SML2 (dS m^{-1})			
	SMS1 wet 0-100 cm	SMS1 dry 0-100 cm	SMS2 wet 0-100 cm	SMS2 dry 0-100 cm
Mean	3.28	3.78	1.30	1.48
Median	3.35	3.86	0.89	1.09
Min.	0.97	2.21	0.38	0.52
Max	6.38	5.41	4.79	3.62
Variance	1.31	0.62	0.99	0.78
St. dev.	1.14	0.78	0.99	0.89
Var. coeff.	0.35	0.21	0.76	0.60

Since soil sensors were installed in mid-November 2020, results for first seven months are presented (to June 2021). On SMS1 average daily value of EC_b was 3.89 dS m^{-1} with maximum value of 6.16 dS m^{-1} at 75-100 cm (Table 2). SMS2 had lower daily average of 2.28 dS m^{-1} with maximum of 4.77 dS m^{-1} also at 75-100 cm (Table 2). Variation coefficient was significantly lower at SMS1 indicating low soil EC_b variation in all horizons. At SMS2 variation coefficient ranged from 29 % to 39 % indicating high EC_b variability in all horizons (Table 2).

Table 2. Soil electrical conductivity at monitoring stations SMS1 and SMS2 obtained by FDR sensors within NRV

	Electrical conductivity at SMS1 (dS m^{-1})					Electrical conductivity at SMS2 (dS m^{-1})				
	0-25 cm	25-50 cm	50-75 cm	75-100 cm	0-100 cm	0-25 cm	25-50 cm	50-75 cm	75-100 cm	0-100 cm
Mean	2.44	3.57	4.19	5.15	3.89	1.85	2.57	2.66	2.05	2.28
Median	2.42	3.49	4.00	5.10	3.91	1.59	2.42	2.65	1.94	2.13
Min.	1.89	3.20	3.80	4.57	1.89	1.35	0.99	0.47	0.55	0.47
Max	3.26	4.58	5.63	6.16	6.16	4.36	4.46	4.50	4.77	4.77
Var.	0.06	0.08	0.18	0.12	1.08	0.53	0.80	0.67	0.35	0.70
St. dev.	0.24	0.29	0.42	0.34	1.04	0.73	0.90	0.82	0.59	0.84
Var. coeff.	0.10	0.08	0.10	0.07	0.27	0.39	0.35	0.31	0.29	0.37

Daily variation of soil EC_b at both SMS1 and SMS2 along with precipitation is given at Chart 1. As shown in the Chart 1.the EC_b varied differently on each location, but on both locations rainfall caused changes in EC_b values indicating the movement of salt through the profile. After the end of May, values of EC_b stabilised in all horizons at both locations.

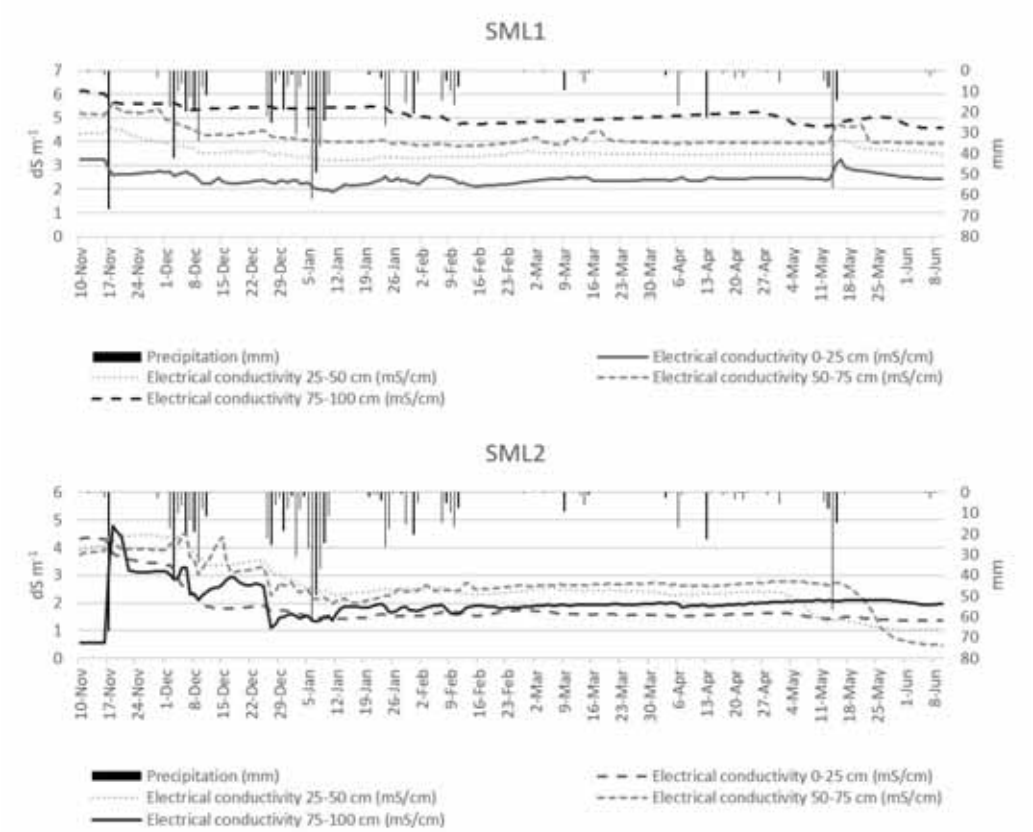

Chart 1. Daily variation of soil EC_b at monitoring stations SMS1 and SMS2 obtained by FDR sensors and daily rainfall (November 2020 – June 2021)

Conclusion

Classical soil monitoring with sampling and laboratory analysis gives us most accurate results, but it is costly and labor intensive. Implementing modern technologies like soil sensors can give us an insight at data in high temporal resolution. This can make decision making much more accurate and precise, especially in vulnerable agro environments such as

river deltas. At SMS1 mean EC values were similar in both monitoring approaches and higher then at SMS2. On the other hand, variability of EC in the soil profile was higher at SMS2 in both approaches compared to SMS1. The similarity in obtained results indicate that soil sensor data may be used as an alternative to classical soil salinity monitoring.

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Literature

Official Gazette 46/2020 (2020). Strategy for adaptation to climate change in the Republic of Croatia for the period up to 2040 with a view to 2070

Official Gazette 66/2019 (2019). The Water Act

Romić D., Romić M., Zovko M., Bakić H., Ondrašek G. (2012). Trace metals in the coastal soils developed from estuarine floodplain sediments in the Croatian Mediterranean region. Environmental Geochemistry and Health. 34 399–416.

Romić D., Romić M., Zovko M., Bubalo M., Ondrašek G., Husnjak S., Maurović N., Bakić H., Matijević L., Vranješ M. (2014). Monitoring of soil and water salinity in the Neretva River valley-five-year (2009-2013) report.

Romić D., Ondrašek G., Zovko M., Bubalo Kovačić M., Romić M., Filipović L., Maurović N., Igrc M.D., Kranjčec F., Vranješ M., Srzić V. (2017). Monitoring of Water and Soil Salinisation in the Neretva River Valley. Annual report. University of Zagreb Faculty of Agriculture, Department of soil amelioration.

Romić D., Castrignano A., Romić M., Buttafuoco G., Bubalo Kovačić M., Ondrašek G., Zovko M. (2020). Modelling spatial and temporal variability of water quality from different monitoring stations using mixed effects model theory. Science of The Total Environment. 704:135875

Zovko M., Romić D., Colombo C., Di Iorio E., Romić M., Buttafuoco G., Castrignano A. (2018). A geostatistical Vis-NIR spectroscopy index to assess the incipient soil salinization in the Neretva River valley, Croatia. Geoderma. 332, 60-72.

PREGLEDNI RAD

Mikoremedijacija policikličkih aromatskih ugljikovodika saprotrofnim i ektomikoriznim gljivama

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Sažetak

Među različitim dostupnim tehnologijama sanacije onečišćenja ekosustava poznato je da su metode bioremedijacije pomoću mikroorganizama prepoznate kao isplative i ekološki prihvatljive. U postupku primjene gljiva u sanaciji onečišćenja koriste se dvije ekološke skupine, saprotrofne i ektomikorizne bazidiomicete. Vrlo dobar kapacitet za razgradnju kontaminanata, kao što su policiklički aromatski ugljikovodici (PAH), postojana organska zagađivala (POPs), halogenirani ugljikovodici, fenoli, eksplozivi i bojila utvrđen je za mnoge vrste bazidiomiceta. Međutim, potrebno je provoditi daljnja istraživanja o praktičnoj primjeni saprotrofnih i ekotmikoriznih bazidiomiceta u procesu mikoremedijaciji.

Ključne riječi: bioremedijacija, saprotrofi, ektomikoriza, održivo gospodarenje

Uvod

Porast broja stanovnika, intenzivna industrijalizacija i urbanizacija uvjetovali su onečišćenje životne sredine, prije svega tla i vode. Sve veća prisutnost štetnih supstanci u okolišu (policiklički aromatski ugljikovodici, teški metali, bojila...) postao je nezaobilazni problem današnjice. Rast proizvodnje i rafiniranja nafte popraćen je povećanjem razmjera onečišćenja naftom, što dovodi do narušavanja ekosuava, bioraznolikosti, smanjenje površine poljoprivrednog zemljišta, smanjenjeplodnosti tla, ali može imati negativne učinke na zdravlje ljudi. U kontaminiranim ekosustavima procesi sanacije mogu se odvijati od nekoliko mjeseci do nekoliko desetljeća ovisno o stupnju onečišćenja, karakteristikama tla, klimatskim uvjetima i nizu drugih čimbenika. U Europi 2,5 milijuna mjesta sa onečišćenjem tla svjedoči o naslijeđu duge industrijalizacije, kako u prošlosti tako i sadašnjosti, a oko 340.000 lokacija zahtijeva žurnu sanaciju (Van Liedekerke i sur., 2014.). Većina razvijenih zemalja ima ekološke propise kojima se utvrđuju mogućnosti i načine sanacije kontaminiranih ekosustava. Konvencionalna sanacija često uključuje uklanjanje onečišćenog tla i njegovo skladištenje na odlagalištu, zatvaranje zagađenih mjesta zemljom ili drugim materijalom, nakon čega slijede mjere revegetacije, ako je navedeno moguće provesti, a što se pokazalo kao jako skupa metoda (Rowlnd i sur., 2009.). Zbog značajnih nedostataka, kao što su visoki troškovi i dodatni negativni učinici na ekosustave, korištenje mehaničkih, toplinskih i fizikalno-kemijskih metoda sanacije onečišćenih ekosustava doseglo je granice (Cunningham i sur., 2021.). Tehnologije biološke remedijacije onečišćenih tala i voda postaju sve važnije i široko rasprostranjene, jer su ekološki prihvatljive i učinkovite te su ekonomski isplativije (Cunningham i sur., 2020.). Bioremedijacija se sve više smatra održivom metodom i predstavlja „zeleni“ biotehnološki alat za sanaciju kontaminiranih tala i vode te može biti konkurentna konvencionalnim nerijetko skupim metodama sanacije (Cunningham i sur., 2021.). Bioremedijacija predstavlja mogućnosti korištenja organizama (bakterije, gljive, biljke, alge i dr) za čišćenje kontaminiranih ekosustava. Navedeni organizmi imaju mogućnost razgradnje, akumulacije ili imobilizacije raznih organskih i anorganskih toksičnih tvari, a što rezultira u značajnom

smanjenju razine onečišćujućih tvari u ekosustavu (Alexander, 1999.). Dodavanje korisnih organizama na onečišćena mjesta naziva se bioaugmentacija, dok se biostimulacija odnosi na dodavanje hranjivih tvari s ciljem poboljšanja rasta i konkurentnosti autohtone mikroflore na onečišćenojlokaciji (Kumar i sur., 2011.). Na mjestima s onečišćenim tlima, bioremedijacija može rezultirati smanjenjem razine onečišćujućih tvari ili može dovesti do djelomičnog smanjenja toksičnosti što dopušta dodatne mjere kao što je revegetacija koja će s vremenom rezultirati daljnjim smanjenjem razine toksičnosti (Alexander, 1999.). Policiklički aromatski ugljikovodici i njihovi derivati predstavljaju jednu od najvećih klasa organskih molekula. Građeni su od većeg broja spojenih aromatskih prstena, a najjednostavniji predstavnik je naftalin, sačinjen od dva prstena (Loick i sur., 2009.). Najčešće su mutageni, kancerogeni, toksični i u prirodi teško razgradivi spojevi koji nastaju nepotpunim izgaranjem fosilnih goriva i drugih organskih tvari. Iako ovi spojevi nastaju i u prirodnim uvjetima npr. u šumskim požarima njihova je prisutnost u okolišu većinom uzrokovana antropogenom aktivnošću. Kada dospiju u okoliš policiklički aromatski ugljikovodici se vežu na čestice tla i postaju slabo dostupni brojnim organizmima koji bi ih mogli razgraditi. Jedno od rješenja je mikoremedijacija odnosno korištenja gljiva u sanaciji raznih kontaminanata koji su teško dostupni drugim organizmima.U ovu svrhu najčešće se upotrebljavaju bazidiomicete, ektomikorizne i saprotrofne gljive. Stoga, cilj ovoga rada je dati pregledan prikaz uloge gljiva u remedijaciji onečišćenih ekosustava te prikazati vrste bazidiomiceta koje imaju mogućnost korištenja u procesu mikoremedijacije.

Bioremedijacija policikličkih aromatskih ugljikovodika

Policiklički aromatski ugljikovodici (PAH) su velika skupina organskih spojeva s dva ili više spojenih aromatskih prstena (benzenski prsten) na linearnom ili kutnom rasporedu (Loick i sur., 2009.). Oni su relativno neutralni do stabilni s niskom topljivošću u vodi. Kemijska svojstva pojedinačnih policikličkih aromatskih ugljikovodika dijelom ovise o njihovoj molekularnoj veličini (tj. broju aromatskih prstenova) kao i o njihovoj molekularnoj topologiji (tj. njihovom obrascu aromatskog povezivanja). Povećanje veličine i kuta molekule policikličkih aromatskih ugljikovodika općenito rezultira povezanim povećanjem njihove hidrofobnosti i elektrokemijske stabilnosti, što pridonosi njenoj postojanosti (Loick i sur., 2009.). Policiklički aromatski ugljikovodici se ispuštaju tako da pokazuju toksične učinke ili posjeduju mutagena, teratogena ili kancerogena svojstva, a kao posljedica toga, neke su klasificirane kao prioritetne onečišćujuće tvari (USEPA, 2021.). Policiklički aromatski ugjikovodici nastaju i unose se u okoliš prirodno ili antropogeno. Uglavnom nastaju kao rezultat pirolitičkog procesa, posebno nepotpunog izgaranja organskih materijala tijekom različitih ljudskih aktivnosti, kao što su prerada ugljena i sirove nafte, izgaranje prirodnog plina, šumski požari, izgaranje otpada, promet vozila. Znatno manje nastaju u prirodnim procesima kao što su karbonizacija i vulkansko ispuštanje (WHO, 2000.). Antropogeni izvori poslicikličkih aromatskih ugljikovodika imaju značajno povećanje u svjetskim industrijama, posebice tijekom 20. stoljeća. S obzirom da predstavljaju jako velike zagađivače u ekosustavu, u tablici 1. prikazano je 16 prioritetnih zagađivača policikličkih aromatskih ugljikovodika.

Tablica 1. Odabrana svojstva 16 prioritetnih zagađivača policikličkih aromatskih ugljikovodika (Mackay i sur., 1992.)

PAH	Broj prstenova	Molekularna težina	Topljivost u vodi (mg L^{-1})
Naftalin	2	128	31
Acenaftalin	3	152	16
Acennaften	3	154	3,8

Fluoren	3	166	1,9
Fenantren	3	178	1,1
Antracen	3	178	0,045
Piren	4	202	0,13
Fluoranten	4	202	0,26
Benzo (*a*) antracene	4	228	0,011
Krizen	4	228	0,006
Benzo (*b*) fluoranten	5	252	0,0015
Benzo (*k*) fluoranten	5	252	0,0008
Benzo (*a*) piren	5	252	0,0038
Dibezno (*a, h*)antracen	5	278	0,0006
Indeno (*1, 2, 3-cd*) piren	6	276	0,00019
Benzo (*ghi*) perilen	6	276	0,00026

Uloga gljiva u remedijaciji

Gljive koriste različite metode za dekontaminiranje zagađenih ekosustava. Metode dekontaminacije zagađenih prostora u prirodi, mikoremedijacijom, uključuju biorazgradnju, biosorpciju i biokonverzija. Biorazgradnja podrazumijeva potpunu degradaciju kemijskog spoja do njegovih najosnovnijih mineralnih komponenti, poput CO_2, H_2O, NO_3 i drugih. Za biorazgradnju su bitni ekstracelularni enzimi koje gljive proizvode kao što su peroksidaza, ligninaza, celulaza, pektinaza, oksidaza i ksilanaza (Nyanhongo i sur., 2007.). Navedeni enzimi su pokazali sposobnost razgradnje širokog spektra polutanata uključujući policikličke aromatske ugljikovodike (Johannes i sur., 1996.). Biosorpcija je proces primanja metalnih iona, polutanata i ksenobiotika, živom ili neživom biomasom (Gavrilescu, 2004.). Primanje polutanata može biti aktivno, odnosno ovisno o funkcionalnom metabolizmu ili pasivno bez uloge metabolizma navesti literaturni navod. Biokonverzija je proces pretvaranja otpada u proizvod, a u slučaju korištenja gljiva to je plodno tijeloNavesti literaturni navod Za proces biokonverzije se koristi poljoprivredni i neke vrste industrijalnog otpada (Kulshreshtha i sur., 2013.). Budući da je krajnji proizvod odnosno mesnatoto tijelo gljive namijenjeno ljudskoj prehrani, mnoge vrste otpada na kojima bi gljive mogle rasti i dati plod nisu prikladne za biokonverziju. Međutim, posljednjih godina, potencijal gljiva za bioremedijaciju odnosno mikoremedijaciju sve se više prepoznaje (Gadd, 2001.). Nekoliko je razloga zašto su gljive pogodni organizmi za mikoremedijaciju i zbog čega mogu biti superiorniji od bakterija ili biljaka u različitim uvjetima rasta i razvoja. Njihova mreža micelija jako je pogodna za prodiranje u tlo i pristupanje svim porama tla. Višestanični micelij gljiva za razliku od jednostaničnih bakterija mogu djelovati u više pravaca (Dix i Webster, 1995.)pojasniti detaljnije Za razliku od ostalih eukariota, stanice hifa gljiva pokazuju neograničen rast, stoga nema ograničenja staničnih dioba u hifama micelija sve dok su resursi dostupni u supstratu. Osim toga, gljivični micelij mnogih vrsta može se formirati i rasti kroz neadekvatne okolišne uvjete te kolonizirati područja daleko od izvora micelija. U usporedbi s bakterijama, gljive su također otpornije na visoke koncentracije toksina (Treu i Falandysz, 2017.). Hife gljiva imaju veliku površinu i biomasu, a iste u nekim tlima mogu doseći duljinu od 1 km g^{-1} tla (Osono i sur., 2003.). Osim toga, gljive proizvode mnoge bioaktivne spojeve, a u svrhu bioremedijacije enzimi gljiva su jako bitni, jer mnogima od njih nedostaje specifičnosti supstrata te se otpuštaju u supstrat kao egzoenzimi. Budući da gljive općenito ovise o ekstracelularnoj razgradnji makromolekula, stvara se velika količina enzima i izlučuje se u supstrat (Harms i sur., 2011.). Sanacija ugljikovodika općenito se temelji na biorazgradnji i stoga uključuje djelomičnu ili potpunu razgradnju odgovarajućih onečišćujućih tvari (Alexander, 1999.). Ovaj mehanizam se bitno razlikuje od bioakumulacije, koja opisuje sposobnost nekih gljiva da akumuliraju ili hiperakumuliraju različite toksične spojeve iz tla.

Pokazalo se da brojni rodovi bazidiomiceta razgrađuju ugljikovodike, a glavni tipovi onečišćujućih tvari uključuju policikličke aromatske ugljikovodike, aromatske ugljikovodike, fenole, halogenirane ugljikovodike, boje i dr. (Treu i Falandysz, 2017.). Policiklički aromatski ugljikovodici su onečišćujuće tvari jako velikih kancerogenih i mutagenih svojstava (Collins i sur., 1998.). Novija istraživanja o bioremedijaciji bazidiomiceta prikazuju korištenje brojnih vrsta gljiva u sanacijama onečišćenja policikličkim aromatskim ugljikovdicima, poljoprivrednom proizvodnjom i sl. (Tran i sur., 2021.). Navedeno može biti rezultat lake dostupnosti polutanata vrstama za koje se prethodno utvrdilo da uspješno razgrađuju iste. Međutim, s druge strane, može značiti da postoje i brojne druge vrste gljiva za koje još uvijek nije utvrđen bioremedijacijski potencijal (Tran i sur., 2021.)

Saprotrofne i mikorizne bazidiomicete

Bazidiomicete, odnosno gljive općenito, dijele se na tri skupine s obzirom na način ishrane: saprotrofne, simbiontske i parazitske gljive (Dix i Webster, 1995.), a prve dvije skupine su pogodne za bioremedijaciju. Saprotrofne bazidiomicete koriste mrtve organske tvari kao izvor ugljika, a uključuju gljive bijele truleži koje razgrađuju lignocelulozu i gljive koje razgrađuju celulozu tj. gljive smeđe truleži. Simbiontske bazidiomicete primaju ugljik od mutualističkog biljnog partnera, a ova skupina uključuje mikorizne gljive (Treu i Falandysz, 2017.). U tablici 2. prikazan su vrste saprotrofnih i simbiontskih (ektomikoriznih) bazidiomiceta pogodne za postupak mikoremedijacije.

Tablica 2. Popis saprotrofnih i ekotomikoriznih vrsta gljiva pogodnih za mikoremdijaciju (Treu and Falandysz, 2017.)

Saprotrofne vrste gljiva	Simbiontske vrste gljiva
Agrocybe perfecta (Rick.) Singer	*Suillus bovinus* (L.) Roussel
Xerotus discolor Mont.	*Suillus luteus* (L.) Roussel
Bjerkandera adusta (Willd.) P. Karst.	*Suillus tomentosus* (Kauffman) Singer
Coprinopsis cinerea (Schaeff.) Redhead, Vilgalys & Moncalvo	*Hebeloma crustuliniforme* (Bull) Quel.
Ganoderma lucidum P. Karst.	*Gomphidius viscidus* (L.)
Irpex lacteus (Fr.) Fr.	*Boletus edulis* Bull.
Phanerochaete chrysosporium Burds.	*Laccaria bicolor* (Marie)
Phanerochaete sordida (P. Karst.)	*Leccinum scabrum* (Bull.) Gray
Peniophora cinerea (Pers.) Cooke	*Pisolithus tinctorius* (Pers.) Coker & Couch
Pleurotus ostreatus (Jacq.) P. Kumm.	
Schizophyllum commune Fr.	
Stropharia coronilla (Bull.) Quel.	
Trametes versicolor (L.) Lloyd	
Trametes coccinea (Fries)	
Phellinus sp.	

Temeljem ranije navedenog može se reći da postoji nekoliko razloga zašto mikomedijacija sa saprotrofnim i ektomikoriznim gljivama može biti pogodna metoda izbora za sanaciju brojnih zagađenih ekosustava. Korištenjem gljiva mogu se uspješno razgraditi razne onečišćujuće tvari na kontaminiranom mjestu. Sinergijski učinak, nekoliko povezanih organizama rizosfere također može doprinijeti razgradnji onečišćujućih tvari ili degradirati neke od primarnih i sekundarnih proizvoda razgradnje. Pored toga, korištenjem mikoriznih gljiva, ugljik nije potencijalno ograničavajući čimbenik, budući da mikorizni sustav uključuje izvor ugljika preko biljaka.

Zaključak

Na temelju pregledane literature može se zaključiti da bazidiomicete posjeduju enzime što ih čini pogodnim organizmima za sanaciju onečišćenih ekosustva sa sposobnošću razgradnje raznih ugljikovodika. Za uspješnu sanaciju onačišćenja potrebno je osigurati izvor ugljika za saprotrofne gljive, dok je kod mikoriznih gljiva pridružena biljna vrsta izvor ugljika. Vrste gljiva koje se koriste za mikoremedijaciju moraju biti pažljivo odabrane i njihov potencijal potrebno je procijeniti interakcijom s drugim organizmima u tlu. Također, autohtone vrste gljiva koje su prilagođene lokalnoj klimi mogu biti pogodnije za sanaciju ekosustava, iako postoji potreba za više studija bioremedijacije u lokalnim terenskim uvjetima s autohtonim vrstama.

Literatura

Collins J.F., Brown J.P., Alexeeff G.V., Salmon A.G. (1998). Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. Regulatory Toxicology and Pharmacology. 28: 45–54.

Cunningham C.J., Kuyukina M.S., Ivshina I.B., Konev A., Peshuv T.A., Knapp C.W. (2020). Potential risks of antibiotic resistance genes and bacteria in bioremediation of petroleum hydrocarbon contaminated soils. Environmental Science: Processes & Impacts. 22: 1110−1124.

Cunninghama C.J., Peshkura T.A., Kuyukinab M.S., Ivshina I.B. (2021). Sustainable Bioremediation of Hydrocarbon Contaminated Soils: Opportunities for Symbiosis with Organic Waste Management? Russian Journal of Ecology. 52 (6): 463-469.

Dix N. J., Webster J. (1995). Fungal Ecology. Chapman and Hall: London, UK.

Gadd G.M. (2001). Fungi in Bioremediation. Cambridge University Press: Cambridge, UK, str. 481.

Gavrilescu M. (2004.). Removal of heavy metals from the environment by biosorption. Engineering in Life Science. 4: 219–232.

Ghosal D., Ghosh S., Dutta T.K., Ahn Y. (2016). Current state of knowl1edge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Frontires in Microbiology. 7: 1369.

Harms H., Schlosser D., Wick L.Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nature Reviews Microbiology 9: 177–192.

Johannes C., Majcherczyk A., Hüttermann A. (1996). Degradation of anthracene by laccase of Trametes versicolor in the presence of different mediator compounds. Applied in Microbiology and Biotechnology. 46: 313–317.

Kulshreshtha S., Mathur N., Bhatnagar P., Kulshreshtha S. (2013). Cultivation of Pleurotus citrinopileatus on handmade paper and cardboard industrial wastes. Industrial Crops and Products. 41: 340–346.

Kumar A., Bisht B. S., Joshi V.D., Dhewa T. (2011). Review on bioremedi1ation of polluted environment: a management tool. International Journal of Environmental Science. 6: 1079–1093.

Loick N., Hobbs P.J., Hale M.D.C., Jones D.L. (2009). Bioremediation of Poly-Aromatic Hydrocarbon (PHA) Contaminated Soil by Composting. Critical Reviews in Environmental Science and Technology. 39: 271-332.

Mackay D., Shiu W.Y., Ma K.C. (1992). Illustrated handbook of physical-cheical propertis and environmental fate for organic chemicals, Vol II. Lewis Publishers, Michigan, USA.

Nyanhongo G.S., Gübitz G., Sukyai P., Leitner C., Haltrich D., Ludwig R. (2007). Oxidoreductases from Trametes spp. in biotechnology: A wealth of catalytic activity. Food Technology and Biotechnology. 45: 250–268.

Osono T., Ono Y., Takeda H. (2003). Fungal ingrowth on forest floor and decomposing needle litter of Chamaecyparis obtusa in relation to resource availability and moisture condition. Soil Biology and Biochemistry. 35: 1423–1431.

Rowland S.M., Prescott C.E., Grayston S.J., Quideau S.A., Bradfield G.E. (2009). Recreating a functioning forest soil in reclaimed oil sands in northern alberta: an approach for measuring success in ecological restoration. Journal of Environmental Quality. 38(4):1580-1590.

Shah A.A., Hasan F., Hameed A., Ahmed S. (2008). Biological degrada1tion of plastics: a comprehensive review. Biotechnology Advances. 26: 246–265.

Tran H.T., Lin C., Bui X.T., Ngo H.H., Kiportich Cheruiyot N., Hoang H.G., Vu C.T. (2021). Aerobic composting remediation of petroleum hydrocarbon-contaminated soil: Current and future perspectives. Science of Total Environment. 753: 142250.

Treu R., Falandysz J. (2017). Mycoemediation of hydrocarbons with basidiomycetes - a review. Journal of Environmental Science and Health, Part B. 52 (3): 148-155.

USEPA (2021). United States Environmental Protection Agency.

WHO (2000). World Health Organization.

Van Liedekerke M., Prokop G., Rabl-Berger S., Kibblewhite M., Louwagie G. (2014). Progress in the Management of Contaminated Sites in Europe. Report EUR 26376; European Union, Luxembourg 1–68.

Microremediation of polycyclic aromatic hydrocarbons by saprotrophic and ectomycorrhizal fungi

Abstarct

Among the various technologies available for remediation of ecosystem pollution, it is well-known that bioremediation methods using microorganisms (fungi) are recognized as cost-effective and environmental-friendly methods. Two ecological groups of fungi are used in the application of fungi for pollution remediation: saprotrophic and ectomycorrhizal basidiomycetes. Many species of basidiomycetes have been found to have a very good ability to degrade pollutants such as polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), halogenated hydrocarbons, phenols, explosives and dyes. However, further research is needed on the practical application of saprotrophic and ecotomycorrhizal basidiomycetes in microremediation.

Key word: bioremediation, saprotrophs, ectomycorrhiza, sustainable management

IZVORNI ZNANSTVENI RAD

Oštećenja tla uvjetovana dugogodišnjom intenzivnom proizvodnjom - prilog zaštiti tla

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Sažetak
Cilj ovog rada bio je utvrditi utjecaj dugotrajnog korištenja nekalcificiranih i kalcificiranih tala u intenzivnoj poljoprivrednoj proizvodnji na njihove bitnije značajke. Istraživanja su provedena na području Slatine na deset pedoloških profila. Uspoređene su značajke tla u oraničnom sloju iz 1988. i 2020. godine. Utvrđeno je veće smanjenje sadržaja čestica gline i praha te povećanje sadržaja krupnog i sitnog pijeska te izrazitija degradacija strukture kod nekalcificiranih tala. Kod ovih tala smanjila se pH vrijednost u vodi i u 1 M KCl-u, dok je kod kalcificiranih ona bila veća. Također je zabilježeno nešto veće smanjenje sadržaja humusa kod nekalcificiranih tala. Intenzivna poljoprivredna proizvodnja uzrok je utvrđenim oštećenjima tla, a kalcifikacijom se ona mogu ublažiti.

Ključne riječi: intenzivna proizvodnja, kalcifikacija, degradacija tla

Uvod

Promjene značajki tla koje dovode u pitanje njegove uloge u intenzivnoj poljoprivrednoj proizvodnji, smatraju se oštećenjem tla (Bašić, 2009.). Naime, pod oštećenjem tla podrazumijeva se svaki proces ili utjecaj, odnosno skup procesa ili utjecaja, kojima se prirodne ili antropogenim utjecajem uvjetovane značajke tla mijenjaju na način da se tlu smanjuje plodnost. Ukoliko se tla u intenzivnoj biljnoj proizvodnji ne koriste na održivi način, može doći do njihovog oštećenja. To se poglavito odnosi na smanjenje sadržaja humusa, zakiseljavanje, smanjenje stupnja stabilnosti strukturnih agregata te ispiranje čestica tla, uz pogoršanje vodozračnih odnosa (Husnjak i sur., 2011.). Zbog toga se gospodarenje tlom u poljoprivredi oduvijek nametalo kao izuzetno bitno pitanje, pri čemu bi osnovni zadatak trebao biti njegova zaštita, odnosno očuvanje njegove kvalitete i plodnosti. Tijekom 1988. godine na širem području Slatine obavljena su istraživanja na nekoliko tipova tala. Kako se prema postojećim podacima poljoprivredno zemljište od tada pa do 2020. godine neprekidno koristilo u poljoprivredi, na istim lokacijama 2020. godine ponovljena su istraživanja u oraničnom sloju tla. Cilj je bio, usporedbom rezultata istraživanih značajki nakon trideset i dvije godine korištenja tala, utvrditi utjecaj intenzivne poljoprivredne proizvodnje na moguća oštećenja nekalcificiranih i kalcificiranih površina.

Materijal i metode

Istraživanje je obavljeno na deset pedoloških profila tijekom 1988., a ponovljeno je 2020. godine, (Slika 1). Za referentnu 1988. godinu korišteni su podaci iz postojećih studija (Bogunović i sur., 1991.a i 1991.b). Na proizvodnoj površini 1 otvoreno je 1988. godine pet pedoloških profila. Na tri lokaliteta utvrđen je hipoglej mineralni, nekarbonatni, pretežno praškasto ilovaste teksture, slabo kisele reakcije i dobre humoznosti. Na preostala dva lokaliteta utvrđen je amfiglej mineralni, nekarbonatni, praškasto glinasto ilovaste teksture, slabo kisele do neutralne reakcije i dobre humoznosti. Navedena tla su hidromeliorirana i na njima nije provedena kalcifikacija.

Slika 1. Položaj proizvodnih površina (arkod parcela) na topografskoj karti

Tijekom 1988. godine na proizvodnim površinama 2 i 3 također je otvoreno pet pedoloških profila. Na tri lokaliteta utvrđeno je lesivirano tlo na praporu tipično, praškasto ilovaste teksture, jako kisele do kisele reakcije te slabe humoznosti. Jedan pedološki profil determiniran je kao distrično smeđe tlo na pjeskovito ilovastom nanosu, praškasto ilovaste do pjeskovito ilovaste teksture, kisele reakcije i vrlo slabe humoznosti. Također je utvrđen stagnoglej, dijelom hidromeliorirani, praškasto ilovaste teksture, jako kisele reakcije i slabe humoznosti. Na navedenim tlima provedena je kalcifikacija 1990. godine i to s većim količinama materijala za neutralizaciju kiselosti. U narednim godinama kalcifikacija se provodila povremeno, s manjim količinama materijala.Na svakoj lokaciji 2020. godine ponovno je otvoren pedološki profil i obavljeno je uzorkovanje tla u antropogenom horizontu. Iz pojedinačnih uzoraka tla analizirane su sljedeće fizikalne i kemijske značajke tla: mehanički sastav tla (HRN ISO 11277:2011), stabilnost strukturnih mikroagregata na temelju indeksa flokulacije (Špoljar, 2019.), reakcija tla (HRN ISO 10390:2005), sadržaj humusa prema Tjurinu i adsorpcijski kompleks tla prema Kappenu. Teksturne klase određene su prema FAO metodologiji (FAO, 2006.).S obzirom na to da je na proizvodnim površinama 2 i 3 prvi put kalcifikacija izvršena 1990. godine, promjene fizikalnih i kemijskih značajki tala nakon trideset i dvije godine korištenja u intenzivnoj poljoprivrednoj proizvodnji obrazložene su posebno za nekalcificirano (varijanta 1) i kalcificirano tlo (varijanta 2).

Rezultati i rasprava

Nakon trideset i dvije godine korištenja nekalcificiranog tla u intenzivnoj poljoprivrednoj proizvodnji, utvrđene su promjene mehaničkog sastava tla, kao i indeksa stabilnosti mikroagregata, (Tablica 1). Tijekom navedenog razdoblja smanjio se sadržaj čestica gline i praha, a povećao se sadržaj čestica krupnog i sitnog pijeska. Sadržaj čestica gline u prosjeku se smanjio za 2,2 %, a sitnog praha za 1,4 %. Sadržaj krupnog praha također se smanjio za 0,6 %. Dugogodišnje korištenje tla uzrokovalo je povećanje sadržaja pijeska u oraničnom sloju. Sadržaj čestica sitnog pijeska u prosjeku se povećao za 1,9 %, a krupnog za 2,8 %. Indeks stabilnosti mikroagregata na kraju istraživanja također je u odnosu na početak bio manji za 4,3. Na kalcificiranom tlu također je došlo do promjene mehaničkog sastava tla kao i indeksa stabilnosti mikroagregata. Međutim, te promjene znatno su manje izražene u odnosu na tlo koje nije kalcificirano, (Tablica 2). Sadržaj čestica gline smanjio se u prosjeku za 1,4 %, a sitnog praha za 0,9 %. Utvrđeno je manje smanjenje sadržaja čestica krupnog praha, u prosjeku za 0,4 %. Dugogodišnje korištenje tla uzrokovalo je povećanje sadržaja pijeska u oraničnom sloju prosječno za 1,1 %, a krupnog pijeska za 1,4 %. Tijekom razmatranog razdoblja također je utvrđeno smanjenje indeksa stabilnosti mikroagregata za 2,5.

Tablica 1. Promjena mehaničkog sastava (%) i indeksa stabilnosti mikroagregata u uzorcima nekalcificiranog tla za 1988. i 2020. godinu

	Krupni pijesak, 2,0-0,2 mm	Sitni pijesak, 0,2-0,063 mm	Krupni prah, 0,063-0,02 mm	Sitni prah, 0,02-0,002 mm	Glina, <0,002 mm	Stabilnost mikro-agregata
Min.	1,9	1,3	-0,4	-0,8	-1,3	-3,1
Max.	3,8	2,8	-1,0	-1,9	-2,7	-8,9
Prosjek	2,8	1,9	-0,6	-1,4	-2,2	-4,3

Tablica 2. Promjena mehaničkog sastava (%) i stabilnosti mikroagregata u uzorcima kalcificiranog tla za 1988. i 2020. godinu

	Krupni pijesak, 2,0-0,2 mm	Sitni pijesak, 0,2-0,063 mm	Krupni prah, 0,063-0,02 mm	Sitni prah, 0,02-0,002 mm	Glina, <0,002 mm	Stabilnost mikro-agregata
Min.	0,9	0,6	-0,2	-0,4	-0,7	-1,7
Max.	2,4	1,7	-0,8	-1,2	-2,0	-4,2
Prosjek	1,4	1,1	-0,4	-0,9	-1,4	-2,5

Manje promjene mehaničkog sastava tla i indeksa stabilnosti mikroagregata koje su utvrđene kod kalcificiranog tla, ukazuju na to kako provedena kalcifikacija ima stanoviti utjecaj na promjene mehaničkog sastava tla. Na kalcificiranom tlu utvrđena je neznatna degradacija strukture u odnosu na nekalcificirano. Husnjak i sur. (2011.) utvrđuju kako korištenje tla u intenzivnoj poljoprivrednoj proizvodnji dovodi do ispiranja čestica tla i smanjenja stupnja stabilnosti mikroagregata. Promjene reakcije tla i sadržaja humusa na nekalcificiranom tlu prikazuje Tablica 3., a na kalcificiranom tlu Tablica 4. Na istraživanoj proizvodnoj površini 1, na lokacijama s pet pedoloških profila, smanjila se pH vrijednost tla i sadržaj humusa. Reakcija tla u vodi smanjila se u prosjeku za 0,58, a u 1 M KCl-u za 0,76. Sadržaj humusa u prosjeku je bio manji za 0,89 %.

Tablica 3. Promjene reakcije tla i sadržaja humusa u uzorcima nekalcificiranog tla za 1988. i 2020. godinu

	Reakcija tla u		Humus
	H_2O	1 M KCl-u	(%)
Min.	-0,31	-0,63	-0,73
Max.	-0,76	-0,81	-1,01
Prosjek	-0,58	-0,76	-0,89

Na kalcificiranom tlu, na proizvodnim parcelama 2 i 3, zbog višekratno provedene kalcifikacije došlo je do povećanja reakcije tla te stupnja zasićenosti tla bazama, odnosno do smanjenja vrijednosti hidrolitskog aciditeta i stanovitog smanjenja sadržaja humusa. Reakcija tla u vodi u uzorcima ranije kalcificiranog tla bila je, u odnosu na početak istraživanja prosječno veća za 1,09, a u 1M KCl-u za 0,68. Vrijednost hidrolitske kiselosti tla bila je manja prosječno za 6,5. Stupanj zasićenosti adsorpcijskog kompleksa tla se povećao u prosjeku za 20,5 %. Sadržaj humusa se smanjio za 0,08 %, što je nešto povoljnije u odnosu na nekalcificirano tlo. Pretpostavlja se da je to rezultat formiranja organsko-

mineralnog kompleksa nakon provedene kalcifikacije te zbog toga veće stabilnosti humusa prema mineralizaciji.

Tablica 4. Promjene reakcije tla u vodi i promjene sadržaja humusa u uzorcima kalcificiranog tla za 1988. i 2020. godinu

	Reakcija tla u				Adsorpcijski kompleks tla*			
	H_2O	1M KCl-u	Humus %	Hidro-litska kiselost, Y_1	T-S m mol ekv.	S m mol ekv.	T m mol ekv.	V (%)
Min	0,24	0,19	-0,03	-4,6	-3,0	0,5	-1,7	10,9
Max	1,88	1,19	-0,16	-9,5	-6,2	4,7	-5,4	36,8
Prosjek	1,09	0,68	-0,08	-6,5	-4,2	2,4	-3,1	20,5

* Izvorni podaci o značajkama tla za referentnu 1988. godinu odnose se na stanje prije kalcifikacije

Buni (2014.) pri provedbi kalcifikacije na kiselim tlima utvrđuje porast pH vrijednosti tla s 5,03 na 6,72 te statistički opravdano smanjenje zamjenjive kiselosti tla. Autor također dobiva značajan porast kapaciteta izmjenjivih kationa i pristupačnog fosfora te smanjenje sadržaja bakra u tlu. Sultana i sur., (2009.) isto tako utvrđuju pozitivan učinak primijenjene kalcifikacije na istraživane kemijske značajke tla. Pri primjeni različitih količina materijala za kalcifikaciju utvrđuju postupno povećanje pH vrijednosti tla te pristupačnog fosfora, kalcija i magnezija. Špoljar (2011.) pri uzgoju usjeva u plodoredu uz upotrebu kombinirane organske i mineralne gnojidbe, s kalcifikacijom, dobiva uglavnom povoljnije stanje većine istraživanih fizikalnih i kemijskih značajki tla. Povoljan utjecaj provedene kalcifikacije na istraživane značajke tla i prinose kultura dobivaju i drugi autori (Kovačević i sur., 2012.; Rastija, 2009.; Yaduvanashi, 2003.). Iz izloženoga proizlazi kako primijenjena kalcifikacija povoljno utječe na većinu istraživanih značajki tla pa se u sklopu održivog gospodarenja tlom preporuča njena veća primjena.

Zaključak

Nakon trideset i dvije godine korištenja tla u intenzivnoj priozvodnji, smanjio se sadržaj čestica gline i praha, a povećao se sadržaj čestica krupnog i sitnog pijeska, s time da su utvrđene promjene nešto veće kod nekalcificiranog tla u odnosu na kalcificirano. Do većeg smanjenja stabilnosti strukturnih agregata, odnosno degradacije strukture tla, došlo je kod nekalcificirane varijante. Nakon trideset i dvije godine korištenja nekalcificiranog tla, smanjila se pH vrijednost tla u vodi i u 1 M KCl-u, a kod kalcificiranih tala na kraju istraživanja ona je bila veća u odnosu na početne vrijednosti. Nešto veće smanjenje sadržaja humusa zabilježeno je kod nekalcificiranih tala. Pretpostavlja se da je razlog tome formiranje organsko-mineralnog kompleksa u tlima s provedenom kalcifikacijom. Iz izloženoga proizlazi kako u intenzivnoj poljoprivrednoj proizvodnji dolazi do ubrzanog oštećenja tla, posebno zakiseljavanja tla i degradacije humusa. Kalcifikacija je mjera kojom se može ublažiti intenzitet oštećenja tla.

Literatura

Bašić F. (2009). Oštećenja i tehnologije zaštite tala Hrvatske - otvorena pitanja. Zbornik radova znanstveno stručnog skupa „Tehnologije zbrinjavanja otpada i zaštite tla“, str. 179-201, Zadar, Hrvatska: Agronomski fakultet Sveučilišta u Zagrebu.

Bogunović M., Husnjak S., Kisić I., Sraka M., Špoljar, A. (1991. a). Studija za potrebe izrade projekta agromelioracija R.J. Bukovica. Sveučilište u Zagrebu Agronomski fakultet, arhiva Zavoda za pedologiju, 313 str., Zagreb, Hrvatska.

Bogunović M., Husnjak S., Kisić I., Špoljar A., Sraka M. (1991. b). Agropedološka studija za potrebe izrade projekta agromelioracija R.J. Višnjica. Sveučilište u Zagrebu Agronomski fakultet, arhiva Zavoda za pedologiju, 208 str., Zagreb, Hrvatska.

Buni J. (2014). Effects of Liming Acidic Soils on Improving Soil Properties and Yield of Haricot Bean. Journal of Environmental & Analytical Toxicology. 4:248. doi: 10.4172/2161-0525.1000248.

FAO (2006). Guidelines for Soil Profile Description - fourth edition. Publishing Management Service, 97 p.p., Rome, Italy.

Husnjak S., Romić M., Poljak M., Pernar N. (2011). Recommendations for Soil Management in Croatia. Agriculturae Conspectus Scientificus. 76 (1): 1-8.

Kovačević V., Rastija D., Sudar R., Iljkić D. (2012). Učinak kalcizacije karbokalkom na tlo, prinos i kvalitetu zrna kukuruza. Glasnik zaštite bilja, (6):54-60, Osijek, Hrvatska: Sveučilište J.J. Strossmayera u Osijeku, Poljoprivredni fakultet.

Rastija D., Lončarić Z., Škripek Ž., Japundžić-Palenkić B., Varošić A. (2009). Utjecaj kalcizacije i gnojidbe na promjene kemijskih svojstava tla i prinos kukuruza. 44. hrvatski i 4. međunarodni simpozij agronoma u Opatiji, str. 83 – 88, Opatija, Hrvatska: Fakultet agrobiotehničkih znanosti.

Sultana B.S., Main M.M.H., Islam M.R., Rahman M.M., Bikash C., Zoha M.S. (2009). Effect of liming on soil properties, yield and nutrient uptake by wheat. Current World Environment. 4(1): 39-47.

Špoljar A., Kisić I., Birkas M., Gunjača J., Kvaternjak I. (2011). Influence of crop rotation, liming and green manuring on soil properties and yields. Journal of environmental protection and ecology. 12 (1): 55-72.

Špoljar A. (2019). Konzervacija i remedijacija tla. Visoko gospodarsko učilište u Križevcima, udžbenik, 209 str., Križevci, Hrvatska: Visoko gospodarsko učilište.

Yaduvanashi N.P.S. (2003). Substitution of organic fertilizers by organic manuresand effect on soil fertility in a rice - wheat rotation on reclaimed soil in India. Journal of Agricultural Science. 140 (2): 161-168.

HRN ISO 10390:2005. Soil quality - Determination of pH. International standard Zagreb Croatia: Croatian Standards Institute.

HRN ISO 11277:2009. Soil quality – Determination of particle size distribution (mechanical composition) in mineral soil – Method by sieving and sedimentation. International standard. Zagreb Croatia: Croatian Standards Institut.

Soil damage caused by many years of intensive production - a contribution to soil protection

Abstract

The main goal of this paper was to determine the impact of long - term use of uncalcified and calcified soils in intensive agricultural production on their more important characteristics. The research was conducted in the area of Slatina on ten pedological profiles. Soil characteristics from 1988 and 2020 were compared. A larger decrease in the content of clay and powder particles and an increase in the content of coarseand fine sand and a more pronounced deterioration of the structure in uncalcified soil were found. In these soils the pH value in water and in 1 M KCl decreased, while in calcified soils it was higher. A slightly greater decrease in humus content was also observed in non-calcified soils. Intensive agricultural production is the cause of established soil damage, and calcification can alleviate it.

Key words: intensive production, calcification, soil degradation

IZVORNI ZNANSTVENI RAD

Utvrđivanje kationskog izmjenjivačkog kapaciteta tala Istočne Hrvatske amonij acetat metodom

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Sažetak

Kationski izmjenjivački kapacitet (KIK) predstavlja jedan od najvažnijih pokazatelja produktivnosti i plodnosti tla te je važan pokazatelj za procjenu fizikalno-kemijskog stanja tla. Mjerenje KIK-a se ne provodi u laboratorijima uvijek direktnim metodama, nego se može izračunavati na temelju ekstrahirane količine baznih kationa (indirektna metoda; Ca^{2+}, Mg^{2+}, K^{+} i Na^{+}). U istraživanje su uključena 4 tipa tla (lesivirano tlo, eutrično smeđe tlo, ritska crnica i aluvijalno tlo) čiji su uzorci prikupljeni na 60 lokacija u Istočnoj Hrvatskoj, iz oraničnog horizonta (0-30 cm). Na prikupljenim uzorcima su provedene standardne laboratorijske analize u svrhu utvrđivanja aktualne i supstitucijske kiselosti, sadržaja organske tvari, sadržaja gline, hidrolitičke kiselosti i baznih kationa ekstrahirani amonij aceta metodom. Provedenim istraživanjem utvrđena je izrazita heterogenost vrijednosti po svim ispitivanim svojstvima i tipovima tala, prosječno utvrđene količine humusa u tlu su se kretale u rasponu od 1,97 do 4,94 %, supstitucijske kiselosti od 4,22 do 7,48 pH jedinica, sadržaj gline od 7,14 do 34,92 %, te prosječna vrijednosti KIK-a od 14,16 na lesiviranom tlu do 36,98 cmol (+) kg^{-1} na ritskoj crnici. Utvrđeni niz kationa na adsorpcijskom kompleksu je Na<K<Mg<Ca.

Ključne riječi: bazni kationi, indirektna metoda, tip tla

Uvod

Jedan od najznačajnijih procesa u tlu je zamjena iona koja obuhvaća adsorpciju, zamjenu i desorpciju aniona i kationa u otopini tla (Tomašić i sur., 2013.) na negativno ili pozitivno nabijene površine čestica tla. Ketterings i sur. (2007.) definiraju KIK kao vrijednost koja predstavlja slobodna mjesta na adsorpcijskom kompleksu za koji se mogu vezati slobodni ioni. Sposobnost zadržavanja kationa u tlu ovisi o negativno nabijenim česticama gline i količini organske tvari u tlu jer se kationi za njih drže pomoću elektrostatskih sila (Japundžić-Palenkić, 2016.). Vukadinović i Vukadinović (2016.) navode da je adsorpcijski kompleks neutralnog tla (pH 7) većinom popunjen s Ca^{2+} ionima (80 %), Mg^{2+} ionima (10-15 %), dok ostatak adsorpcijskog kompleksa zauzimaju ostali bazni kationi, te da popunjenost tla tim kationima može biti mjerilo vrijednosti nekog tla. Uz koncentracije pojedinih kationa u tlu važno je poznavati i sadržaj orgaske tvari, pH reakciju tla i sadržaj gline. Najveći utjecaj na adsorpcijski kompleks nekog tla ima organska tvar, čiji utjecaj na vrijednost KIK-a može biti od 5 do 20 puta veći od sadržaja gline (Ketterings i sur., 2007.). Uz sadržaj organske tvari važna je disocijacija organskih kiselina koja je ovisna o pH vrijednosti nekog tla, odnosno tla s nižim pH vrijednostima će imati proporcionalno niži KIK (Ciesielski i Sterckeman, 1997.). Vrijednosti KIK-a na tlima istočne Hrvatske se najčešće kreću od 15 cmol (+) kg^{-1} do 45 cmol (+) kg^{-1}, dok je raspon vrijednosti od 5 cmol (+) kg^{-1} tla do 200 cmol (+) kg^{-1} tla (Vukadinović i Lončarić, 1998.).

Materijal i metode

Na istraživanom području Istočne Hrvatske prikupljeni su uzorci oraničnog horizonta (0-30 cm) na ukupno 60 lokacija. Terenska istraživanja su uključivala određivanje ekto i endomorfoloških svojstava tla u svrhu determinacije tipa tla (Škorić, 1991.). Na prikupljenim uzorcima provedena su laboratorijska istraživanja: elektorometrijsko određivanje aktualne i supstitucijske kiselosti tla (H_2O i 1 M KCl) pomoću pH metra (HRN ISO10390:2005.). Sadržaj humusa u tlu je utvrđen metodom mokrog spaljivanja organske tvari kalijevim bikromatom (HRN ISO14235:1994.). Hidrolitička kiselost tla je određena prema metodi, koja uključuje neutralizaciju tla višebaznim solima i predstavlja nezasićenost adsorpcijskog kompleksa lužnatim ionima. Teksturni sastav tla, tj. udio pojedinih mehaničkih elemenata u tlu, određen je prema ISO metodi (HRN ISO 11277:2004). Koncentracija baznih kationa u tlu je utvrđena uz trostruko centrifugiranje tla metodom ekstrakcije tla s 1 M amonijevim acetatom (AA metoda) (Jones, 2001.): preliti 10 g tla s 50 ml otopine za ekstrakciju, mućkati na rotacijskoj mućkalici 30 minuta. Nakon mućkanja uzorke centrifugirati 5 minuta te supernatant profiltrirati u odmjernu tikvicu od 100 ml. Isti postupak ponoviti 2 puta uz dodavanje 20 ml ekstrakcijskog sredstva (AA) i na kraju dopuniti odmjerne tikvice do oznake 100 ml. Sadržaj ili koncentracija pojedinog elementa je dobivena supstitucijom amonijevim ionom (NH_4^+) iz ekstrakcijske otopine amonijevog acetata, te izmjerena na AAS-u Perkin Elmer Analyst 200. Utvrđene koncentracije pojedinog elementa su izražene kao mg elementa 100 g^{-1} tla, te se vrijednost KIK-a preračunala u ekvivalentnu vrijednost prema formuli:

$$KIK\ (cmol\ (+)\ kg^{-1} = \frac{Ca\ (mg100g^{-1})}{40{,}08/2} + \frac{Mg\ (mg100g^{-1})}{24{,}305/2} + \frac{K\ (mg100g^{-1})}{39{,}098} + \frac{Na\ (mg100g^{-1})}{22{,}99} + \frac{Hk\ (mg100g^{-1})}{1}$$

Utvrđenoj vrijednosti KIK-a, na kiselim tlima pridodana je vrijednosti hidrolitičke kiselosti, te su vrijednosti KIK-a izražene u cmol (+) kg^{-1} tla.

Rezultati i rasprava

Na temelju provedenih istraživanja utvrđena je prosječna vrijednost supstitucijske kiselosti svih uzoraka u iznosu od 6,30 pH jedinica. Raspon vrijednosti se kretao od 4,77 (lesivirano tlo) do 8,51 (ritska crnica) pH jedinica (Tablica 1), što je karakteristično za tla u Istočnoj Hrvatskoj (Zebec i sur., 2015.). Prosječne vrijednosti utvrđene supstitucijske kiselosti na svim istraživanim tlima prikazane su u Tablici 1. Najniža vrijednost sadržaja humusa je izmjerena na aluvijalnom tlu (1,14 %), dok je najviša vrijednost izmjerena na ritskoj crnici (11,80 %). Prosječni sadržaj organske tvari na eutrično smeđem tlu iznosi 2,51 %, lesivirano tlo 1,93 %, aluvijalno tlo 2,43 % i na ritskoj crnici 4,94 % (Tablica 1). Najniža izmjerena prosječna vrijednost organske tvari na lesiviranom tlu je povezana s burnim i ubrzanim procesima premještanja (lesivaže) unutar profila tla (Vukadinović i Vukadinović, 2016.), te samim time i nižih vrijednost KIK-a u istraživanom horizontu tla (Grafikon 1). Izmjerene vrijednosti hidrolitičke kiselosti su se kretale u rasponu od 0,79 cmol (+) kg^{-1} (ritska crnica) do 6,04 cmol (+) kg^{-1} (lesivirano tlo), a prosječna vrijednost hidrolitičke kiselosti svih ispitivanih uzoraka iznosi 2,85 cmol (+) kg^{-1} (Tablica 1). Minimalne vrijednosti sadržaja minerala gline su izmjerene na aluvijalnom tlu (1,83 %), dok su maksimalne vrijednosti izmjerene na uzorku ritske crnice (46,81 %) što je vidljivo u Tablici 2. Prosječne vrijednosti sadržaja gline na eutrično smeđem tlu iznose 23,59 %, lesiviranom tlu 20,69 %, aluvijalnom tlu 8,03 %, te na ritskoj crnici u iznosu od 34,92 %. Slične vrijednosti teksturnog sastava su dobil u istraživanju Zebec i sur. (2015.), što je ujedno i karakteristika tipa tla (Vukadinović i Vukadinović, 2016.).

Tablica 1. Osnovna agrokemijska svojstva istraživanih tala

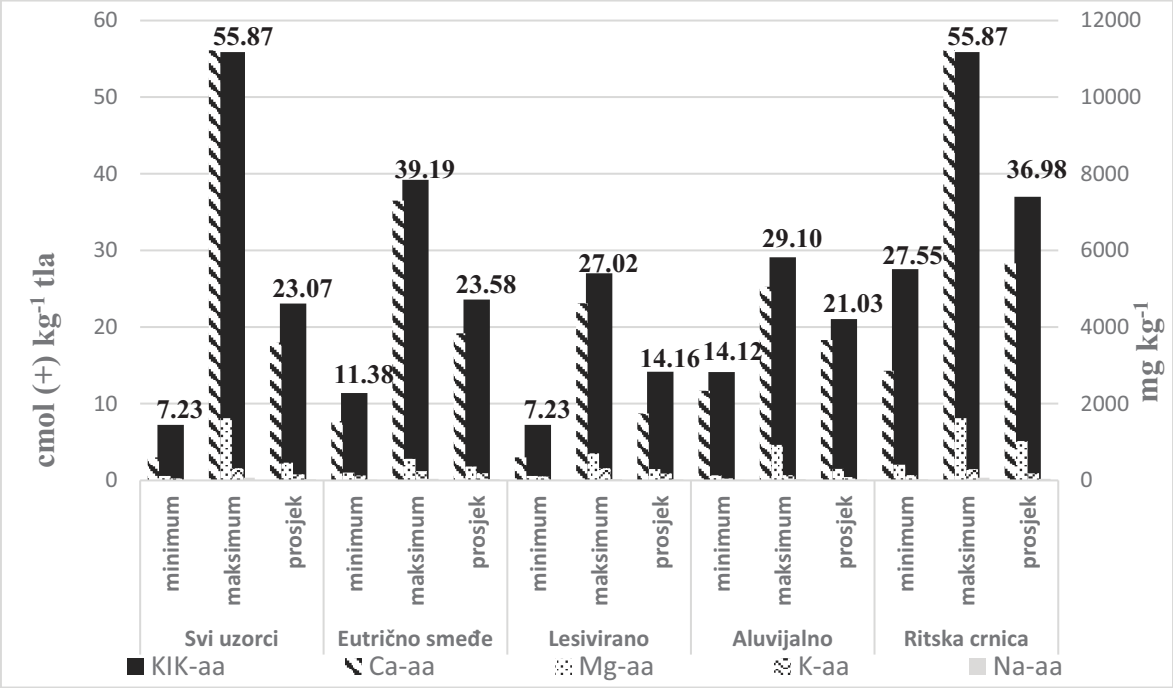
		Svi uzorci	Eutrično smeđe	Lesivirano	Aluvijalno	Ristka crnica
pH (H_2O)	min.	4,77	5,48	4,77	8,04	6,61
	maks.	8,51	8,47	8,32	8,51	8,4
	prosjek	7,34	7,36	6,27	8,28	7,51
pH (KCl)	min.	3,69	4,22	3,69	7,02	5,30
	maks.	7,91	7,91	7,43	7,72	7,54
	prosjek	6,30	6,35	5,03	7,44	6,42
Humus (%)	min.	1,14	1,21	1,31	1,14	2,62
	maks.	11,80	3,17	3,10	5,58	11,80
	prosjek	2,82	2,51	1,93	2,43	4,94
Hidrolitička kiselost	min.	0,79	1,23	1,53	-	0,79
	maks.	6,04	3,68	6,04	-	1,84
	prosjek	2,85	2,57	3,13	-	1,32

Tablica 2. Teksturni sastav istraživanih tala

		Svi uzorci	Eutrično smeđe	Lesivirano	Aluvijalno	Ristka crnica
Krupni pijesak	min	0,13	0,13	0,51	0,39	0,18
	maks.	26,61	2,12	4,57	26,61	1,61
	prosjek	1,69	0,57	1,73	3,58	0,60
Sitni pijesak	min	0,51	0,68	0,78	4,85	0,51
	maks.	60,25	10,72	9,27	60,25	12,35
	prosjek	10,91	2,78	2,97	32,48	3,10
Krupni prah	min	10,06	37,53	35,75	15,38	10,06
	maks.	56,27	45,69	56,27	44,06	39,26
	prosjek	36,87	41,85	43,59	31,98	27,80
Sitni prah	min	7,84	24,67	17,43	7,84	25,63
	maks.	62,92	35,16	35,37	62,92	40,34
	prosjek	29,60	31,21	31,02	23,58	33,58
Glina	min	1,83	6,86	12,18	1,83	26,01
	maks.	46,81	29,97	27,11	22,76	46,81
	prosjek	20,93	23,59	20,69	8,03	34,92

Na temelju provedenih istraživanja prosječna vrijednost KIK-a svih istraživanih uzoraka iznosi 23,07 cmol (+) kg^{-1}. Vrijednosti KIK-a su se kretale u rasponu od 7,23 (lesivirano tlo) do 55,87 (ritska crnica) cmol (+) kg^{-1} (Grafikon 1). Na uzorcima lesiviranog tla izmjerena je minimalna vrijednost KIK-a na pojedinačnom uzorku u iznosu od 7,23 cmol (+) kg^{-1}, ali i najniža prosječna vrijednost od 14,16 cmol (+) kg^{-1}. Najniže vrijednosti KIK-a pratile su najniže prosječne vrijednosti organske tvari (1,93 %) i supstitucijske kiselosti (5,03 pH jedinica), što također u istraživanju potvrđuju Zebec i sur. (2015.). Kod uzorka čija vrijednost KIK-a iznosi 7,23 cmol (+) kg^{-1} utvrđen je sadržaj organske tvari 1,48 % i sadržaj gline 12,18 %, i najniže izmjerene vrijednosti baznih kationa. Na uzorcima eutrično smeđeg tla vrijednosti KIK-a su se kretale u rasponu od 11,38 do 39,19 cmol (+) kg^{-1} dok je prosječna vrijednost KIK-a na eutrično smeđem tlu iznosila 23,58 cmol (+) kg^{-1} (Grafion 1). Najviša izjmerena vrijednost KIK-a na pojedinačnom uzorku eutrično smeđeg tla je povezana s visokim izmjerenim vrijednostima Ca (7298 mg kg^{-1} tla) što je rezultat povećane količine karbonata, odnosno priustnosti konkrecija u uzorku tla. Prosječna vrijednost KIK-a na aluvijalnom tlu iznosila je 21,03 cmol (+) kg^{-1}, dok je minimalna vrijednost iznosila 14,12

cmol (+) kg^{-1}, a maksimalna 29,10 cmol (+) kg^{-1}. Niže izmjerene vrijednosti KIK-a na aluvijalnom tlu su povezane s niskim sadržajem organske tvari i gline u uzorcima tla.



Grafikon 1. Vrijednosti KIK-a i koncentracije baznih kationa na istraživanim tlima

Na temelju provedenih istraživanja na ritskoj crnici izmjerene su srednje vrijednosti KIK-a u iznosu od 36,98 cmol (+) kg^{-1}, dok se raspon kretao od 27,55 do 55,87 cmol (+) kg^{-1}. Najveća izmjerena vrijednost KIK-a je povezana s najvećim izmjerenim vrijednostima Ca u pojedinačnom uzorku 11221 mg kg^{-1} čija je vrijednost rezultat visokog sadržaja karbonata u tlu. Uz visoke koncentracije Ca, na istom uzorku je također izmjerena i najviša vrijednost humusa (11,80 %).

Zaključak

Na temelju provedenog istraživanja i dobivenih rezultata može se zaključiti da je prisutna izrazita heterogenost fizikalno kemijskih svojstava tla između i unutar pojedinih sistematskih jedinica. Istraživana fizikalna i kemijska svojstva tla su pedosistematska svojstva pojedinog tipa tla te je stoga utvrđena i varijabilnost KIK-a unutar pojedinih tipova tala. Prosječno utvrđena vrijednost KIK-a iznosila je 23,07 cmol (+) kg^{-1} s utvrđenim rasponom od 14,16 na teksturno lakšem i kiselijem tlu (lesivirano tlo) do 36,98 cmol (+) kg^{-1} na teksturno teškom tlu. Rezultati provedenog istraživanja potvrđuju da bi analitički utvrđene vrijednosti KIK-a trebale biti važan parametar u monitoringu stanja poljoprivrednog zemljišta te bitan indikator plodnosti nekog tla.

Literatura

Ciesielski H., Sterckeman T., (1997.). A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. Agronomie. 17 (1997) 9-16, DOI: 10.1051/agro:19970102

HRN ISO (11277:2004.). Kvaliteta tla - Određivanje raspodjele veličine čestica (mehaničkog sastava) u mineralnom dijelu tla. Hrvatski zavod za norme, Zagreb

HRN ISO (10693:2004.). Kakvoća tla - Određivanje sadržaja karbonata - Volumetrijska metoda. Hrvatski zavod za norme, Zagreb

HRN ISO (14235:1994.). Kakvoća tla - Određivanje organskog ugljika sulfokromnom oksidacijom Hrvatski zavod za norme, Zagreb

HRN ISO (10390:2005.). Kakvoća tla - Određivanje pH-vrijednosti. Hrvatski zavod za norme, Zagreb.

Japundžić-Palenkić B. (2016.). Usporedba metoda određivanja kationskog izmjenjivačkog kapaciteta tala istočne hrvatske, Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti, Osijek, 1-33

Jones J.B. (2001.). Laboratory Guide for Conducting Soil Tests and Plant Analysis. Boca Raton, London, New York, Washington D.C., CRC Press, 79-93

Ketterings Q., Reid S., Rao R. (2007.). Cation Exchange Capacity (CEC), Nutrient Management Spear Program, Cornell Nutrient Analysis Laboratory, Cornell Universtiy Cooperative Extensio, 20-45

Škorić A. (1991.). Sastav i svojstva tla; Pedološko i biljnoekološko značenje, Fakultet poljoprivrednih znanosti, Zagreb, 5-29

Tomašić M., Zgorelec Ž., Jurišić A., Kisić I. (2013.). Cation Exchange Capacity of Dominant Soil Types in the Republic of Croatia, Journal of Central European Agriculture, 2013, 14(3), 84-98

Vukadinović V., Vukadinović, V. (2016.). Tlo, gnojidba i prinosi, Što uspješan poljoprivrednik mora znati o tlu, usjevima, gnojidbi i tvorbi prinosa, Poljoprivredni fakultet Osijek, Osijek, 282

Vukadinović V., Bertić B. (1998.). Praktikum iz agrokemije i ishrane bilja, Poljoprivredni fakultet Osijek, Osijek, 7-46

Vukadinović V., Lončarić Z. (1998.). Ishrana bilja, Poljoprivredni fakultet Osijek, Osijek, 25-34

Zebec V., Rastija D., Kerovec D., Semialjac Z., Lončarić Z. (2015.). Cation exchange capacity of some acid soils in Eastern Croatia, 9th International Symposium on Plant-Soil Interactions at Low pH, Lončarić, Zdenko ; Kochian, Leon (ur.). Osijek: Poljoprivredni fakultet u Osijeku, 36-36

Determination of cation exchange capacity on eastern croatiasSoils by ammonium acetate method

Abstract

Cation exchange capacity (CEC) is one of the most important indicators of soil productivity and fertility and an important indicator for assessing the physico-chemical condition of soil. The measurement of CEC in laboratories is usually not performed by direct methods, but is calculated on the basis of chemical methods, ie the extracted amount of base cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}). The study included 4 soil types (leached soil, eutric brown soil, marsh black soil and alluvial soil) whose samples were collected at 60 locations in Eastern Croatia, from the arable horizon (0-30 cm). Standard laboratory analyzes were performed on the collected samples in order to determine the actual and substitution acidity, organic matter content, clay content, hydrolytic acidity and base cations extracted with ammonium acetate method. The conducted research determined a distinct heterogeneity of values in all soil properties and types. The average determined amounts of humus in the soil ranged from 1.97 to 4.94%, substitution acidity from 4.22 to 7.48 pH units, clay content from 7.14 to 34.92%, and average CEC values from 14.16 on leached soil to 36.98 cmol (+) kg^{-1} on marsh black soil. The determined cation composition on the adsorption complex is Na<K<Mg<Ca.

Key words: base cations, indirect method, soil type

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Agroekonomika i ruralni razvoj

02

Agricultural Economics and Rural Development

PREGLEDNI RAD

Cjenovna konkurentnost smještaja u ruralnim područjima

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Sažetak
Ruralni turizam se često spominje kao jedan od pridonositelja moguće gospodarske i demografske revitalizacije Republike Hrvatske. Međutim, brojna istraživanja pokazuju kako ovaj oblik turizma zaostaje za onim obalnim u Republici Hrvatskoj, a razlog je nedovoljna iskorištenost brojnih i raznolikih prirodnih resursa koji se nude na tome području. Cilj ovoga rada je usporediti ponudu smještaja cijena u ruralnim područjima Osječko-baranjske županije i središnje Istre i u ruralnim područjima Republike Hrvatske i njezinih susjednih zemalja (Mađarske, Slovenije, Srbije i Bosne i Hercegovine). Rezultati istraživanja pokazali su kako su smještaji u OBŽ skuplji u odnosu na središnju Istru, dok je u usporedbi sa susjednim zemljama Republika Hrvatska na drugom mjestu, odmah iza Slovenije.

Ključne riječi: ruralni turizam, smještaj, Osječko-baranjska županija, središnja Istra, Republika Hrvatska

Uvod

Turizam ima vrlo značajnu ulogu u modifikaciji ruralnih prostora jer kroz svoje izravne i neizravne utjecaje, generira značajne efekte, kao što su dodatni prihodi lokalnog stanovništva, priljev deviznih sredstava, povećanje investicija i zapošljavanja, čime potiče i osnažuje razvoj lokalnog gospodarstva. Ruralni turizam je najširi pojam koji obuhvaća sve turističke usluge / aktivnosti / vidove turizma unutar ruralnih područja, uključujući seoski, lovni, ribolovni, turizam u parkovima prirode, zimski, seoski, ekoturizam, zdravstveni, kulturni itd. Ruralni turizam nije nužno dopunska djelatnost na svojem gospodarstvu kojom se stvara dodatni prihod, već može biti i profesionalna djelatnost kao što su to npr. mali obiteljski hotel, konjički centar itd. (Baćac, 2011.). Ruralna područja najčešće obilježava vrlo bogata prirodna, kulturna i tradicijska baština, ali i problemi depopulacije i napuštanja tradicionalnih djelatnosti i proizvodnje. Iz toga razloga, danas ruralne prostore najčešće karakterizira malen broj uglavnom starijeg stanovništva, malena poljoprivredna obiteljska gospodarstva i nerazvijena infrastruktura. Prema definiciji, ruralni turizam ostvaruje se u različitim oblicima među kojima se posebno ističe seoski turizam (agroturizam) na seljačkim gospodarstvima. Osnovne djelatnosti u ruralnom turizmu su ugostiteljstvo (pripremanje hrane i pružanje usluga prehrane, pripremanje i usluživanje pića i napitaka i pružanje usluga smještaja te pripremanje hrane za potrošnju na drugom mjestu i opskrba tom hranom) i turistička djelatnost (pružanje usluga u turizmu) (Demonja i Ružić, 2010.). Za pokretanje i razvoj agroturizma dostupna su bespovratna sredstva iz EU fondova i to u sklopu Mjere 6 „Razvoj poljoprivrednih gospodarstava i poslovanja“, Podmjere 6.2 „Potpora ulaganju u pokretanje nepoljoprivrednih djelatnosti u ruralnim područjima“ i Podmjere 6.4 “Ulaganja u stvaranje i razvoj nepoljoprivrednih djelatnosti”. Osim što im je cilj omogućiti mladima zapošljavanje i izvan poljoprivrednih zanimanja, ove podmjere potiču i cjenovnu konkuretnost objekata. Korisnici podmjera mogu biti poljoprivredna gospodarstva upisana u Upisnik poljoprivrednih gospodarstava te fizičke osobe u svojstvu nositelja ili člana

obiteljskog poljoprivrednog gospodarstva koji pokreću novu nepoljoprivrednu djelatnost u ruralnim područjima.

Materijal i metode

Cilj ovoga rada je usporediti ponudu smještaja cijena u ruralnim područjima Osječko-baranjske (u daljnjem tekstu OBŽ) i Istarske županije i u ruralnim područjima Republike Hrvatske i njezinih susjednih zemalja (Mađarske, Slovenije, Srbije i Bosne i Hercegovine) te skrenuti pažnju na mogućnosti i potrebne mjere koje bi dovele do veće potražnje za smještajem u ruralnome području Republike Hrvatske. U radu su korišteni sekundarni izvori podataka. Na temelju podataka sa stranica za oglašavanje smještaja (booking.com) primijenjena je metoda analize i komparacije za prikaz kretanja i strukture cijena smještaja u ruralnim područjima OBŽ-a, središnje Istre, Slovenije, BIH, Srbije i Mađarske. Svi analizirani podaci odnose se na cijene smještaja u 2022. godini, a analizirana su tri godišnja doba, točnije mjeseci travanj, srpanj i listopad.

Rezultati i rasprava

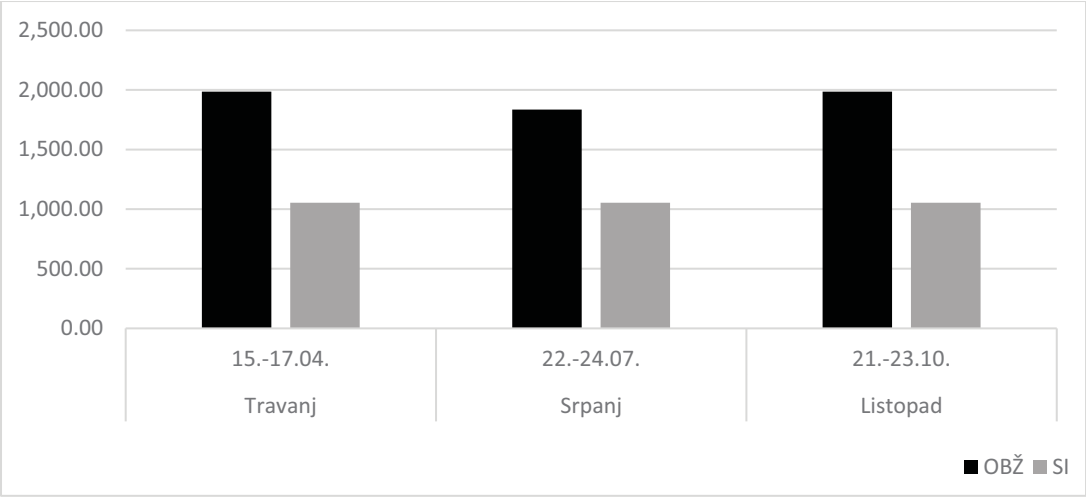
Nalaženje i odabir smještaja u ruralnom području za ovo istraživanje provedeno je na temelju podataka sa stranice booking.com. U radu su analizirane i uspoređene cijene smještaja klasificirani kao apartmani, kuće/ville te bed&breakfast ponude u OBŽ u odnosu na središnju Istru, a analizira se jedna vrsta objekta iz svake skupine analiziranih objekata. Kriterij po kojima su se izabrali smještaji je ponuda sadržaja. Također, analizirane su i uspoređene cijene smještaja klasificirani kao apartmani i kuće za odmor u ruralnom području u Hrvatskoj u odnosu na Sloveniju, Bosnu i Hercegovinu, Mađarsku i Republiku Srbiju. Prema autorima Song i sur. (2010.) na turističku potražnju mogu utjecati brojni čimbenici: cijene, stavovi potrošača, političko okruženje i slično, ali odlučujuću ulogu ima cijena koja definira turističku potražnju kroz raspoloživi dohodak pojedinca.

Usporedba cijene smještaja između OBŽ i središnje Istre

U usporedbi cijene smještaja u OBŽ i središnjoj Istri kao prvi primjer uzeti su apartmani za četiri osobe. Ukupan broj noćenja je dva, a promatrani termini su od 22. travnja do 24. travnja (proljeće), od 22. srpnja do 24. srpnja (ljeto) i od 21. listopada do 23. listopada (jesen).

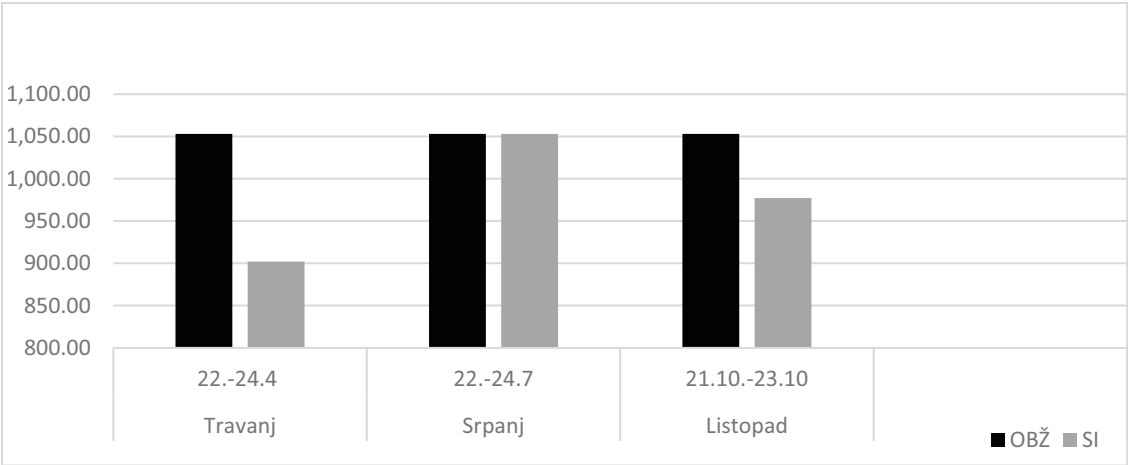
Za OBŽ kao primjer korišten je „Apartment Tri Mudraca“ koji se nalazi u mjestu Karanac, svega 1,1 km od etno sela Karanac. Sadrži dvije spavaće sobe, jednu kupaonicu, blagovaonicu, potpuno opremljenu kuhinju i terasu s pogledom na vrt koji je na raspolaganju gostima kao i besplatan WiFi. Cijena na stranici booking.com za četiri osobe u proljeće i jesen iznosi 1.985,00 HRK, dok je preko ljeta 1.835,00 HRK. S druge strane, analiziran je u središnjoj Istri „Apartman Snješka“ koji se nalazi u Buzetu, na 28 km od Opatije. Sadrži dvije spavaće sobe, jednu kupaonicu, kuhinju, dnevni boravak kao i balkon s pogledom na vrt koji je na raspolaganju gostima kao i besplatan WiFi. Cijena za četiri osobe jednaka je cijelu godinu i iznosi 1.053,00 HRK u 2022. godini.

U Grafikonu 1 možemo vidjeti kako je u svim promatranim razdobljima cijena apartmana bila veća u OBŽ. Kada je riječ o cijenama apartmana u OBŽ možemo primijetiti kako su one različite po sezonama. Tako je u proljeće i jesen ona nešto veća u odnosu na ljetni period. Također, dolazimo do pretpostavke kako se vlasnici odlučuju u ljetnom periodu na nižu cijenu jer znaju da će se većina ljudi odlučiti na boravak u obalnom dijelu Hrvatske pa nižom cijenom žele pridobiti onaj dio turista koji je neodlučan ili pak želi svoj odmor iskoristiti u kontinentalnom dijelu zemlje.



Grafikon 1. Usporedba cijene apartmana u OBŽ u odnosu na središnju Istru

Kao drugi primjer, u radu su uspoređene cijene bed&breakfast ponude između OBŽ i središnje Istre. Grafikon 2 prikazuje razlike u cijenama ponude apartmani Ivica i Marica (OBŽ) koji se nalaze u Karancu, 30-tak kilometra od Osijeka. Gostima je osim apartmana na raspolaganju vrt i besplatan WiFi kao i doručak u okviru kojeg se poslužuju domaća jela. Cijena na stranici booking.com dvokrevetne sobe s bračnim krevetom i privatnom kupaonicom + doručak (domaća jela) za dvije osobe jednaka je za sva promatrana razdoblja (travanj, srpanj i listopad) i iznosi 1.053,00 HRK. Za usporedbu u središnjoj Istri odabran je objekt Skalameri, u Kozljaku koji se nalazi 20-tak km od Opatije. Isto kao i kod prethodnog, gostima je osim apartmana na raspolaganju vrt i besplatan WiFi kao i doručak u okviru kojeg se poslužuju domaća jela. Cijena je prema booking.com-u kod ovoga smještaja po sezonama drugačija. Tako je u proljeće 902,00 HRK, u ljeto 1.053,00 HRK, dok u jesen iznosi 977,00 HRK. Iz grafikona se može vidjeti kako su cijene kao i u prethodnom primjeru veće u OBŽ za proljetno i jesensko razdoblje, dok su u ljetnom periodu jednake. Također, možemo primijetiti kako su cijene ponude u središnjoj Istri mijenjaju po sezonama, a najviše su u ljetnom periodu.

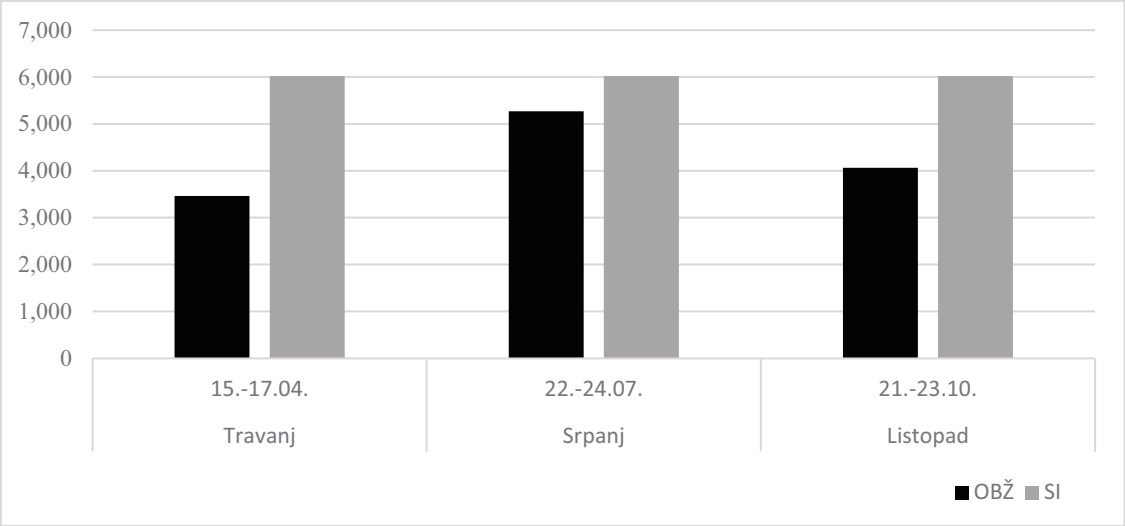


Grafikon 2. Usporedba cijene ponude B&B u OBŽ u odnosu na središnju Istru

Idući su primjer kuće/vile za odmor s bazenom. U OBŽ kao primjer uzet je objekt Sport's Resort. Nalazi se u Feričancima, a nudi klimatiziran smještaj s pogledom na jezero i popločanim dijelom dvorišta, sezonski vanjski bazen, sadržaje za vodene sportove te

besplatan WiFi. Mlađi gosti mogu se zabaviti na dječjem igralištu. Za središnju Istru odabrana je Istria Estate. Objekt se nalazi u Roču te u ponudi ima smještaj s vlastitim bazenom, pogledom na planinu i popločanim dijelom dvorišta. U okviru objekta gostima su također na raspolaganju besplatan WiFi i privatno parkiralište.

Na Grafikonu 3 prikazano je kretanje cijena objekata po sezonama te je uspoređeno koliko su one više/niže u jednoj u odnosu na drugu ponudu. Cijena Sport's Resort-a za osam osoba za dva noćenja u proljeće iznosi 3.461,00 HRK, u ljeto 5.267,00 HRK, a u jesen 4.063,00 kuna (booking.com). S druge strane, cijena Istria Estate-a za osam osoba za dva noćenja jednak je u svim terminima i iznosi 6.019,00 HRK u 2022. godini. Iz prikazanog grafa možemo vidjeti kako su cijene u Istri nešto više, čak gotovo duplo više u travnju u odnosu na objekt u OBŽ.

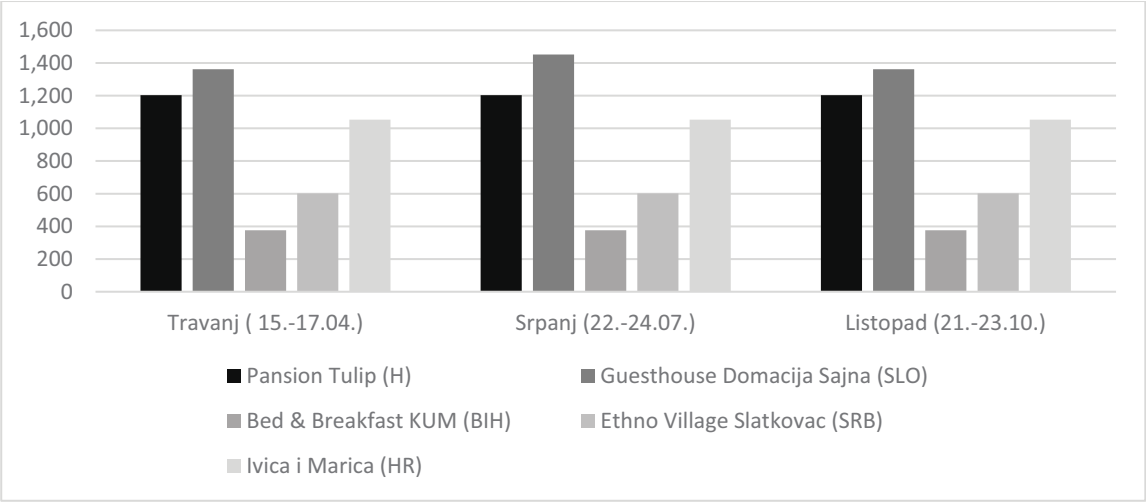


Grafikon 3. Usporedba cijena kuće/vile za odmor u OBŽ u odnosu na središnju Istru

Usporedba cijene smještaja u ruralnom području Republike Hrvatske s BIH, Srbijom, Mađarskom i Slovenijom

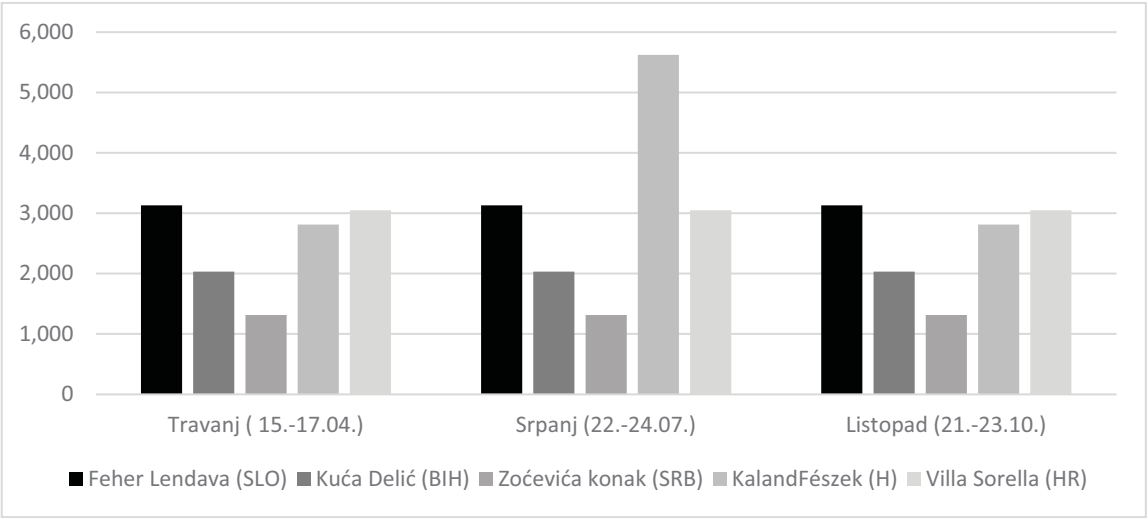
Drugi dio rada odnosi se na usporedbu cijena smještaja u ruralnom području Hrvatske u odnosu na Bosnu i Hercegovinu, Srbiju, Mađarsku i Sloveniju. Kao prvi primjer uspoređene su cijene bed&breakfast ponude Apartmana Ivica i Marica (u svim sezonama 1.053,00 HRK) s Pansion Tulip iz Mađarske, zatim slovenskim Guesthouse Domacija Sajna, Bed & Breakfast KUM iz BIH te Ethno Village Slatkovac iz Srbije. Promatrano razdoblje također je isto kao i kod prethodnog istraživanja, cijena je za dvije osobe i uključuje doručak. Pansion Tulip je za sva promatrana razdoblja jednak, točnije 1.203,00 HRK, Guesthouse Domacija Sajna je od u proljeće i jesen 1.362,00 HRK, a preko ljeta 1.452,00 HRK, Bed & Breakfast KUM ima istu cijenu koštanja u cijeloj sezoni, 376,00 HRK kao i Ethno Village Slatkovac koje je 602,00 HRK. U svim navedenim objektima na raspolaganju je besplatno privatno parkiralište kao i besplatni WiFi.

Iz prikazanog grafikona možemo vidjeti kako su cijene smještaja u Sloveniji najviše, dok su one u BIH najniže.



Grafikon 4. Usporedba cijene ponude B&B u RH u odnosu na Mađarsku, Srbiju, BIH i Sloveniju

Cijena kuće za odmor s bazenom drugi je primjer koji je analiziran i uspoređen između navedenih zemalja. Podaci o cijenama također su preuzeti sa stranice booking.com. Kao slovenski primjer u radu je korišten objekta Feher Lendava, bosanski Kuća Delić, Zoćevića konak iz Srbije te kao mađarski primjer KalandFészek (Avanturističko gnijezdo). Svi navedeni smještaji uspoređeni su s cijenama hrvatskog smještaja Ville Sorella. U svim navedenim objektima gostima je na raspolaganju kuhinja, dnevni boravak, vrt, privatno parkiralište, bazen, oprema za roštilj i besplatan WiFi. Cijena noćenja u kući Feher Lendava koja se nalazi u Lendavi, Sloveniji za četiri odrasle osobe i dva noćenja u svim terminima je jednaka i iznosi 3.129,00 HRK, Kuća Delić u Račićima, BIH također ima istu cijenu 2.033,00 HRK, kao i Zoćevića konak, Petrovići, Srbija 1.314,00 HRK, dok je cijena u mađarskom KalandFészek-u (Avanturističko gnijezdo) koje se nalazi u Mecsekszakáli utca u proljeće i jesen 2.812,00 HRK, dok je preko ljeta duplo veća i iznosi 5.624,00 HRK. Villa Sorella u Karancu, Hrvatska u svim navedenim terminima cijena smještaja iznosi 3.048,00 HRK. U promatranim razdobljima slovenski smještaj u travnju i listopadu ima najveću cijenu, dok u srpnju s cijenom prednjači Mađarska i to za duplo više u odnosu na ostale smještaje.



Grafikon 5. Usporedba cijene ponude kuće/vile za odmor u RH u odnosu na Mađarsku, Srbiju, BIH i Sloveniju

Kako navode autori Sudarić i sur. (2021.) veću cijenu svojih usluga gospodarstva bi mogla ponuditi osmišljavnjem paketa usluga. Jedinstvena i prepoznatljiva ponuda koja bi obuhvaćala smještaj, hranu i druge aktivnosti utjecala bi na formiranje ponude doživljaja gdje se cijene ne iskazuju pojedinačno za svaku uslugu koja je sadržana u paketu, već kroz ukupnu cijenu.

Zaključak

Brojna istraživanja pokazuju kako ruralni turizam zaostaje za obalnim turizmom u Republici Hrvatskoj, a razlog je nedovoljna iskorištenost brojnih i raznolikih prirodnih resursa koji se nude na tome području. Ipak, „skok“ u razvoju, primjetan je 2020. godine pojavom pandemije Covid-19 te primoranim zatvaranjem u svoje županije koje je potaknulo ljude na boravak u prirodi i želju za odmorom u smještajima na selu. Rezultati usporedbe OBŽ sa središnjom Istrom pokazali su kako su cijene objekata, osim kuća/vila za odmor, veće u OBŽ u odnosu na one u Istri. Razlog tome je što vlasnici vjerojatno nižom cijenom žele privući turiste i u središnji dio Istre jer polaze od pretpostavke da turisti koji idu u ovaj dio Hrvatske radije uzimaju apartmane bliže ili uz more nego u unutrašnjosti. Cijene kuće/vila za odmor nešto su veće nego one u OBŽ, a razlog tome je što takvi objekti između ostaloga sadrže i bazen te time konkuriraju onima koji se nalaze na moru, a nalaze se u mirnijem mjestu okruženi prirodom. Kada je riječ o usporedbi cijene smještaja u ruralnom području Republike Hrvatske s BIH, Srbijom, Mađarskom i Slovenijom, najveće cijene imaju smještaji u Sloveniji što ni ne čudi jer ova zemlja već godinama pokazuje visoku razinu uspješnosti u razvoju ruralnog turizma. S druge strane, najmanju cijenu imaju smještaji u BIH što također nije iznenađujuće s obzirom na njihov životni standard, ali i razvijenost ponude ruralnog turizma. Iz analiziranog stanja, Hrvatska se po cijeni smještaja nalazi odmah iza Slovenije, a ispred Mađarske. Iz svega navedenog vidljivo je kako cjenovno i sadržajem ruralni smještaji u Hrvatskoj mogu konkurirati navedenim susjednim zemljama, no prostora za razvoj ima na pretek s obzirom na to da je samo 2 % od ukupno 93 % koliko zauzima ruralni prostor zastupljeno ovim vrstom turizma. Također, bolja organizacija marketinških djelatnosti približila bi ruralna područja prema većem tržištu, što bi direktno utjecalo na modeliranje cijena prema kvaliteti, ali i boljoj ekonomskoj stabilnosti stanovništva koje se bave turizmom.

Literatura

Baćac R. (2011). Seoski turizam: pojašnjenje pojmova i termina. Objavljeno u Priručnik za bavljenje seoskim turizmom, Mujo I., Gredičak T., Škunca O. (ur.), 16-17. Zagreb, Hrvatska: Ministarstvo turizma Republike Hrvatske.

Demonja D., Ružić P. (2010). Ruralni turizam u Hrvatskoj, s hrvatskim primjerima dobre prakse i europskim iskustvima, 12-16. Zagreb, Hrvatska: Meridijani.

Song H., Kim J.H., Yang S. (2010). Confidence intervals for tourism demand elasticity. Annals of Tourism Research. 37 (2): 377-396.

Sudarić T., Zmaić K., Deže J. (2018). Identifikacija i vrednovanje činitelja razvoja ruralnog turizma istočne Hrvatske. Objavljeno u Zbornik radova 4. međunarodnog kongresa o ruralnom turizmu, Smoločić Jurdana D., Milohnić I. (ur.), 267-277. Rijeka, Hrvatska: Sveučilište u Rijeci, Fakultet za menadžment u turizmu i ugostiteljstvu.

www.booking.com (pristup 11.-15.01.2022.)

Price competitiveness of accommodation in rural areas

Abstract
Rural tourism is often mentioned as one of the contributors to the possible economic and demographic revitalization of Croatia. However, numerous studies show that this form of tourism lags behind the coastal one in Croatia, and the reason is the insufficient use of numerous and diverse natural resources offered in this area. The aim of this research is to compare the offer of price accommodation in rural areas of Osijek-Baranja County and central Istria and in rural areas of Croatia and its neighboring countries (Hungary, Slovenia, Serbia and Bosnia and Herzegovina). The results of the research showed that accommodation in Osijek-Baranja County is more expensive than in central Istria, while in comparison with neighboring countries, Croatia is in second place, just behind Slovenia.

Key words: rural tourism, accommodation, Osijek-Baranja County, central Istria, Croatia

IZVORNI ZNANSTVENI RAD

Bruto marža proizvodnje mlijeka u Hrvatskoj

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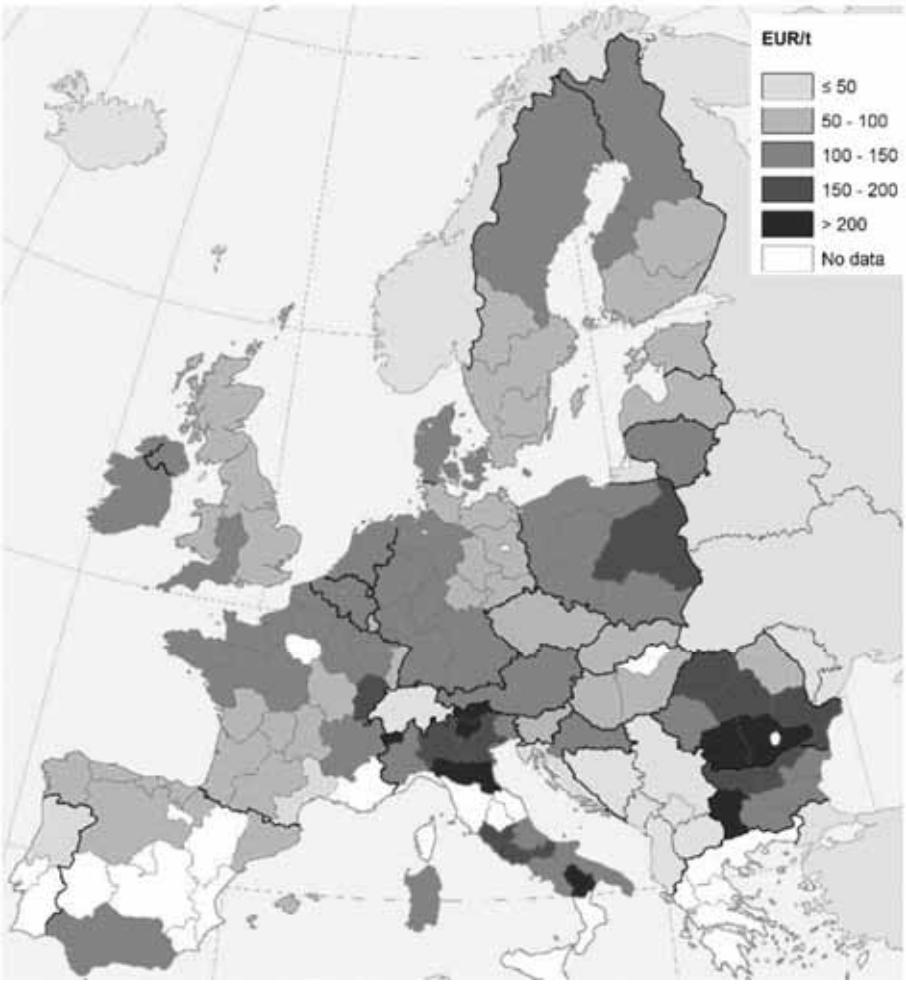
Sažetak
U radu je analizirana vrijednost marže prema ekonomskim veličinama farme i proizvedenoj količini mlijeka. U istraživanom uzorku bruto marža proizvodnje mlijeka, analizirana po grlu stoke, pokazuje pravilno povećanje srednjih vrijednosti s rastom broja grla, odnosno prema ekonomskim veličinama poljoprivrednih gospodarstava u skupinama. Slično se ponašaju i maksimalne bruto marže po skupinama gospodarstava, dok za gospodarstva s minimalnim maržama zakonitost ekonomije razmjera ne vrijedi potpuno, budući je u skupini od 50 do 100 tisuća eura utvrđena niža vrijednost nego u dvije skupine manje veličine.

Ključne riječi: bruto marža, proizvodnja mlijeka, FADN

Uvod

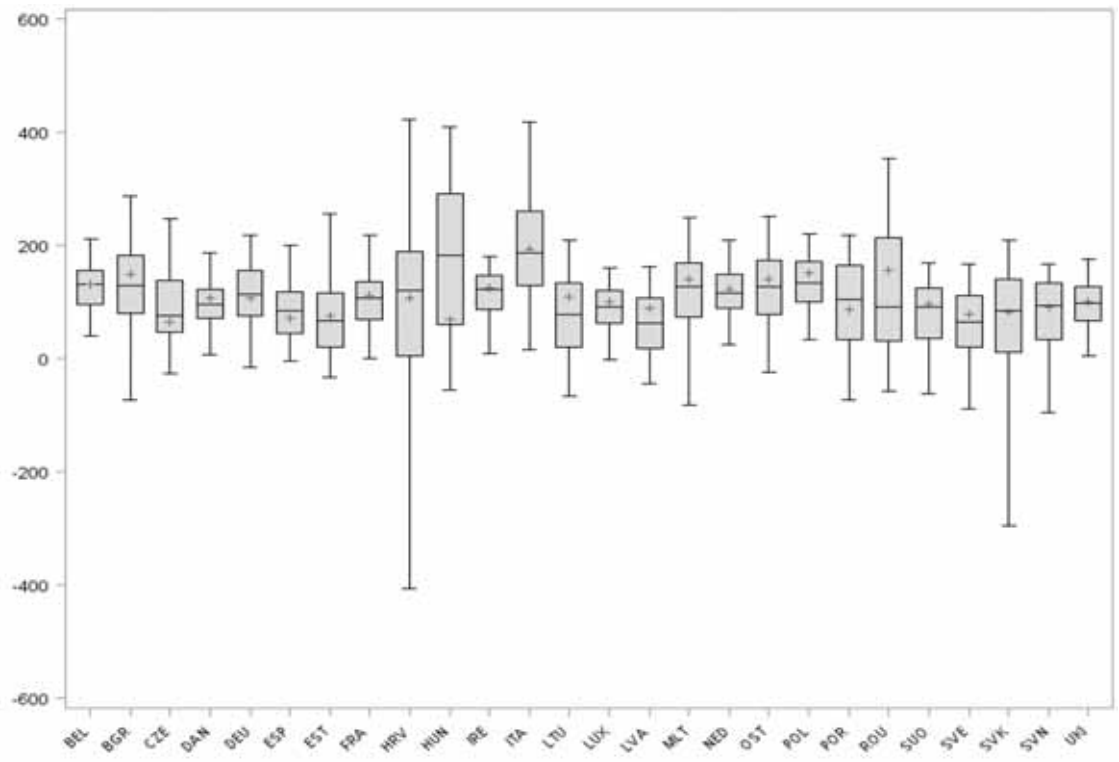
Kretanja marži u proizvodnji mlijeka po članicama EU prikazana su prema EU Dairy report based on FADN. U razdoblju 2020. - 2021. godine marže za Italiju (194 € t^{-1}) bile su daleko najviše u EU. Slijedila je Rumunjska (154 € t^{-1}), koja je unatoč niskoj cijeni mlijeka imala velike koristi od dobrovoljnih plaćanja. Operativni troškovi u Rumunjskoj također su bili među najnižima u EU. Poljska i Bugarska imale su slične vrijednosti prihoda od mlijeka i operativnih troškova kao i Rumunjska, a njihove bruto marže bile su odmah iza Rumunjske sa 151 i 150 € t^{-1}. Bruto marža s povezanim plaćanjima je na Malti i u Austriji na razini od 140 € t^{-1}. U Belgiji, koja slijedi odmah iza ove dvije države članice, ne samo da je cijena pala od 2017. do 2018., nego su i troškovi porasli uzrokujući pad bruto marže od 21 % (na 134 € t^{-1}). U Irskoj, Nizozemskoj i Francuskoj bruto marže su također smanjene u odnosu na prethodnu 2019. godinu (za 10-21 %), ali su i dalje bile zadovoljavajuće u usporedbi s ostalim državama članicama. U Litvi (109 € t^{-1}) prosječna bruto marža s vezanim plaćanjima smanjena je u 2018. u odnosu na 2017. zbog pada cijene mlijeka i povećanja operativnih troškova. Međutim, pad je donekle ublažen povećanjem vezanih plaćanja. U Latviji (89 € t^{-1}) je situacija bila slična, uz značajnu razliku u razini troškova, što je rezultiralo maržom za 20 €/t manjom nego u susjednoj državi članici. Estonija je zabilježila sličnu razinu prihoda kao Litva i Latvija (povezana potpora bila je marginalna, samo 7 € t^{-1}), no njezini su troškovi bili najveći među ove tri zemlje članice, pa je stoga njena bruto marža iznosila samo 76 € t^{-1}. Finska i Hrvatska bile su države članice s najvećom netržišnom potporom proizvodnji mlijeka u 2018. (84 € t^{-1}, odnosno 69 € t^{-1}). Hrvatska je uspjela održati razine prihoda i operativnih troškova slične prethodnoj godini, te su stoga marže bile tek nešto manje od 109 € t^{-1}. Slična razina bruto marže s povezanim plaćanjem, između 107-100 € t^{-1}, zabilježena je u Finskoj, Danskoj, Njemačkoj, Luksemburgu i Ujedinjenom Kraljevstvu. Zajednički je čimbenik bio da su prihodi za sve njih ovisili isključivo o cijenama mlijeka, jer nisu imali koristi od netržišne potpore u obliku dobrovoljno vezanih potpora.



Slika 1. Prosječna bruto marža s povezanim plaćanjima po regijama FADN-a u 2018. (€ t^{-1} mlijeka)
Izvor: https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/fadn-dairy-report-2021_en.pdf

Prosjeci EU ne otkrivaju velike razlike među farmama po zemljama članicama i regijama unutar pojedinih zemalja. Neke zemlje, kao npr. Rumunjska s relativno visokim prosječnim bruto maržama jednog dijela proizvođača, imaju niže srednje bruto marže u odnosu na EU. To znači da više od polovice farmi spada ispod prosječne bruto marže EU. Nasuprot tome, u drugim državama članicama kao što je Hrvatska prosjek EU je niži od medijana, odnosno više od polovice poljoprivrednih gospodarstava dostiže prosječnu bruto maržu. Hrvatska također ima najširi raspon bruto marže od oko -400 do preko 400 € t^{-1}.

Mađarska i Češka imaju najniže bruto marže u razdoblju 2018. - 2021. godine, a obje ove članice imaju relativno niske razine specijalizacije (64 % u Češkoj i 80 % u Mađarskoj u odnosu na prosjek EU-28 od 93 %), pa su njihovi operativni troškovi visoki u odnosu na prihod od mlijeka. Njihovi prihodi nisu niski u usporedbi s drugim državama članicama i imaju značajne koristi od dobrovoljno vezanih potpora, ali troškovi umanjuju dobit, smanjujući marže na jedva 72 € t^{-1} u Mađarskoj i 65 € t^{-1} u Češkoj.



Grafikon 1. Ponderirane vrijednosti bruto marže s povezanim plaćanjima po zemljama članicama EU (€ t^{-1}mlijeka)
Izvor: https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/fadn-dairy-report-2021_en.pdf

Materijal i metode

Podaci su dobiveni anketiranjem 1.717 poljoprivrednih gospodarstava s proizvodnjom mlijeka tijekom razdoblja 2017. - 2019. godine, pri čemu su za analizu korišteni podaci o veličini farme, proizvodnji mlijeka, prihodima i varijabilnim troškovima proizvodnje. Najveći broj gospodarstava (458) je s područja Bjelovarsko-bilogorske županije, a slijede Sisačko-moslavačka (210), Vukovarsko-srijemska (202) i Osječko-baranjska županija (144), što odgovara razmještaju isporučitelja mlijeka u Hrvatskoj. Ukupni broj farmi i grla na njima čini gotovo 1/3 ukupnog broja stada i krava pod kontrolom mliječnosti koju provodi Hrvatska agencija za poljoprivredu i hranu. Farme su veličine od 5 do 300 grla, a u analizi su grupirane po visini prihoda (do 10, 10-20, 20-50, 50-100 i preko 100 tisuća €). Bruto marža kao pokazatelj usporedbe gospodarskog položaj i konkurentnosti domaćih proizvođača je izračunata prema shemi u Tablici 1.

Tablica 1. Shema izračuna doprinosa pokriću

Ukupno prihod (UP)	Ukupno varijabilni troškovi (VT)
+Vrijednost proizvodnje mlijeka	+Vlastita stočna hrana (trošak inputa: sjeme, gnojivo, zaštitna sredstva…)
+Izravna plaćanja za proizvodnju mlijeka	+Kupljena stočna hrana
	+Veterinarske usluge i lijekovi
+Prihodi izvan proizvodnje mlijeka	+Usluge rada ljudi i strojeva
	+Troškovi vlastite mehanizacije (trošak goriva i tekućeg održavanja)
Doprinos pokrića (marža profita, bruto marža pokrića) = UP-VT	

Rezultati i rasprava

Promatrano po grlu, bruto marža proizvodnje mlijeka ispitivanih gospodarstava pokazuje pravilno povećanje srednjih vrijednosti s povećanjem broja grla, odnosno po skupinama gospodarstava prema njegovoj ekonomskoj veličini. Slično se ponašaju i maksimalne bruto marže po skupinama ekonomskih veličina, dok za gospodarstva s minimalnim maržama ne vrijedi zakonitost ekonomije razmjera, budući je u skupini od 50-100 tisuća eura zabilježena niža vrijednost nego u dvije skupine manje veličine. Analizirano s obzirom na količinu proizvedenog mlijeka (po toni) utvrđene su promjene koje se mogu objasniti samo velikim odstupanjima u proizvodnji mlijeka po grlu kod ispitivanih farmi. S obzirom da su prosječne proizvodnje mlijeka po skupinama od 4.100 do 6.900 kg/grlu/god., a odstupanja unutar skupina od minimalnih do maksimalnih proizvodnji od 700 do čak 3.800 kg/grlu/god., jasne su i razlike unutar i između skupina. Odstupanja od minimuma do maksimuma su manja kako se povećava ekonomska vrijednost skupine unatoč povećanju količine mlijeka po grlu unutar njih.

Tablica 2. Bruto marže proizvodnje mlijeka po ekonomskoj veličini poljoprivrednog gospodarstva (u € t^{-1})

Ekonomska veličina (1000 €)		do 10	10-20	20-50	50-100	preko 100
Prosječni broj grla na farmi		7	14	21	32	67
Po farmi						
Proizvodnja mlijeka (lit)	min	19.000	36.000	65.000	130.000	250.000
	max	45.000	96.000	162.500	337.500	880.000
	srednja vrijednost	28.700	64.400	113.400	198.400	462.300
Bruto marža	min	3.060	5.226	8.939	18.531	41.824
	max	8.931	19.457	24.321	77.223	134.381
	srednja vrijednost	5.510	11.391	17.504	27.659	72.743
Po grlu						
Proizvodnja mlijeka (lit)	min	3.800	4.500	5.000	5.000	5.000
	max	4.500	4.800	6.500	7.500	8.800
	srednja vrijednost	4.100	4.600	5.400	6.200	6.900
Bruto marža	min	611,96	653,21	687,59	712,73	836,48
	max	893,09	952,84	972,84	1.716,07	1.343,81
	srednja vrijednost	787,08	813,66	833,52	864,36	1.085,72
Po toni mlijeka						
Bruto marža	min	161,04	145,16	137,52	139,41	152,71
	max	198,46	202,67	154,36	228,81	167,30
	srednja vrijednost	191,97	176,88	149,67	142,55	157,35

Vrijednosti maksimalne bruto marže su kod istraživanih gospodarstava dvostruko manje nego je to u FADN izvješćima EU, pri čemu su maksimalne vrijednosti kod svih skupina

ekonomskih veličina osim one od 20-50 tisuća (od oko 20 do nešto manje od 50 grla u stadu) veće od one preko 100 tisuća eura. Istodobno, srednje vrijednosti pokazuju bolji gospodarski položaj i konkurentnost manjih i srednjih veličina farmi. Gotovo sva gospodarstava se nalaze iznad EU prosjeka, jer su i najniže zabilježene bruto marže veće od prosječnih u EU. Preliminarni podaci o neto marži (bruto marža umanjena za fiksne troškove uključujući naknadu za rad) ukazuju na negativne vrijednosti, odnosno gubitke kod proizvođača u svim skupinama do ekonomske veličine od 50 tisuća eura (ili do 30 grla u stadu i niže proizvodnosti), a nakon toga sve farme u svim razinama proizvodnje po grlu ostvaruju dobit.

Zaključak

Bruto marža ili doprinos pokrića je jedno od osnovnih mjerila gospodarske sposobnosti i konkurentnosti poljoprivrednih gospodarstava EU korištenjem FADN sustava. Analiza bruto marže u istraživanim obiteljskim gospodarstvima pokazuje manja odstupanja bruto marže promatrano po ekonomskim veličinama gospodarstva po količinama proizvedenog mlijeka (t), ali veća odstupanja promatrano po grlu, jer su i veće razlike u proizvodnosti grla. Negativne bruto marže, odnosno gubici se u istraživanim gospodarstvima javljaju do ekonomske veličine od 50.000 eura. Razlike i odstupanja minimalnih i maksimalnih bruto marži po skupinama proizvođača uzrokovane su odstupanjima u visini proizvodnje mlijeka po grlu, a unatoč najvećim razlikama proizvodnosti kod ekonomski najveće veličine gospodarstva su odstupanja između minimalnih i maksimalnih iznosa marži najmanja.

Napomena

U radu su korišteni podaci anketnog ispitivanja za doktorski rad dr.sc. Mate Čačića na poslijediplomskom doktorskom studiju iz Ekonomike poljoprivrede na Agronomskom fakultetu Sveučilišta u Zagrebu. Anketni upitnik izrađen je u Zavodu za menadžmentu i ruralno poduzetništvo Agronomskog fakulteta Sveučilišta u Zagrebu (izv.prof.dr.sc. Branka Šakić Bobić).

Literatura

European Milk Board (2021). What is the cost of producing milk? Available from: https://mcusercontent.com/91ae04a80d4a90ffd66b56980/files/16617d79-200a-1b46-a2ef-2fb1284f6ad7/2021_Cost_study_EN.03.pdf

European Commission (2021). EU Dairy Farms Report based on 2018 FADN data. Available from: https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/fadn-dairy-report-2021_en.pdf

European Commission (2021). Milk Market Observatory. Available from: https://ec.Europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eu-milk-prices-margins_en.pdf

European Commission (2020). EU Agricultural and Farm Economics Briefs. Available from: https://ec.Europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/agri-farm-economics-milk-margin-estimate-2020_en.pdf

Grgić Z., Šakić Bobić B., Očić V. (2005). Prag rentabilnosti proizvodnje mlijeka u različitim proizvodnim sustavima obiteljskih gospodarstava. Stočarstvo. 59(6): 425-432.

Grgić Z., Čačić M., Pavičić Z., Šakić Bobić B. (2020). Osnovni činitelji ekonomike proizvodnje mlijeka na farmama različitog kapaciteta. Objavljeno u Zbornik radova 55. hrvatskog i 15. međunarodnog simpozija agronoma, Mioč B., Širić I. (ur.), 153-156. Vodice, Hrvatska: Agronomski fakultet Sveučilišta u Zagrebu.

Hanisch M., Malvido A., Rommel J., Sagebiel J. (2018). The structural effect of cooperatives on price volatility in the European dairy sector. Applied EconomicsLetters. 25 (8): 576-579.

Gross margin of Croatian dairy production

Abstract
The paper analyzes the value of the gross margin by economic size of the farm and per ton of milk produced. The gross margin of milk production at the surveyed farms observed per head shows a regular increase in mean values with an increase in the number of heads, i.e. by groups of farms according to its economic size. Maximum gross margins by groups of farms behave similarly, while for farms with minimum margins the regularity of economies of scale does not apply completely, since in the group 50-100 thousands euros, lower value was recorded than in two smaller groups.

Key words: gross margin, milk production, FADN

IZVORNI ZNANSTVENI RAD

Ocjena važnosti pojedinih oblika društvene infrastrukture ruralnog područja Hrvatske

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Sažetak

Cilj rada je, korištenjem anketnog istraživanja, ocijeniti pojedine oblike društvene infrastrukture (DI) ruralnog prostora Hrvatske te utvrditi podudarnost ocjena ispitanika s alokacijom sredstva iz Podmjere 7.4. Programa ruralnog razvoja. Ispitanici su važnijom ocijenili društvenu infrastrukturu zdravstvene (ocjena 4,64) i obrazovne namjene (4,65) u odnosu na infrastrukturu sa sportskom (3,68), kulturnom (3,35) i vjerskom namjenom (3,29). U alokaciji potpora iz trećeg natječaja Podmjere 7.4. prevladavaju sredstva za izgradnju i opremanju društvenih domova, sportskih i kulturnih centara pa se ovakva alokacija ne podudara s ocjenama ispitanika.

Ključne riječi: društvena infrastruktura, ruralni razvoj, podmjera 7.4., anketa

Uvod

Infrastruktura je kapitalno intenzivno ulaganje u materijalne resurse i usluge bitne za omogućavanje, odvijanje ili poboljšanje gospodarskih i društvenih uvjeta nekog prostora (Fulmer, 2009.). Infrastruktura se može podijeliti u dvije skupine na: (a) gospodarsku i (b) društvenu ili socijalnu infrastrukturu. Bottini i sur. (2012.) definiraju gospodarsku infrastrukturu kao ulaganje u gospodarske djelatnosti kao što su transport, komunikacije, elektroenergetski i vodni sustav koji poboljšavaju graničnu korisnost drugih oblika fizičkog kapitala, dok je društvena infrastruktura ulaganje koje poboljšava graničnu produktivnost ljudskog kapitala.

Društvena infrastruktura predstavlja različite objekte, sadržaje i usluge iz područja zdravstva, obrazovanja, kulture i drugih društvenih djelatnosti namijenjenih zadovoljavanju društvenih potreba stanovništva. Društvena infrastruktura tako, u materijalnom smislu, uključuje škole, knjižnice, sportska igrališta, crkve, poštanske urede, vrtiće, društvene domove, kulturne i slične objekte. U slučaju ruralne društvene infrastrukture ovi su sadržaji locirani na ruralnom području i njihova prisutnost doprinosi ukupnoj atraktivnosti sela kao mjesta rada i života.

Ciljevi rada su ocijeniti važnost pojedinih oblika društvene infrastrukture na ruralnim područjima Hrvatske i utvrditi u kojoj se mjeri te ocjene podudaraju s alokacijom sredstava iz Programa ruralnog razvoja (Podmjera 7.4.) namijenjenih jačanju društvene ruralne infrastrukture. Pretpostavke istraživanja su:

- gospodarska i društvena infrastruktura podjednako su važne za razvoj ruralnih područja (H1),
- raspoloživost društvene infrastrukture važan je preduvjet odluke o ostanku stanovništva u ruralnom području (H2),
- među društvenom infrastrukturom važnija je ona sa zdravstvenom i obrazovnom namjenom dok je manje važna društvena ruralna infrastruktura s kulturnom i sportskom namjenom (H3),

- škole i dječji vrtići važniji su ispitanicima mlađih dobnih skupina dok su vjerski objekti važniji ispitanicima starije dobne skupine (H4).

Materijal i metode

U radu je dan pregled dosadašnjih istraživanja društvene infrastrukture kao uvod u anketno istraživanje kojim su prikupljeni primarni podaci za potrebe izrade rada. Anketno istraživanje provedeno je online u razdoblju od 1. lipnja do 5. kolovoza 2021. godine na uzorku od 98 ispitanika, stanovnika ruralnih područja Hrvatske. Uzorak ispitanika bio je prigodan i odabran je većim dijelom temeljem osobnih poznanstava autora rada. Pri uzorkovanju nastojalo se u što većoj mjeri obuhvatiti stanovništvo ruralnih područja. Tehnikom „grude snijega" inicijalni ispitanici prosljeđivali su anketu do ostalih sudionika istraživanja. Sekundarni podaci, o rezultatima dosad održanih natječaja za podmjeru 7.4. preuzeti su iz javnih podataka Ministarstva poljoprivrede.

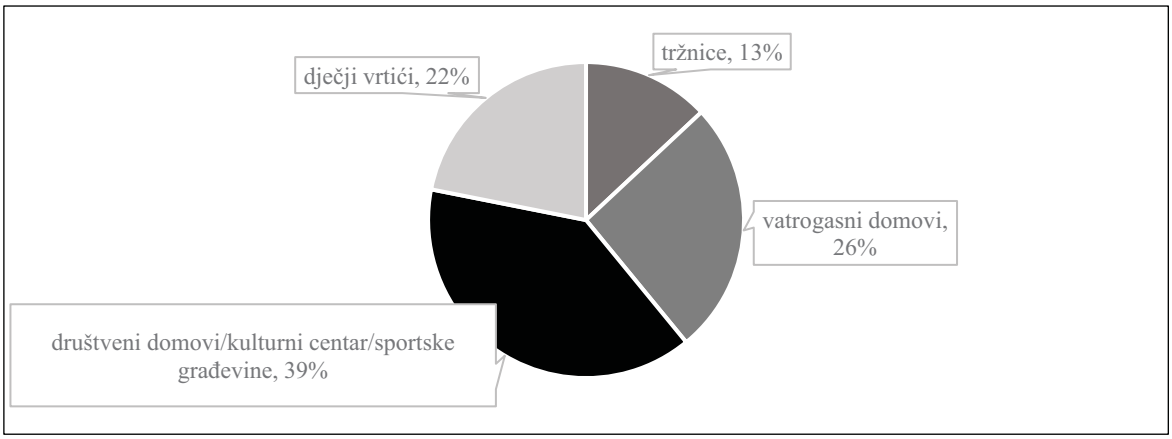
Rezultati i rasprava

Uloga društvene infrastrukture je osigurati društveni razvoj i ostvarenje ukupnog društvenog blagostanja nekog prostora (Stawicki i Vaznoniene, 2019.). Društvena infrastruktura osigurava razvoj lokalnih jedinica, zadovoljenje osnovnih društvenih potreba i interesa stanovništva te stvaranje uvjeta za njegov opstanak i reprodukciju (Frolova i sur., 2016.). U tom smislu društvena infrastruktura ruralnog prostora važan je element njegovog ukupnog razvoja i relevantan čimbenik zadovoljstva ruralnog stanovništva s utjecajem na demografske pokazatelje. Društvena i gospodarska infrastruktura najčešće su pozitivno korelirane pa se tako uz dobre makroekonomske i sociodemografske pokazatelje nekog područja (BDP, zaposlenost, prirodno kretanje stanovništva) pojavljuje i zadovoljavajuća kvaliteta obrazovnih, zdravstvenih, kulturnih i ostalih društvenih usluga (Vinogradova i sur., 2015.). Neovisno o kojim se dijelovima svijeta radi, ruralni prostori često raspoloživošću društvene infrastrukture zaostaju za urbanim dijelovima zemalja (Satish, 2007.). U istraživanju autora Frolova i sur. (2016.) predstavnici lokalnih jedinica vlasti iz ruralnih područja Rusije iskazali su dvostruko veće nezadovoljstvo stanjem društvene infrastrukture od svojih kolega iz urbanih dijelova zemlje. Vaznonienė i Pakeltienė (2017.) temeljem objavljenih istraživanja i statističkih podataka zaključuju da količina i kvaliteta društvene infrastrukture i usluga koje se nude u ruralnim područjima Litve ne odgovaraju zahtjevima koje postavlja većina seoskog stanovništva. Zaostajanje infrastrukturne opskrbljenosti u ruralnim dijelovima Kanade uočava institucija koja predstavlja poljoprivrednike ove zemlje - Canadian Federation of Agriculture. Na njihovim mrežnim stranicama navodi se da mnoga područja u ruralnim dijelovima Kanade pate od nedostataka infrastrukture, bilo da se radi o digitalnoj povezanosti, prometnoj infrastrukturi ili pristupu socijalnim uslugama.

Trenutna ulaganja u socijalnu infrastrukturu na razini Europske unije procijenjena su na oko 170 milijardi eura godišnje (Fransen i sur., 2018.). Autori zaključuju da je to nedovoljno u odnosu na aktualne potrebe europskog stanovništva.

Zbog važnosti društvene infrastrukture javnim se financijskim izvorima u čitavoj EU potiče njezin razvoj pri čemu se prednost daje ruralnim područjima u kojima je ona naročito deficitarna. U Programu ruralnog razvoja Republike Hrvatske (2014-2020) predviđena je podmjera 7.4. kojom je predviđeno financiranje pokretanja, poboljšanja te proširenja brojnih društvenih usluga za zadovoljavanje društvenih potreba ruralnog stanovništva. Sukladno Pravilniku o provedbi mjere 7 „temeljne usluge i obnova sela u ruralnim područjima" (NN 48/2018) prihvatljivi troškovi u korištenju podmjere 7.4. su gradnja i/ili opremanje objekata 18 različitih oblika društvene infrastrukture, od vatrogasnih domova, društvenih domova, dječjih vrtića, rekreacijskih zona do biciklističkih staza, groblja i tržnica. Iz navedenog je razvidno da su osim društvene infrastrukture ovom podmjerom uključeni i neki oblici

gospodarske infrastrukture. S druge strane, neprihvatljivo je financiranje škola i zdravstvenih ustanova koje se financiraju iz drugih javnih izvora. Naime, Republika Hrvatska nije u prvoj financijskoj omotnici (2014.-2020.) uspjela ispregovarati financiranje izgradnja škola iz europskih fondova, ali se to očekuje u drugoj europskoj omotnici (2021.-2027.). Do izrade ovog rada objavljena su dva nacionalna natječaja za podmjeru 7.4. te je ukupno zaprimljeno 1.078 prijava s ukupnim traženim iznosom potpore od 611.877.161 eura. Do kraja 2019. godine odobrene su 343 prijave s ukupnim iznosom potpore od 234.245.611 eura (Ministarstvo poljoprivrede, 2020.). Krajem 2021. završile su prijave na treći natječaj na kojem je, prema uvjetima natječaja, predviđena alokacija sredstava prikazana na Grafikonu 1.



Grafikon 1. Predviđena alokacija sredstava iz trećeg natječaja Podmjera 7.4.

U anketnom istraživanju sudjelovalo je 98 ispitanika, stanovnika ruralnog području trinaest hrvatskih županija. Regionalna raspodjela ispitanika je neujednačena pa je tako gotovo polovica svih ispitanika iz ruralnih područja triju županija (Koprivničko-križevačka, Zagrebačka i Grad Zagreb) što je posljedica prigodnog uzorkovanja. Pojedinačno najviše ispitanika (22 %) je iz naselja s manje od 1.000 stanovnika. Među ispitanicima prevladavaju žene (67 %) i mlađe dobne skupine pa je njih 68 % u dobi do 40 godina. Najveći je udio ispitanika (46 %) sa završenim srednjoškolskim obrazovanjem i dolaze iz domaćinstva s ukupnim mjesečnim primanjima između 8 i 12 tisuće kuna.

Prvo pitanje odnosilo se na usporedbu važnosti gospodarske i društvene infrastrukture. Prema očekivanju i postavljenoj prvoj hipotezi rada, gospodarska i društvena infrastruktura ocijenjene su podjednako važne za kvalitetu života ruralnog područja

Tablica 1. Koja vrsta infrastrukture je važnija za kvalitetu života u ruralnom području

Vrsta infrastrukture	Udio odgovora (%)
Gospodarska	7,1
Društvena	9,2
Podjednako su važne	83,7

U sljedećoj tablici prikazani su odgovori ispitanika na pitanje u kojoj mjeri njihova odluka o ostanku na selu ovisi o dostupnosti društvene infrastrukture. Tek 18 % ispitanika navelo je da takva odluka ne ovisi ili slabo ovisi o dostupnoj društvenoj infrastrukturi.

Tablica 2. U kojoj mjeri vaša odluka o ostanku na selu ovisi o postojanju kvalitetne ruralne društvene infrastrukture

Jako ovisi	48,6 %
Umjereno ovisi	33,4 %
Slabo ovisi	9,7 %
Ne ovisi	8,3 %

Nadalje, ispitanici su ocjenama 1 - nevažno do 5 - izuzetno važno ocijenili doprinos pojedinih oblika društvene infrastrukture kvaliteti života na selu. Pri tome su najčešćim oblicima društvene infrastrukture pridodani i oni koji su prihvatljivi za financiranje iz pomjere 7.4. Najveću važnost dali su školama i liječničkim ambulantama uz najmanje odstupanje odgovora. Visoka ocjena važnosti pridodana je i dječjim igralištima, ali uz veću varijabilnost odgovora iskazanu standardnom devijacijom. Manje važni oblici društvene infrastrukture ruralnog prostora su društveni domovi, sportski, kulturni i vjerski objekti pri čemu je za ove posljednje iskazana nešto veća varijabilnost odgovora.

Tablica 3. Važnost pojedinih vrsta društvene infrastrukture za kvalitetu života na selu

Vrsta infrastrukture	**Srednja vrijednost**	**Standardna devijacija**
Škole	4,65	0,73
Liječnička ambulanta	4,64	0,76
Dječji vrtići	4,45	0,92
Dječja igrališta	4,23	1,96
Vatrogasni/Društveni dom	3,69	1,09
Sportsko-rekreativni sadržaji	3,68	0,98
Kulturni centar	3,35	1,08
Vjerski objekti	3,29	1,34
Planinarski dom	2,79	1,17
Prosječna vrijednost	**3,88**	**1,09**

Svi prethodni odgovori ispitanika obrađeni su i s obzirom na njihova socioekonomska obilježja (dob, stručna sprema, mjesečna primanja). Među ispitanicima uglavnom ne postoje značajnije razlike odnosno odstupanja u ocjenama važnosti pojedinih oblika društvene infrastrukture. Nešto veće oscilacije odgovora utvrđene su u pogledu:

a. doprinosa dostupnosti društvene infrastrukture odluci o ostanku na selu prema mjesečnim primanjima ispitanika. Pri tome ispitanici s manjim mjesečnim primanjima (do 6 tisuće kuna) kao i oni u rasponu od 12-16 tisuće kuna svoj ostanak na selu u manjoj mjeri uvjetuju raspoloživosti društvene infrastrukture u odnosu na ispitanike drugih dohodovnih kategorija.
b. ocjeni važnosti vjerskih objekata prema stručnoj spremi ispitanika. Pri tome ispitanici sa završenom osnovnom školom ocjenjuju ovaj oblik društvene infrastrukture prosječnom ocjenom 4,33 dok je ukupna prosječna ocjena 3,29.

Zaključak

Istraživanje je potvrdilo hipotezu istraživanja o podjednakoj važnosti gospodarske i društvene infrastrukture, kao i o utjecaju raspoloživosti društvene infrastrukture na odluku o ostanku na selu. Također, potvrđena je i hipoteza o većoj važnosti infrastrukture zdravstvene (ocjena 4,64) i obrazovne namjene (ocjena 4,65) u odnosu na infrastrukturu s

kulturnom i sportskom namjenom. Škole i dječji vrtići najvažniji su ispitanicima u dobnoj skupini od 16-26 godina dok su vjerski objekti važniji ispitanicima starije dobne skupine (60+). Rezultati istraživanja ne korespondiraju s alokacijom sredstava iz predviđene alokacije potpora iz trećeg natječaja za podmjeru 7.4. kod kojeg je najviše sredstva predviđeno za izgradnju i opremanje društvenih domova, sportskih i kulturnih centara.

Napomena

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Literatura

Bottini N., Coelho M., Kao J. (2012). Infrastructure and growth, 1-45. London, UK: London School of Economics.

Fransen L., Del Bufalo G., Reviglio E. (2018). Boosting investment in social infrastructure in Europe: Report of the high-level task force on investing in social infrastructure in Europe. Publications Office of the European Union. Raspoloživo: https://data.europa.eu/doi/10.2765/794497

Frolova E.V., Vinichenko M.V., Kirillov A.V., Rogach O.V., Kabanova E.E. (2016). Development of social infrastructure in the management practices of local authorities: trends and factors. International Journal of Environmental and Science Education. 11 (15): 7421-7430.

Jeffrey F. (2009). What in the world is infrastructure, 30-32. PEI Infrastructure Investor.

Ministarstvo poljoprivrede (2019). Godišnje izvješće o provedbi programa ruralnog razvoja Republike Hrvatske. Raspoloživo: https://ruralnirazvoj.hr/files/Implementation-report_2014HR06RDNP001_2019_0_hr.pdf

Narodne novine (48/2018): Pravilnik o provedbi mjere 7 „Temeljne usluge i obnova sela u ruralnim područjima“.

Satish P. (2007). Rural Infrastructure and Growth: An Overview. Indian Journal of Agricultural Economics. 62 (1): 32-51.

Stawicki M., Vaznonienė G. (2019). Assessment of rural social infrastructure services in Lithuania and Poland in the context of green economy. Proceedings of the 9th International scientific conference Rural Development. Kaunas, Litva: Agriculture Academy of Vytautas Magnus University.

Vaznonienė G., Pakeltienė R. (2017). Methods for the assessment of rural social infrastructure needs. European Countryside. 9 (3): 526-540.

Vinogradova M., Kulyamina O., Koroleva V., Larionova A. (2015). The Impact of Migration Processes on the National Security System of Russia. Mediterranean Journal of Social Sciences. 5: 161-168.

Assessment of the importance of social infrastructure in rural Croatia

Abstract

The aim of this paper is to assess certain forms of social infrastructure of rural areas of Croatia and to determine the concordance of respondents' assessments with the allocation of funds from Submeasure 7.4. Rural Development Program. Respondents rated the social infrastructure for health (grade 4.64) and educational purposes (4.65) more important than the infrastructure with sports (3.68), cultural (3.35) and religious purposes (3.29). In the allocation of grants from the third tender of Sub-measure 7.4. sports and cultural centers predominate, so this allocation does not coincide with the respondents' assessments.

Key words: social infrastructure, rural development, submeasure 7.4., survey

PROFESSIONAL PAPER

Kalahari truffle: An underprized delicacy

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Abstract
Mushrooms can be edible and are used as food and nutraceuticals. They are mostly collected from the wild and sold as is. Buyers wash and cook them before consumption. This piece assessed the prices of Kalahari truffles mostly at the largest open market where, when they are in season in Namibia, a high concentration of truffles sales occur. It intelligently argues for the creation of veld-food supply chains to improve rural development. The prices of truffles increased towards the end of the growing season. In Namibia and Botswana, the Kalahari truffles are sold in the informal markets at € 4.75 per kg in Namibia, which is significantly lower than € 1496.5 per kg in developed nations. The Kalahari truffles value should be unleashed to enhance the incomes of rural communities via the development of veld-food supply chains.

Key words: veld-food, *Kalaharituber pfeilli*, price, weight, rural development

Introduction

The livelihoods of most rural people in developing nations depend on subsistence farming, fishing, collection of veld and forest resources. In terms of foods, veld/forest products include edible wild fruits, melons, tubers, roots, nuts, insects, lerps, and mushrooms. In Namibia, edible mushrooms are used as food and a source of income. In developed nations, edible mushrooms are regarded as a delicacy with high nutritional and functional value (Valverde et al., 2015). They are also valued for their exceptional sensory attributes including unique aroma and flavour (Villares et al., 2012). Kalahari truffles and *Termitomyces schimperi* are commonly harvested for domestic consumption and/or selling in the informal markets in Namibia. This should be understood within the context of high economic inequality in Namibia, where the GINI coefficient is high at 0.56 as compared to that of 0.25 in the Scandinavian countries (Namibia Statistics Agency, 2018a). More (53.1%) people live in rural areas. About 28% of all households, mostly those in rural and informal shanty locations within the towns in Namibia are poor or severely poor based on their costs of basic needs compared to the poverty lines.

With little value-addition and manufacturing in Namibia, the unemployment rate is 33.4% (Namibia Statistics Agency, 2018b). It is suspected to be as high as 50% among the youths Truffles are fungi that form fruit bodies below the ground (hypogenous) (Danesh, 2015). They are therefore telluric and seasonal (Trappe et al., 2008). They are not only harvested for their culinary properties but for their nutritional value as well (Kagan-Zur and Roth-Bejerano, 2008; Shavit and Volk, 2007). Kalahari truffles (Figure 1) including *Kalaharituber pfeilli,* the common truffle found in Namibia are underprized as compared to other truffles in other parts of the world. For instance in Europe, the market price for truffles ranged from € 187.5 to € 2810.5 per kg (Álvarez-Lafuente et al., 2018), whereas in Namibia, they are harvested for household consumption and/or for selling in the informal market

where prices generally low. Participation of wild food resources collectors in veld-food supply chains has the potential to improve the development in rural communities. This could become true provided that the collected veld-food such as truffles can be afforded market opportunities at the formal national and international levels. Increased incomes can be returned to the collectors through improved product value through enhanced veld-food supply chains. Currently, there is little or no literature on the prices of truffles collected from the veld in Namibia. This study therefore investigated the price of Kalahari truffle in Namibia.

Figure 1. Washed fresh Kalahari truffles

Materials and methods

Typical at any given open market in Namibia, the manner of sale, display and pricing of the products often did not differ among the vendors. This was also the case at the studied open markets (Ondangwa and Omuthiya). To determine the prices of Kalahari truffle, about 15 vendors who were present at the Omuthiya market at the initial time of sampling were asked how much they were selling the truffle batches for. At the Ondangwa open market, there was only one vendor who had only one batch available. In all cases, a customer had the liberty to choose the batch or batches that they want to buy. Truffles were sold fresh in subjectively vendor-determined batches (groups of individual truffles, Figure 2). Vendors did not have weighing balances to weigh the truffles. Therefore, the truffles were sold per batch. Batches were therefore subjectively formed by the vendor. A batch in this study was considered as a sub-population of the purchased truffles.

A total of seven batches of fresh Kalahari desert truffles (*Kalaharituber pfeilli*) were purchased from different vendors at the informal market in Omuthiya and Ondangwa. The batches were chosen to represent those that have large but fewer number and those with small but large number of truffles. One batch was obtained from each of a total of eight vendors. Six batches (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) of truffles were purchased during the peak of the growing season (mid May 2018) while batches T_6 and T_7 were purchased towards the end of growing season (around June). At the end of the growing season, there were only 2 vendors found at the Omuthiya open market. All the truffle batches were bought from different vendors at Omuthiya gwIipundi in Oshikoto region, except for batch T_5 which was bought from Ondangwa in Oshana region. The truffles in their respective batches were then brought in the same day to the Department of Food Science and Technology at the University of Namibia in Windhoek, Namibia. The number of truffles in each batch was counted. The weight of all individual truffle in the different batches was determined using an analytical

balance. Note that the vendors at the time of the study did not have an objective method to constitute a batch. They thus did not know the weight of the truffles in each batch or the weight of the batches.

Figure 2. Representative picture of the batches (11) of truffles sold at the open market during the 2018 growing season. These are batches on display for one vendor.

Results and discussion

In Namibia, truffles are sold at open markets (Figure 2) in vendor-determined batches. Table 1 shows the number of Kalahari truffles, weight and cost of Kalahari truffles sold in open market in Namibia.

Table 1: Prices, weights of Kalahari truffles sold in open markets (Ondangwa and Omuthiya) in Namibia in 2018. Exchange rate was based on the Euro to Namibian Dollar Exchange Rate History For 20 May 2018 (20/05/18) (exchangerates.org.uk).

Batch	Count	Total weight (g)	Average weight (g)	Median weight (g)	Minimum weight (g)	Maximum Weight (g)	Price (€ per batch)	Price (€ kg^{-1})
T_1	7	336.6	48.1	38.7	21.8	117.8	1.35	4.02
T_2	24	506.3	21.1	20.8	8.0	42.0	1.35	2.67
T_3	39	621.9	16.0	14.0	2.7	48.4	1.35	2.18
T_4	11	695.1	63.2	52.0	21.6	184.4	1.35	1.95
T_5	12	647.4	54.0	27.5	8.3	165.5	2.71	4.18
T_6	22	874.0	39.7	23.0	12.0	270.0	4.06	4.65
T_7	15	856.0	57.1	52.0	30.0	122.0	4.06	4.75

It was found that the different batches of truffles had different number of truffles. The number of truffles per batch ranged between 7 and 39 (Table 1). It is important to note that the price per batch is the actual amount that was paid to the vendors based on the vendors-determined pricing. The price/kg is the value that was calculated after the batches of truffles were taken to the laboratory and the weights of all truffles in each batch were measured. The batches with visibly small sized truffles tended to have more counts (e.g. T_3) whereas batches with visibly large sized truffles had fewer counts (e.g T_1). Upon converting the vendor-determined subjective prices to the price per kg, it became clear that batches that were priced the same had in fact different weights and thus the price per kg were different. Customers were therefore not getting the same truffle amount per euro. This means that some customers fetch more truffles per euro (e.g. T_4) while others get less per euro (T_1). This highlights the importance of objective methods of setting prices of truffles sold at the open markets in Namibia.

The prices of truffles followed the general trend of supply and demand. This is evidenced by the fact that the batches T_6 and T_7 that were bought towards the end of the growing season had relatively high prices of € 4.65-4.75 per kg, respectively (Euro to Namibian Dollar Exchange Rate History For 20 May 2018 (20/05/18) (exchangerates.org.uk)) per batch. Based on the prices, it is clear that the truffles in Europe are highly prized and expensive when compared to the Kalahari truffles sold in Namibia. For example, the price of truffle in developed nations can be as high as 1108.5 times than the market price of Kalahari truffles that were sold at the main open market in north-central regions of Namibia. This is based on the market price of truffles in Europe that has been reported to be € 22.1 to € 1496.5 per kg (Morte et al., 2012; Bonito et al., 2013; US Dollar to Euro Exchange Rate History For 20 May 2018 (20/05/18) (exchangerates.org.uk)) in comparison to the market prices of truffles in Namibia (€ 1.95 to € 4.75 per kg). The low prices of the Kalahari truffles in Namibia could be attributed to the general economic status of people living in the study area, which is mostly rural with an average annual consumption per capita of € 1140.90 (Namibia Statistics Agency, 2018a).

Kalahari truffles in rural communities in Namibia are harvested for use at household level and also for selling in the informal markets. Truffle collectors could earn more if they had better access to even low-level technology such as simple packaging equipment or refrigeration technology. Preservation and processing of Kalahari truffles into high value export products and marketing have the potential to penetrate the world market and contribute to the economic growth of countries where truffles currently are underutilised and not well researched. Improving the veld-food supply chains and accessing the international markets can yield increased income for the rural truffle collectors and other actors. This in turn can reduce poverty and lead to improved livelihoods and development of rural communities.

The production of Kalahari truffles is reported to be declining mostly in places where livestock is concentrated (Trappe et al., 2008). Some land practices such as ploughing may also pose a threat to the production of the Kalahari truffles. Studies geared towards sustainable harvesting and possibility of cultivation of the Kalahari truffles are essential to future exploitation of this nutritious food resource.

Conclusions

This piece reports for the first time the price per weight of Kalahari truffles sold at the open market in north-central Namibia. Based on the price, it is concluded that the Kalahari truffles in Namibia are underprized and are sold at significantly low prices (€ 1.35 per kg) when compared to truffles in Europe (€ 1496.5 per kg). It appeared that the pricing of Kalahari Desert truffle depends on the vendor's intuition as the prices were not consistent per count

or weight. Consumers are not guaranteed the same purchase value for their money. Development of a veld-food supply chain of the Kalahari truffles locally and internationally can improve the Namibian rural communities that collect the truffles. Improving the Kalahari truffles quality via value-addition processing, standardisation of pricing per weight and safe packaging, storage and distribution to access the high value market opportunities in developed nations can lead to the high return of income to the Kalahari truffles harvesting communities and thus increase their standard of living, create jobs, reduce unemployment and steps closer to the eradication of poverty in Namibia.

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References

Álvarez-Lafuente A., Benito-Matías L. F., Peñuelas-Rubira J. L., Suz L. M. (2018). Multi-cropping edible truffles and sweet chestnuts: production of high-quality Castanea sativa seedlings inoculated with *Tuber aestivum*, its ecotype *T. uncinatum*, *T. brumale*, and *T. macrosporum*. Mycorrhiza. (28): 29-38.

Bonito G., Smith M. E., Nowak M., Healy R. A., Guevara G., Cázares E., Tedersoo L. (2013). Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. Public Library of Science One. Vol (8).

Kagan-Zur V., and Roth-Bejerano N. (2008). Desert truffles. Fungi. (1): 32-37.

Morte A., Andrino A., Honrubia M., Navarro-Ródenas A. (2012). Terfezia cultivation in arid and semiarid soils. In Edible ectomycorrhizal mushrooms. 241-263.

Namibia Statistics Agency. (2018a). Namibia household income and expenditure survey 2015/2016 report. Windhoek: Namibia Statistics Agency.

Namibia Statistics Agency. (2018b). The Namibia Labour Force Survey 2018 Report. Windhoek: Namibia Statistics Agency

Shavit E., and Volk, T. (2007). Terfezia and Tirmania, desert truffles (terfez, kama, p/faqa). Delicacies in the sand or manna from heaven. Zugriff. (14): 2010.

Trappe J. M., Claridge A. W., Arora D., Smi, W. A. (2008). Desert truffles of the African Kalahari: ecology, ethnomycology, and taxonomy. Economic botany. (62): 521.

Valverde M. E., Hernández-Pérez T., Paredes-López O. (2015). Edible mushrooms: improving human health and promoting quality life. International journal of microbiology. 2015: 1-4.

Villares A., Mateo-Vivaracho L., Guillamón E. (2012). Structural features and healthy properties of polysaccharides occurring in mushrooms. Agriculture. (2): 452-471.

PREGLEDNI RAD

Analiza tržišta suncokreta u razdoblju od 2010. do 2020. godine u Republici Hrvatskoj

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Sažetak
Cilj rada je pregled proizvodnje i analiza vanjskotrgovinske razmjene suncokreta u Republici Hrvatskoj za razdoblje od 2010. do 2020. godine. U radu je analizirano tržište sjemena suncokreta u Republici Hrvatskoj te su prikupljeni podatci iz različitih statističkih baza podataka o proizvodnji, otkupnim i veleprodajnim cijenama, vrijednosti izvoza i uvoza na temelju kojih je izračunata trgovinska bilanca. Prosječna proizvodna površina pod suncokretom u Republici Hrvatskoj u promatranom razdoblju od 2010. do 2020. godine iznosila je 35.425 ha te je zabilježen trend povećanja istih kao i proizvodnje i prinosa. Kod otkupnih i veleprodajnih cijena bilježe se oscilacije iz godine u godinu te je u 2020. godini zabilježeno smanjenje otkupne cijene za 4,13 % i povećanje veleprodajne cijene za 4,94 % u odnosu na razdoblje od 2010. - 2019. godine. U promatranome razdoblju (2010. - 2020.) izvoz suncokreta bio je veći od uvoza što je rezultiralo pozitivnom trgovinskom bilancom od 14.743.234,00 €. Varijabilnost izvoza (68,21 %) je dva puta veća u odnosu na varijabilnost uvoza (34,16 %) što pogoduje trgovinskoj bilanci.

Ključne riječi: Hrvatska, suncokret, proizvodnja, vanjskotrgovinska razmjena, cijene

Uvod

Prema podatcima Državnog zavoda za statistiku (Statistička izvješća 2010., 2020.) vidljivo je povećanje udjela industrijskog bilja u ukupnim zasijanim površinama. U 2010. godini industrijsko bilje bilo je zasijano na svega 14,0 % površina dok se u 2020. godini taj udjel promijenio te je bilo zasijano na 20,6 % površina. U petogodišnjem razdoblju od 2015. do 2020. godine u ukupnoj proizvodnji uljarica dominanto mjesto ima soja (50,6 %), zatim uljana repica (24,7 %) i suncokret s udjelom od 23,7 % (Ministarstvo poljoprivrede, 2021.). Sektor uljarica bilježi snažan rast proizvodnog volumena posebno nakon pristupanja Republike Hrvatske u EU (Kranjac i sur., 2019.). Prema podatcima Ministarstva poljoprivrede (2021.) Republika Hrvatska je samodostatna u proizvodnji uljarica te je u 2020. godini u vanjskotrgovinskoj razmjeni uljarica ostvarila suficit u vrijednosti od 122,1 milijun eura. Pozitivne pokazatelje bilježi proizvodnja suncokreta u 2020. godini s povećanjem proizvodnih površina kao i vanjskotrgovinska razmjena koja također bilježi suficit (Ministarstvo poljoprivrede, 2021.). Suncokret (*Helianthus annus* L.) je jedna od glavnih uljarica u svijetu koja se uzgaja za proizvodnju jestivog i biodizelskog ulja (de Oliveira Filho i Egea, 2021.). Nusproizvodi od ekstrakcije suncokretovog ulja predstavljaju vrijednu sirovinu s potencijalom za nekoliko primjena u području ishrane ljudi i životinja. Zbog svog kemijskog sastava uključujući visok sadržaj proteina i fenolnih spojeva uporaba sjemenki i nusproizvoda sjemenki suncokreta i njegovih frakcija koristi se u svrhu obogaćivanja proizvoda npr. u pekarskoj industriji i proizvodnji funkcionalne hrane (de Oliveira Filho i Egea, 2021.). Mijić i sur. (2020.) kao pozitivan pokazatelj u Hrvatskoj navode Tvornicu ulja Čepin koja kontinuirano ulaže u modernizaciju svojih kapaciteta te da

je u mogućnosti otkupiti i preraditi cjelokupnu proizvodnju suncokreta u Hrvatskoj što je svakako pozitivan pokazatelj hrvatskim proizvođačima za dodatno povećanje proizvodnje sjemena suncokreta. Cilj rada je utvrditi i opisati trendove razvoja tržišta suncokreta u razdoblju od 2010. do 2020. godine te na temelju dostupnih podataka utvrditi ponudu, potrošnju i vanjskotrgovinsku razmjenu.

Materijal i metode

Izvori podataka o površinama (ha), prinosima (t ha^{-1}) i proizvodnji suncokreta (t) u Republici Hrvatskoj za razdoblje od 2010. do 2020. godine bila su Statistička izvješća Republike Hrvatske Državnog zavoda za statistiku (različita godišta). Podatci o uvozu i izvozu unutar i izvan EU-27 zemalja za razdoblje od 2010. do 2020. godine prikupljeni su iz statističke baze podataka EUROSTAT, a podatci o cijenama (otkupnim i veleprodajnim) prikupljeni su iz baze podataka Tržišnog informacijskog sustava u poljoprivredi (TISUP). Za statističku obradu podataka korišten je statistički programski paket IBM SPSS Statistic V26. Na temelju prikupljenih podataka u radu su prikazane i izračunate mjere deskriptivne statistike za ispitivane varijable, a veze između ispitivanih varijabli utvrđene su pomoću Spearmanovog koeficijenta korelacije.

Rezultati i rasprava

Prema podatcima iz Tablice 1. proizvodna površina pod suncokretom u Republici Hrvatskoj u promatranom razdoblju od 2010. do 2020. godine iznosila je u prosjeku 35.425 ha. Prosječno odstupanje vrijednosti od aritmetičke sredine za proizvodne površine iznosi 4,32 ha odnosno 12,18 %. Najviše proizvodnih površina pod suncokretom bilo je 2013. godine (40.805 ha), a najmanje 2010. godine (26.412 ha). Ujedno 2013. godine ostvarena je i najveća proizvodnja (130.576 t) i prinos (3,20 t ha^{-1}) suncokreta što je u skladu s istraživanjem Mijić i sur. (2021.) koji navode da se visok prinos uljarica u 2013. godini temelji na velikim rezervama zimske vlage i povoljnoj raspodjeli oborina tijekom vegetacije. Nalazi su u skladu i s istraživanjima Jug i sur. (2018.) i Iljkić i sur. (2019.) koji navode da je po pitanju prinosa, suncokret najvarijabilnija kultura na koju vremenske prilike imaju znatan utjecaj. U razdoblju 2010. – 2020. godine ostvarena je proizvodnja od prosječno 102.247 t suncokreta s prosječnim prinosom od 2,87 t ha^{-1}. Prinos suncokreta u promatranom razdoblju bilježi rast te se kretao 2,34 do 3,20 t ha^{-1}. U 2020. godini zabilježeno je povećanje proizvodnje suncokreta za 19,45 % i prinosa za 8,45 % u odnosu na razdoblje od 2010. do 2019. godine. Površine pod suncokretom u Republici Hrvatskoj u istraživanju Mijić i sur. (2021.) u razdoblju od 1999. do 2018. godine su značajno varirale i ovisile o uvjetima uzgoja, cijenama sirovina (gnojivo, sjeme, zaštitna sredstva), otkupu, poticajima, potporama te primjeni tehnologije i znanju poljoprivrednih proizvođača te su u prosjeku iznosile 32.741 ha s prosječnim prinosom od 2,54 t ha^{-1}. Također prema spomenutim autorima Republika Hrvatska je druga zemlja po prinosu zrna po hektaru u EU prema višegodišnjem prosijeku (1999. – 2018.). Prema istraživanju Kuts i Makarchuk (2020.) u razdoblju od 2019. do 2020. godine zemlje najveći proizvođači sjemena suncokreta u svijetu su Ukrajina s 16,5 milijuna tona (30,0 %), Rusija 15,3 milijuna tona (29,0 %), EU-27 9,6 milijuna tona (17,0 %), Argentina 3,3 milijuna tona (6,0 %), Kina 3,3 milijuna tona (6,0 %) i Turska s 1,8 milijuna tona (3,0 %). S prikupljenim podatcima u ovome istraživanju i istraživanju Mijić i sur. (2020.) podudaraju se i podatci FAOSTATA (2022.) prema kojem spomenutih šest zemalja, iako su najveći proizvođači suncokreta, u prosjeku ostvaruju znatno manje prinose od Republike Hrvatske (Ukrajina 2,04 t ha^{-1}, Rusija 1,42 t ha^{-1} i EU-27 2,09 t ha^{-1}). Prema podatcima Europske komisije (2021.) za razdoblje od 2021. do 2031. godine očekuje se polagano povećanje prinosa i proizvodnje uljarica, posebice suncokreta i soje te se predviđa da će proizvodnja uljarica u EU u 2031. godini iznositi 31.2 milijuna tona. Međutim,

novonastala situacija u Ukrajini značajno će utjecati tržište žitarica (pšenice i kukuruza), suncokreta, ali i gnojiva, čiji su glavni izvoznici bili upravo Ukrajina i Rusija. Cijene osnovnih poljoprivrednih proizvoda već su bile visoke, a njihovo povećanje se očekuje i u budućem razdoblju. Ukrajina je među najvećim svjetskim izvoznicima žitarica i uljarica te je neupitni lider u prodaji suncokretovog ulja (Matuszak, 2021.). EU rafinerije nabavljaju 35 - 45 % suncokretovog ulja iz Ukrajine, pa je neupitno da će globalno potrošači biti pogođeni te će zemlje poput Kine, Indije i EU morati potražiti druge alternative (sojino, repičino, palmino, maslinovo ulje) po znatno višim cijenama (Matuszak, 2021., EEAS, 2022.).

Prema podatcima Državnog zavoda za statistiku (2022.) prosječna ukupna domaća potrošnja u razdoblju od 2010. do 2020. godine iznosila je 51.757 t ,od toga prosječna industrijska potrošnja iznosila je 46.538 t, a ljudska 1.121 t. Republika Hrvatska u promatranome razdoblju bila je samodostatna kada je u pitanju proizvodnja suncokreta, a prosječna potrošnja po glavi stanovnika iznosila je 0,27 kg.

Republika Hrvatska je u razdoblju od 2010. do 2020. u prosjeku izvezla suncokreta u vrijednosti 18.738.049,00 €, a uvezla suncokreta u vrijednosti od 3.994.815,00 €. Najmanji uvoz ostvaren je 2011. godine (1.584.537,00 €), a najveći 2020. godine (11.448.407,00 €). Najveći izvoz suncokreta ostvaren je 2013. godine (30.241.479,00 €) kada je ujedno ostvarena i najveća proizvodnja. U promatranom razdoblju varijabilnost izvoza (68,21 %) je dva puta veća u odnosu na varijabilnost uvoza (34,16 %) (Tablica 1). Uvoz i izvoz suncokreta mogu se smatrati vrlo varijabilnim svojstvom što je potvrđeno i Spearmanovim koeficijentom korelacije (Tablica 2). Utvrđena je statistički značajna veza između izvoza suncokreta izvan i uvoza izvan EU-27 zemalja ($p \leq 0,01$) te negativna statistički značajna veza između izvoza suncokreta izvan i uvoza unutar EU-27 zemalja ($p \leq 0,05$). Prema tome vidljivo je da najveći uvoz i izvoz Republika Hrvatska i dalje ostvaruje sa zemljama u okruženju koje nisu članice EU.

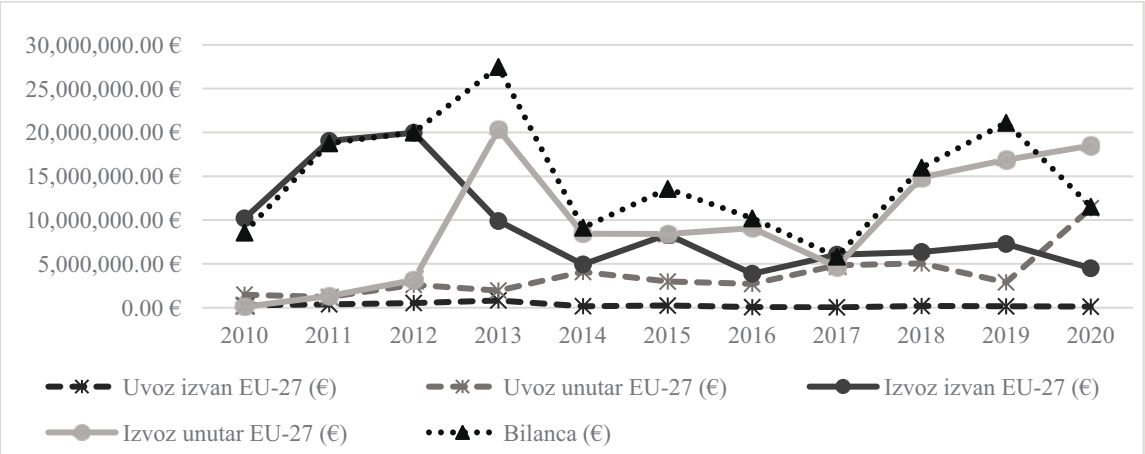
Tablica 1. Površine, prinosi, proizvodnja, vanjskotrgovinska razmjena i cijene suncokreta u Republici Hrvatskoj

	Max.	Min.	Prosjek	St.dev.	CV (%)
Površina (ha)	40.805	26.412	35.425	4.32	12,18
Proizvodnja (t)	130.576	61.789	102.247	19.04	18,62
Prinos (t ha^{-1})	3,20	2,34	2,87	0,24	8,47
Uvoz (€)	11.448.407,00	1.584.537,00	3.994.815,00	2.724.839	68,21
Izvoz (€)	30.241.479,00	10.293.552,00	18.738.049,00	6.400.304	34,16
OC (kn kg^{-1})	3,39	1,76	2,27	0,45	20,05
VPC (kn kg^{-1})	3,54	1,91	2,44	0,46	19,00

Napomena: max.- maksimum, min. – minimum, st. dev. – standardna devijacija, CV – koeficijent varijacije (%), OC – otkupna cijena, VPC – veleprodajna cijena
Izvor: Državni zavod za statistiku (2010., 2016., 2020.), EUROSTAT (2022.) i TISUP (2022).

Razdoblje od 2010. do 2020. godine ne karakteriziraju velike oscilacije iz godine u godinu te je na temelju podatka na Grafikonu 1 vidljivo da je u spomenutome razdoblju uvoz suncokreta konstantan osim blagog porasta u 2019. godini. Izvoz suncokreta iz Republike Hrvatske u promatranom razdoblju pokazuje tendenciju porasta što je posebno izraženo u 2020. godini. Na Grafikonu 1 vidljivo je da se od ulaska Republike Hrvatske u EU smanjio uvoz suncokreta iz zemalja izvan EU-27, a isti se povećao od 2017. do 2020. unutar zemalja EU-27. Isti trend primjetan je i kada je riječ o izvozu suncokreta izvan i unutar zemalja EU-27. U promatranom razdoblju vanjskotrgovinska bilanca je pozitivna i u prosjeku iznosi 14.743.234,00 €. Isti trend primjetan je i u Rumunjskoj u razdoblju od 2007. do 2016. godine

kada je vrijednost izvoza brzo rasla te je pokrivenost uvoza izvozom dovela do pozitivne trgovinske bilance (Popescu, 2018.).



Grafikon 1. Vanjskotrgovinska razmjena suncokreta unutar i izvan EU-27 zemalja
Izvor: EUROSTAT (2022.)

Tablica 2. Spearmanov koeficijent korelacije (rs) između proizvodnje, uvoza, izvoza i cijena suncokreta, 2010. – 2020.

	Proizvodnja	OC	VPC	Uvoz izvan	Uvoz unutar	Izvoz izvan	Izvoz unutar
Proizvodnja	-						
OC	-.630*	-					
VPC	-.573	.932**	-				
Uvoz izvan	-.373	.333	.255	-			
Uvoz unutar	.573	-.571	-.355	-.664*	-		
Izvoz izvan	-.582	.575	.482	.855**	-.709*	-	
Izvoz unutar	.836**	-.703*	-.645*	-.200	.464	-.509	-

** Korelacija je sig. p≤0,01, *korelacija je sig. p≤0,05
Napomena: OC - otkupna cijena, VPC – veleprodajna, uvoz i izvoz unutar i izvan EU-27

Prosječna otkupna cijena suncokreta za razdoblje od 2010. do 2020. godine iznosila je 2,27 kn kg^{-1} dok je veleprodajna cijena u istome razdoblju u prosjeku iznosila je 2,44 kn kg^{-1}. Prosječno odstupanje vrijednosti od aritmetičke sredine za otkupnu cijenu iznosi 0,45 kn kg^{-1} odnosno 20,05 %, a za veleprodajnu 0,46 kn kg^{-1} odnosno 19,00 %. Otkupna cijena suncokreta je u 2020. godini manja za 4,13 % u odnosu na razdoblje od 2010. - 2019. godine, a veleprodajna je cijena veća za 4,94 % u odnosu na isto razdoblje (Tablica 1). Spearmanovim koeficijentom korelacije prikazanim u Tablici 2 utvrđena je negativna statistički značajna veza ($p \leq 0,01$) između proizvodnje suncokreta i otkupnih cijena. Najmanja otkupna cijena (1,76 kn kg^{-1}) i veleprodajna cijena (1,91 kn kg^{-1}) suncokreta zabilježene su 2014. godine, a najveće cijene zabilježene su 2012. godine (otkupna 3,39 kn kg^{-1} i veleprodajna 3,54 kn kg^{-1}) (Tablica 1).

Zaključak

Suncokret ima veliku agrotehničku, ali i gospodarsku vrijednost za poljoprivredu Republike Hrvatske. Prema podatcima prikazanim u radu evidentan je trend porasta proizvodnje suncokreta u analiziranom razdoblju (2010. do 2020.). Proizvodne površine pod suncokretom u 2020. godinu veće su za 11,22 % u odnosu na razdoblje od 2010. do 2019. godine. Također u 2020. godini zabilježeno je i povećanje proizvodnje za 19,45 % i prinosa

suncokreta za 8,45 % u odnosu na razdoblje od 2010. do 2019. godine. Prosječna otkupna cijena suncokreta u razdoblju od 2010. do 2020. godine iznosila je 2,27 kn kg^{-1}, te je zabilježeno njezino smanjenje u 2020. godini dok je veleprodajna cijena u prosjeku iznosila 2,44 kn kg^{-1} te bilježi povećanje u 2020. godini u odnosu na razdoblje od 2010. do 2019. godine. Pozitivne pokazatelje bilježi vanjskotrgovinska razmjena suncokreta koja u promatranome razdoblju od 2010. do 2020. godine bilježi suficit prosječne vrijednosti od 14.743.234,00 €. Velika potražnja za sjemenkama suncokreta koje imaju široku primjenu za ljudsku, ali i industrijsku upotrebu uglavnom na EU tržištu, ali i drugim vanjskim tržištima, prilika je za Republiku Hrvatsku da intenzivira svoju proizvodnju i izvoz.

Literatura

De Oliveira Filho J.G., Egea, M.B. (2021). Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process. Journal of Food Science. 86 (5):1497-1510.

Državni zavod za statistku (2011). Poljoprivredna proizvodnja u 2010. Statistička izvješća. Raspoloživo: https://www.dzs.hr/

Državni zavod za statistku (2017). Poljoprivredna proizvodnja u 2016. Statistička izvješća. Raspoloživo: https://www.dzs.hr/

Državni zavod za statistku (2021). Poljoprivredna proizvodnja u 2020. Statistička izvješća. Raspoloživo: https://www.dzs.hr/

Državni zavod za statistku (2022). Bilanca uljarica. Raspoloživo: https://www.dzs.hr/PXWeb/Selection.aspx?px_path=Poljoprivreda,%20lov,%20%c5%a1umarstvo%20i%20ribarstvo__Bilanca%20usjeva&px_tableid=BU2.px&px_language=hr&px_db=Poljoprivreda,%20lov,%20%c5%a1umarstvo%20i%20ribarstvo&rxid=1dcc9a5d-b5fb-4dc0-ba3c-8188ed4a9a26

EEAS (2022). The war in Ukraine and its implications for the EU. Raspoloživo: https://eeas.europa.eu/headquarters/headquarters-homepage_en/112754/The%20war%20in%20Ukraine%20and%20its%20implications%20for%20the%20EU

EUROSTAT (2022). International Trade. Raspoloživo: http://epp.eurostat.ec.europa.eu/newxtweb/mainxtnet.do

Europska komisija (2021). EU agricultural outlook for markets, income and environment, 2021-2031. European Commission, DG Agriculture and Rural Development, Brussels. Raspoloživo: https://ec.europa.eu/info/food-farming-fisheries/farming/facts-and-figures/markets/outlook/medium-term_en

FAOSTAT (2022). Crops and livestock products. Raspoloživo: https://www.fao.org/faostat/en/#data/QCL

Ministarstvo poljoprivrede (2021). Godišnje izvješće o stanju poljoprivrede u 2020.. 6-222. Raspoloživo: https://poljoprivreda.gov.hr/UserDocsImages/dokumenti/poljoprivredna_politika/zeleno_izvjesce/2021_12_15%20Zeleno%20izvje%C5%A1%C4%87e%202020_final.pdf

Iljkić,D., Kranjac D., Zebec V., Varga I., Rastija M., Antunović M., Kovačević V. (2019). Stanje i perspektiva proizvodnje žitarica i uljarica u Republici Hrvatskoj. Glasnik zaštite bilja. 3: 62-71.

Jug D., Jug,I., Brozović B., Vukadinović V., Stipešević B., Đurđević B. (2018). Uloga konzrvacijske poljoprivrede u ublažavanju i prilagodbi klimatskim promjenama. Poljoprivreda. 24 (1): 35-44.

Kranjac D., Zmaić K., Sudarić T., Grgić I, Zrakić M. (2019). Pregled i perspektive tržišta soje u Republici Hrvatskoj do 2030. godine primjenom modela parcijalne ravnoteže.

Objavleno u *Zborniku radova 54. hrvatskog i 14. međunarodnog simpozija agronoma*, Mioč, B., Širić, I. (ur.), 172-131. Vodice, Hrvatska: Agronomski fakultet Zagreb.
Kuts T, Makarchuk O. (2022). Problems of World Agriculture. Scientific Journal Warsaw University of Life Science – SGGW. 20 (35): 4-15.
Matuszak S. (2021). The breadbasket of the world? Agricultural development in Ukraine. OSW Report. Raspoloživo: https://www.osw.waw.pl/en/publikacje/osw-report/2021-12-09/breadbasket-world
Mijić A., Liović I., Sudarić A., Duvnjak T., Jug D., Kranjac D., Jovović Z., Markulj Kulundžić A. (2021). Status and perspectives of sunflower production in Croatia. Agriculture and Forestry. 67 (1): 35-45.
Popescu A. (2018). Romania's sunflower seeds production, export and import- analysis of the 2007-2017 period and forecast for 2018-2022 Horizon. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development. 18 (4): 261-269.
Tržišni informacijski sustav u poljoprivredi (2022). Baza podataka - trend cijena. Raspoloživo: http://www.tisup.mps.hr/baza_trend.aspx

Overview of sunflower seed market in the period from 2010 to 2020 in Croatia

Abstract
The aim of this research was to evaluate the sunflower production and analyses foreign trade of sunflower seed in the Republic of Croatia from 2010 to 2020. The paper analyses the sunflower seed market in the Republic of Croatia and compile the data on production, purchase and wholesale prices, values of exports and imports based on which the trade balance was calculated. The average production area under sunflower in the Republic of Croatia in the analysed period (2010 to 2020) amounted to 35,425 ha. An increasing trend of production area, production and yield of the sunflower was recorded. Oscillations in purchase and wholesale prices were observed from year to year and in 2020 a decrease in the purchase price by 4.13% and an increase in the wholesale price by 4.94% compared to the period from 2010 to 2019 were recorded. In the observed period (2010 - 2020), sunflower exports were higher than imports, which resulted in a positive trade balance of € 14,743,234.00. The variability of exports (68.21%) is twice as high as the variability of imports (34.16%), which favours the trade balance.

Key words: Croatia, sunflower, production, foreign trade, prices

IZVORNI ZNANSTVENI RAD

Mjere dobrobiti životinja u Hrvatskoj u programskom razdoblju 2014.-2020.

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Sažetak
S ciljem poboljšanja uvjeta držanja i uzgoja u intenzivnim stočarskim sustavima, u Program ruralnog razvoja Republike Hrvatske za razdoblje 2014. – 2020. uvrštena je mjera 14 (M14) Dobrobit životinja, čija je provedba započela u svibnju 2018. godine. Cilj rada je utvrditi koje su to operacije obuhvaćene M14 te njihovo korištenje u Hrvatskoj. Najviše sredstava unutar M14 za 2018. i 2019. godinu isplaćeno je u govedarstvu (68.080.673,37 kn), a najmanje u kozarstvu (941.926,08 kn). Obzirom na razmještaj korisnika prednjače poljoprivrednici iz Osječko-baranjske županije (isplaćeno 46.390.118,34 kn) dok u Dubrovačko-neretvanskoj županiji nije bilo korisnika M14. Iskoristivost sredstava na državnoj razini je vrlo niska (23,00 %) što ukazuje na nisku popularnost promatrane mjere ili na neinformiranost poljoprivrednika.

Ključne riječi: dobrobit životinja, korištenje sredstava, mjera 14, Program ruralnog razvoja

Uvod

Svjetska organizacija za zdravlje životinja (OIE) dala je 2008. godine sljedeću definiciju zadovoljavajuće razine dobrobiti životinja: „Zadovoljavajuća razina dobrobiti životinje postignuta je ako je životinja zdrava, ako joj je udobno, ako je uhranjena i sigurna, može se ponašati na njoj svojstven (prirodan) način te ako ne trpi nelagodno stanje kao što je bol, strah ili nelagoda." Znanstvenici i zakonodavci koriste pojam dobrobit životinja kao znanstveni koncept koji opisuje potencijalno mjerljivu kvalitetu života životinje u određenom trenutku (Broom, 2017.). Rastuća društvena i znanstvena zabrinutost za dobrobit životinja je dovela do promjena u zakonodavstvu diljem svijeta (Broom, 2010.; De la Fuente i sur., 2017.), pa tako i u EU zakonodavstvu o dobrobiti farmskih, laboratorijskih i divljih životinja kojima se trguje (Broom, 2017.). Temeljem razvoja aktivnosti koje osiguravaju dobrobit životinja smatra se „Bambelovih pet sloboda" iz 1979. godine: (1) pravo na hranu i vodu, (2) pravo na udoban smještaj, (3) pravo na život bez boli, ozljeda i bolesti, (4) pravo na izražavanje prirodnog ponašanja i (5) pravo na život bez straha i opasnosti koje su kasnije pretočene u različite neobvezujuće ili obvezujuće dokumente (MP, 2021.). Zakonodavstvo je bilo glavni politički pristup za zaštitu dobrobiti domaćih životinja (Bennett, 1997.).

Cilj promicanja dobrobiti životinja jedan je od prioriteta ruralnog razvoja za razdoblje 2014. - 2020. te je Sud u državama članicama Europske unije (EU) utvrdio pozitivne primjere mjera koje su korisne za životinje. Međutim, mjera za „dobrobit životinja" nije bila u širokoj uporabi. Postojali su određeni nedostatci u pogledu isplativosti predmetne mjere te su države članice rijetko koristile priliku za pružanje potpore dobrobiti životinja u okviru drugih mjera ruralnog razvoja (Europski revizorski sud, 2018.). Provedba mjere 14 – Dobrobit životinja (M14) u Hrvatskoj započela je u svibnju 2018. godine (MP, 2021.), a 31. prosinca 2019. godine aktiviralo ju je 15 europskih regija dok 14 zemalja financira ovu mjeru na nacionalnoj razini unutar odgovarajućeg nacionalnog plana ruralnog razvoja. U Hrvatskoj je za M14 predviđeno 0,8 % sredstava iz PRR-a 2014.-2020. (Italian rural network, 2020.).

Cilj rada je utvrditi koje su to operacije dobrobiti životinja obuhvaćene mjerom 14 Programa ruralnog razvoja 2014.-2020. te njihovo korištenje u Hrvatskoj.

Materijali i metode

U radu je korištena deskriptivna analiza sekundarnih podataka. U izračunima su korišteni interni podatci Agencije za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju dobiveni na zahtjev, stanje na dan 30.11.2020. godine. Za izračun udjela gospodarstva koja sudjeluju u M14 korištene su baze podataka Ministarstva poljoprivrede - JRDŽ o broju posjednika i stoke po kategorijama i županijama (MP, 2020.). Navedeni podatci nisu dostupni za sektor peradarstva tako da za taj sektor nije bilo moguće izračunati obuhvat korisnika mjere.

Rezultati i rasprava

Mjera 14

Ulazak u sustav potpore za ovu mjeru je dobrovoljan, a potpora se isplaćuje s ciljem nadoknade izgubljenog prihoda ili povećanih troškova koji proizlaze iz propisanih obveza u mjeri. Namijenjena je poljoprivrednicima koji dobrovoljno žele obavljati intervencije (mimo minimalnih zakonskih uvjeta), a s ciljem poboljšanja dobrobiti uzgajanih životinja. M14 uključuje primjenu visokih standarda dobrobiti životinja koji su iznad važećih zakonskih propisa ili uobičajene prakse u sljedećim područjima: (1) osiguranje vode, hrane i brige o životinjama u skladu sa njihovim prirodnim potrebama, (2) osiguranje uvjeta držanja na način da imaju dovoljno prostora, odgovarajuću stelju i prirodno osvjetljenje, (3) pristup na otvoreno, (4) prakse koje izbjegavaju sakaćenje i/ili kastraciju životinja, a ukoliko je to zaista neophodno upotrebu anestetika, analgetika te protuupalnih lijekova ili imuno kastraciju.

U sklopu navedene mjere provode se sljedeće operacije:

- 14.1.1. Dobrobit životinja u govedarstvu (mliječne krave, tovna junad, telad)
- 14.1.2. Dobrobit životinja u svinjogojstvu (odbijena prasad, krmače i nazimice, svinje za tov)
- 14.1.3. Dobrobit životinja u peradarstvu (brojleri, nesilice, purani)
- 14.1.4. Dobrobit životinja u kozarstvu i (uzgoj i držanje koza)
- 14.1.5. Dobrobit životinja u ovčarstvu (uzgoj i držanje ovaca).

Korištenje sredstava

U Tablici 1 je vidljivo da je broj gospodarstava obuhvaćen operacijom 14.1.1. u 2019. godini 825 što čini 3,90 % gospodarstava koja su se bavila govedarstvom. Također vidljivo je da je u 2019. u odnosu na 2018. godinu došlo do rasta broja korisnika operacije za 4,43 %, broja uvjetnih grla (UG) za 2,60 % i odobrenog iznosa potpore za čak 67,34 %. Razlog tome je vjerojatno veći broj područja dobrobiti koje su korisnici primjenjivali na svojim gospodarstvima. Najveći broj korisnika mjere u 2019. godini bio je u Bjelovarsko-bilogorskoj županiji (148 odnosno 6,13 % gospodarstava koja se bave govedarstvom), slijede Osječko-baranjska (146 odnosno 13,58 %) i Koprivničko-križevačka (84 odnosno 3,26 %).

U svinjogojstvu je udio gospodarstava koja koriste M14 PRR-a dosta manji nego li u govedarstvu i u 2019. godini iznosi za Panonsku Hrvatsku 0,28 %, Grad Zagreb 0,12 %, Jadransku Hrvatsku 0,09 % te za Sjevernu Hrvatsku 0,26 %. Najveći broj korisnika navedene operacije zabilježen je u Osječko-baranjskoj (37), Vukovarsko-srijemskoj (23) i Brodsko-posavskoj (21) županiji.

Tablica 1. Isplaćene potpore za dobrobit životinja u govedarstvu u 2018. i 2019. godini

Operacija		14.1.1. Dobrobit životinja u govedarstvu				
NUTS 2 regija		Panonska Hrvatska	Grad Zagreb	Jadranska Hrvatska	Sjeverna Hrvatska	Ukupno
2018*	Broj korisnika	495	11	38	246	790
	Ukupni broj gospodarstava	9536	346	4996	8342	23.220
	Udio korisnika	5,19 %	3,18 %	0,76 %	2,95 %	3,40 %
	Broj UG**	59.382,85	514,16	2.821,75	16.912,64	79.631,40
	Odobreni iznos potpore u HRK	20.496.828,73	187.240,05	775.537,33	4.070.611,96	25.530.218,07
2019*	Broj korisnika	545	8	38	234	825
	Ukupni broj gospodarstava	8760	315	4653	7446	21.174
	Udio korisnika	6,22 %	2,54 %	0,82 %	3,14 %	3,90 %
	Broj UG**	62.132,46	337,22	2.775,87	16.454,00	81.699,55
	Odobreni iznos potpore u HRK	34.569.443,12	259.089,95	1.268.147,51	6.625.314,48	42.721.995,06
Ukupno isplaćeni iznos potpore u HRK***		55.002.194,74	445.578,11	2.032.294,77	10.600.605,75	68.080.673,37

* Podaci se odnose na proizvodne godine; **UG=uvjetna grla; *** Stanje na 30.11.2020.

Izvor: Interni podatci APPRRR, 2021.; MP, 2020.

Ukupan broj korisnika u RH je u padu u 2019. u odnosu na 2018. godinu, dok je broj operacijom obuhvaćenih UG i iznos isplaćenih potpora u porastu (Tablica 2).

Tablica 2. Isplaćene potpore za dobrobit životinja u svinjogojstvu u 2018. i 2019. godini

Operacija		14.1.2. Dobrobit životinja u svinjogojstvu				
NUTS 2 regija		Panonska Hrvatska	Grad Zagreb	Jadranska Hrvatska	Sjeverna Hrvatska	ukupno
2018*	Broj korisnika	126	6	3	41	176
	Ukupni broj gospodarstava	26494	356	1671	13913	42434
	Udio korisnika	0,48 %	1,69 %	0,18 %	0,29 %	0,41 %
	Broj UG**	62.738,80	1.248,45	77,92	7.618,47	71.683,64
	Odobreni iznos potpore u HRK	6.193.058,93	111.031,71	13.503,26	1.120.865,67	7.438.459,57
2019*	Broj korisnika	106	1	3	36	146
	Broj gospodarstava u Hrvatskoj	38151	814	3233	13913	56111
	Udio korisnika	0,28 %	0,12 %	0,09 %	0,26 %	0,26 %
	Broj UG**	68.918,91	19,22	85,95	20.557,13	89.581,21
	Odobreni iznos potpore u HRK	11.171.021,65	2.586,64	58.594,76	9.093.032,06	20.325.235,11
Ukupno isplaćeni iznos potpore u HRK***		17.360.852,00	113.584,73	72.098,05	10.191.606,68	27.738.141,46

* Podaci se odnose na proizvodne godine; **UG=uvjetna grla; *** Stanje na 30.11.2020.

Izvor: Interni podatci APPRRR, 2021.; MP, 2020.

U peradarskoj proizvodnji također postoje varijacije između 2018. i 2019. godine u broju korisnika, broja UG i odobrenih iznosa potpore ovisno o regiji (Tablica 3). Tako u Panonskoj Hrvatskoj bilježimo rast broja korisnika i odobrenog iznosa potpore te pad broja UG-a u 2019. u odnosu na 2018. dok u Jadranskoj Hrvatskoj bilježimo pad broja korisnika i UG te povećanje odobrenih iznosa potpore.

Obuhvat korisnika koji koriste M14 u kozarstvu najveći je u Sjevernoj (1,68 %) nakon koje slijede Panonska (0,88 %) i Jadranska Hrvatska (0,42 %) te Grad Zagreb (0,00 %). U ovčarstvu je najveći u Gradu Zagrebu (12,66 %) dok je u ostalim regijama dosta niži (0,78-2,81 %) (Tablica 4). Najviše korisnika M14 u kozarstvu ima u Zadarskoj županiji (18), slijede Međimurska (8) i Splitsko-dalmatinska (7). U ovčarstvu također po broju korisnika

prednjači Zadarska (102), a slijede ju Bjelovarsko-bilogorska (48) i Ličko-senjska županija (44).

Tablica 3. Isplaćene potpore za dobrobit životinja u peradarstvu u 2018. i 2019. godini

Operacija	NUTS 2 regija	2018*			2019*			Ukupno isplaćeni iznos potpore u HRK***
		Broj korisnika	Broj UG**	Odobreni iznos potpore u HRK	Broj korisnika	Broj UG**	Odobreni iznos potpore u HRK	
14.1.3. Dobrobit životinja u peradarstvu	Panonska Hrvatska	16	5.484,99	1.448.586,47	19	4.751,25	2.104.997,30	3.553.582,09
	Grad Zagreb	1	579,35	159.237,55	2	1.363,26	644.725,22	803.962,77
	Jadranska Hrvatska	9	2.679,63	391.919,46	6	1.302,82	576.799,88	968.719,31
	Sjeverna Hrvatska	18	20.560,73	3.114.029,88	19	24.204,38	6.379.982,39	9.481.359,65
	Ukupno	44	29.304,70	5.113.773,36	46	31.621,71	9.706.504,79	14.807.623,82

* Podaci se odnose na proizvodne godine; **UG=uvjetna grla; *** Stanje na 30.11.2020.

Izvor: Interni podatci APPRRR, 2021.

Tablica 4. Isplaćene potpore za dobrobit životinja u kozarstvu i ovčarstvu u 2019. godini

Operacija		14.1.4. Dobrobit životinja u kozarstvu			
NUTS 2 regija		Panonska Hrvatska	Grad Zagreb	Jadranska Hrvatska	Sjeverna Hrvatska
2019.	Broj korisnika mjere	15	0	37	13
	Broj gospodarstava u Hrvatskoj	1707	70	8707	772
	Udio korisnika	0,88 %	0,00 %	0,42 %	1,68 %
	Broj UG**	158,17	0,00	637,75	127,79
	Odobreni iznos potpore u HRK	51.339,20	0,00	364.607,00	56.460,67
Ukupno isplaćeni iznos potpore u HRK***		51.339,19	0,00	363.585,49	56.038,36
		14.1.5. Dobrobit životinja u ovčarstvu			
2019.	Broj korisnika mjere	220	10	193	20
	Broj gospodarstava u Hrvatskoj	9181	79	6868	2552
	Udio korisnika	2,40 %	12,66 %	2,81 %	0,78 %
	Broj UG**	4.227,10	314,19	4.664,08	259,59
	Odobreni iznos potpore u HRK	2.225.755,83	121.000,81	2.345.238,59	134.899,54
Ukupno isplaćeni iznos potpore u HRK***		2.222.088,33	121.000,79	2.344.542,63	134.818,75

* Podaci se odnose na proizvodne godine; **UG=uvjetna grla; *** Stanje na 30.11.2020.

Izvor: Interni podatci APPRRR, 2021.; MP, 2020.

Ukupno gledano, za 2018. i 2019. godinu, najviše potpora za M14 je isplaćeno u Osječko-baranjskoj županiji (46.390.118,34 kn), a slijede Bjelovarsko-bilogorska (12.313.400,57 kn) i Vukovarsko-srijemska (10.180.492,56 kn) (APPRRR, 2021.). Iako su iznosi potpora stimulativni, M14 je u Hrvatskoj vrlo loše iskorištena. Kako se navodi u izvješću Italian Rural Network 2014-2020 (2020.), u Hrvatskoj je iskorišteno svega 23 % sredstava predviđenih za navedenu mjeru. Na začelju se uz Hrvatsku nalaze Cipar (12 %), Bugarska (11 %) i Grčka (0 %), dok je vodeća u iskorištavanju sredstava Mađarska (86 %).

Zaključak

Poljoprivrednici bi u većoj mjeri trebali koristiti M14 jer osim poboljšanja dobrobiti životinja na gospodarstvu povećavaju i svoje prihode. Do 30.11.2020. godine najviše je sredstava isplaćeno u sektoru govedarstva, a najmanje u kozarstvu. Promatrajući po

županijama, najviše je sredstava isplaćeno u Osječko-baranjskoj, dok u Dubrovačko-neretvanskoj nije bilo isplata za M14. Najveći obuhvat korisnika M14 je u govedarstvu (3,90 %), a najmanji u svinjogojstvu (0,26 %).

Napomena

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Literatura

Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (2021). Interni podatci o isplaćenim potporama za M14 Dobrobit životinja.

Bennett R.M. (1997). Farm animal welfare and food policy. Food Policy. 22 (4): 281-288.

Italian Rural Network 2014-2020 (2020). Animal welfare in the Rural development programme for the 2014-2020 period in the European Union. Raspoloživo: https://enrd.ec.europa.eu/sites/default/files/enrd_publications/animal_welfare_in_the_rural_development_programme_for_the_2014_2020_period_in_the_eu.pdf

Broom D.M. (2010). Animal welfare: an aspect of care, sustainability, and food quality required by the public. Journal of veterinary medical education. 37(1): 83-88.

Broom D.M. (2017). Animal Welfare in the European Union. Raspoloživo: https://www.europarl.europa.eu/RegData/etudes/STUD/2017/583114/IPOL_STU(2017)583114_EN.pdf

De la Fuente M.F.C., Souto A., Caselli C.B., Schiel N. (2017). People’s perception on animal welfare: why does it matter? Ethnobiology and Conservation. 6 (18): 1-7.

Europski revizorski sud (2018). Dobrobit životinja u EU-u: premošćivanje jaza između ambicioznih ciljeva i praktične primjene. Raspoloživo: https://op.europa.eu/webpub/eca/special-reports/animal-welfare-31-2018/hr/

Ministarstvo poljoprivrede (2020). JRDŽ – broj domaćih životinja. Raspoloživo: https://hpa.mps.hr/jrdz-izvjestaji/broj-domacih-zivotinja/

Ministarstvo poljprivrede (2021). Kako ostvariti potporu za mjeru M14 dobrobit životinja iz programa ruralnog razvoja Republike Hrvatske za razdoblje 2014. – 2020.? Raspoloživo: https://ruralnirazvoj.hr/files/MPS-Brosura-200x275-Kako-ostvariti-potporu-za-mjeru-14.pdf

World Organization for Animal Health (OIE) (2008). Wildlife. Buletin 2008-3. Raspoloživo: https://www.oie.int/app/uploads/2021/03/bull-2008-3-eng.pdf

Animal welfare measures in Croatia in the programe period 2014-2020

Abstract

In order to improve the conditions of keeping and breeding in intensive livestock systems, the Croatian Rural Development Program for the period 2014-2020 included measure 14 Animal welfare, the implementation of which began in May 2018. The aim of the paper was therefore to determine which operations are covered by the M14 and their use in Croatia. Most funds within the M14 for 2018 and 2019 were paid in cattle breeding (HRK 68,080,673.37), and the least in goat breeding (HRK 941,926.08). Among the counties, Osijek-Baranja leads (HRK 46,390,118.34), while nothing has been paid in Dubrovnik-Neretva. The utilization of funds at the state level is very low (23%), which indicates the low popularity of the observed measure.

Key words: animal welfare, measure 14, Rural development program, use of funds

IZVORNI ZNANSTVENI RAD

Proizvodno ekonomski pokazatelji u tovu brojlera

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Sažetak

Cilj rada bio je vrednovanje utjecaja različitih hranidbenih tretmana u tovu brojlera na efikasnost primijenjene hranidbe. U istraživanju su upotrijebljeni Ross 308 pilići (N=120) koji su bili podijeljeni u 4 skupine. Hranidbeni tretmani razlikovali su se u finišer smjesama koje su osim ostalih krmiva sadržavale i 5 % ulja i to: T1 suncokretovo, T2 sojino, T3 repičino i T4 laneno ulje. Smjese su bile izbalansirane na razini 20 % sirovih proteina i 13,5 MJ Kg/Me. Sve skupine pilića nalazile su se u istom peradnjaku, a hranjenje i napajanje bilo je *ad libitum*. U radu su istraženi proizvodno ekonomski pokazatelji: Indeks proizvodne efikasnosti (PEI), Europski brojlerski indeks (EBI), Indeks ekonomske efikasnosti (EEI) i indeks troškova hrane (CIF). Efikasnost proizvodnih indeksa bila je sljedeća (PEI i EBI): T1 363 i 351, T2 347 i 335, T3 362 i 351 i T4 367 i 359. PEI pokazatelji ovisili su u većoj mjeri o završnoj masi brojlera i konverziji hrane. Vrijednosti EBI slijedile su vrijednosti PEI pokazatelja. Za komparaciju produktivnosti performansi više tretmana (skupina) brojlera prikladni su PEI i EBI pokazatelji. Ekonomska efikasnost i troškovi hrane mogu se prikazati pomoću vrijednosti EEI i CIF pokazatelja.

Ključne riječi: brojleri, proizvodna efikasnost, ekonomska efikasnost, Ross 308

Uvod

Unapređenje proizvodnih obilježja suvremenih hibrida brojlera zahtijeva podmirenje hranidbenih potreba kako bi se proizvodni potencijal u potpunosti iskoristio. Izbor hranidbenog plana ovisi o preporukama selekcijskih tvrtki za svaki hibrid pilića (Martinez i Valdivie, 2021.; Eila i sur., 2012.). Hrana sudjeluje u tovu s najvećim udjelom troškova i direktno utječe na produktivnost i ekonomičnost proizvodnje (Moosavi i sur. 2011.). Na efikasnost rasta brojlera utječu: tjelesna masa, prosječni dnevni prirast, konzumacija i konverzija hrane. Način hranidbe najbitniji je čimbenik pri smanjenju troškova proizvodnje. Troškovi hrane čine 55-70 % ukupnih troškova pri proizvodnji pilećeg mesa (Aggrey i sur., 2010.). Poboljšanje iskorištavanja hrane skraćuje period tova brojlera i smanjuje proizvodne troškove (Marcu i sur., 2013.). Rad predstavlja nastavak ranijih istraživanja o proizvodnim karakteristikama brojlera u tovu pri uporabi različitih hranidbenih tretmana (Kralik, 2007.; Kralik i sur., 2012.). Cilj rada bio je rangiranje brojlera prema proizvodnim i ekonomskim pokazateljima u pokusu s više hranidbenih tretmana.

Materijal i metode

Za istraživanje su upotrijebljeni Ross 308 brojleri (N=120) koji su slučajnim izborom podijeljeni u 4 skupine. Hranidbeni tretmani razlikovali su se u sastavu finišer smjesa koje su osim ostalih krmiva sadržavale i 5 % biljnih ulja i to: T1 suncokretovo, T2 sojino, T3 repičino i T4 laneno ulje. Smjese su bile izbalansirane na razini 20 % sirovih proteina i 13,5 MJ ME/kg. Sve skupine pilića uzgajane su u istom peradnjaku, a hranjenje i napajanje bilo je *ad libitum*. Pri istraživanju se koristio podni sustav tova u trajanju od 42 dana. Tijekom tova istraživani su sljedeći pokazatelji: tjelesna masa pilića (kg), prosječni dnevni prirast (g),

konzumacija smjesa (kg), konverzija hrane u prirast (kg) i preživljavanje pilića (%). Proizvodno ekonomski pokazatelji izračunati su (Marcu i sur., 2013.; Martins i sur., 2016.) na sljedeći način:

Indeks proizvodne efikasnosti (PEI)
PEI=tjelesna masa (kg) x preživljavanje (%) / trajanje tova (d) x konverzija (kg) x100

Europski brojlerski indeks (EBI)
EBI=(preživljavanje (%) x dnevni prirast (g)) / (konverzija hrane (kg) x 10)

Indeks ekonomske efikasnosti (EEI)
EEI=LC_a / CT_{ai} tj. najniža cijena koštanja hrane/kg mase između tretmana / cijena koštanja hrane od $_{ai}$ tretmana.

Indeks troškova hrane (CIF)
CIF=CT_{ai} x 100/LC_a

Rezultati i rasprava

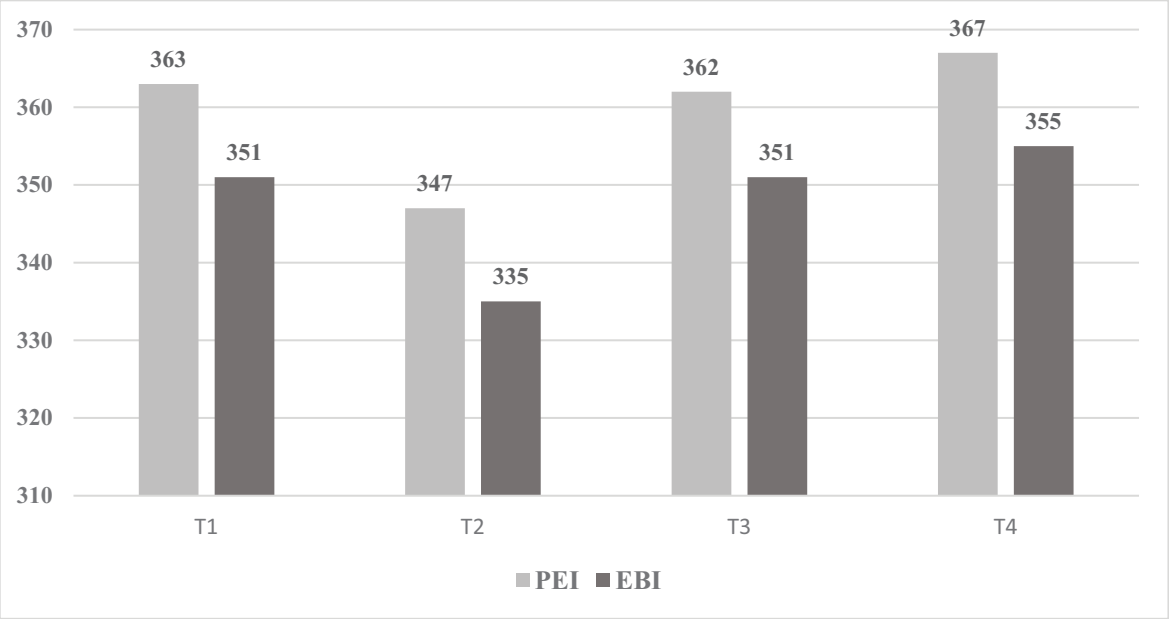
Proizvodni rezultati brojlera prema hranidbenim tretmanima (T1, T2, T3 i T4) prikazani su u Tablici 1. Završne tjelesne mase brojlera razlikovale su se između tretmana. Konzumacija smjesa (starter + finišer) i prosječni dnevni prirasti utjecali su na konverziju smjesa u prirast žive mase.

Tablica 1. Proizvodne karakteristike brojlera

Opis	Tretmani - skupine			
	T1	T2	T3	T4
Živa masa (kg)	2,71	2,68	2,83	2,81
Dnevni prirast (g)	64,90	63,81	67,31	66,83
Konzumacija hrane (kg)	4,19	4,61	4,77	4,57
Konverzija hrane (kg)	1,78	1,84	1,86	1,82
Preživljavanje (%)	96,77	96,77	96,77	96,77

Najveću završnu masu postigli su brojleri T3 tretmana (2,83 kg), zatim slijede T4, T1 i T2 tretmani (2,81, 2,71 i 2,68 kg). Najbolju konverziju hrane u prirast žive mase (kg kg^{-1}) ostvarili su brojleri T1 tretmana (1,78 kg), zatim slijede T4, T2 i T3 (1,82, 1,84 i 1,86 kg) tretmani. Preživljavanje brojlera bilo je jednako kod svih hranidbenih tretmana (96,77 %). Eila i sur. (2011.) navode da selekcioneri peradi nastoje proizvesti genotipove koji će postići u tovu živu masu prema zahtjevima tržišta u što kraćem vremenskom razdoblju. Martinez i Valdivie (2021.) navode da su Ross 308 brojleri pogodni za proizvodnju mesa. Za 35 dana tova postižu 1644-1996 g žive mase uz konverziju hrane 1,50-1,71 kg za kg žive mase, a preživljavanje se kreće 86-95 %. El-Tahawy i sur. (2017.) postigli su s Ross 308 hibridom brojlera bolje rezultate preživljavanja (98 %), lošiju konverziju hrane (2,02 kg) uz prosječnu masu 1884 g za 35 dana tova, u odnosu na rezultate ovog istraživanja. Slične proizvodne rezultate za navedeni hibrid pilića navode Irfan i sur. (2017.) za 35 dana tova. Javid i sur. (2012.) postigli su u tovu Ross 308 pilića za 49 dana, 2256 g žive mase i 2,18 kg konverziju hrane. Komparacija rezultata istraživanja u radu s rezultatima navedenih autora pokazuje različitost očitovanja genetskog potencijala Ross 308 hibrida pilića, ovisno o proizvodnim čimbenicima i primijenjenom managementu. Različiti hranidbeni tretmani rezultirali su odgovarajućim proizvodno ekonomskim pokazateljima. PEI i EBI pokazatelji prema Martinis i sur. (2013.) kreću se od 97,32 -100, odnosno EBI od 100-102,75. PEI vrijednost

je u većoj mjeri ovisila o završnoj masi brojlera i konverziji hrane. Najveća vrijednost ustanovljenja je kod T4 (367) tretmana, zatim slijede T1, T3 i T2 (363, 362 i 347) tretmani. Vrijednosti EBI slijedile su vrijednosti EPI pokazatelja (Grafikon 1).



Grafikon 1. Indeks proizvodne efikasnosti (PEI) i europski brojlerski indeks (EBI)

Cijena troškova kg smjese ovisila je o sastavu smjesa odnosno vrsti upotrijebljenih ulja, obzirom da su sva ostala krmiva u smjesama bila jednaka. Dijeljenjem cijene koštanja hrane (€) i ostvarene završne mase brojlera, utvrđena je cijena koštanja konzumiranih smjesa (Tablica 2).

Tablica 2. Troškovi hrane i indeksi ekonomske efikasnosti

Opis	Tretmani- skupine			
	T1	T2	T3	T4
Smjesa €/kg tjelesne mase	0,85	0,90	0,88	0,89
EEI	100	94,8	96,7	95,4
CIF	100	105,4	103,4	104,6

Vrijednost pokazatelja EEI bila je najveća kod T1 tretmana (100), a najmanja kod T2 tretmana (94,8). Najveća vrijednost CIF pokazatelja bila je kod T2 tretmana, a zatim slijede T4, T3 i T1 tretmani (103,4, 104,6 i 100). Za komparaciju proizvodnih pokazatelja više tretmana u pokusu, prikladni su PEI i EBI pokazatelji. Ekonomska efikasnost konzumirane hrane može se prikladno pokazati pomoću EEI i CEF pokazatelja u cilju međusobne usporedbe tretmana. EEI i CIF pokazatelji su relativne vrijednosti jer se prilikom njihova izračuna stavljaju u odnos troškovi hrane (najniža vrijednost) u odnosu na iste troškove svakog sljedećeg tretmana. Buyakov i sur. (2021.) navode da je prosječna profitabilnost u tovu Ross 308 hibrida brojlera u granicama kao kod ostalih hibrida. Troškovi hrane u tovu brojlera mogu postići i do 70 % od ukupnih troškova (Moosavi i sur., 2011). Marcu i sur. (2013.) preporučuju pri usporedbi efikasnosti različitih tretmana izračun indeksa proizvodne efikasnosti PEI i EBI. Autori su ustanovili da se PEI vrijednosti kreću od 346,09 do 376,61, a EBI vrijednosti od 340,45 do 370,23. EBI pokazatelj je prikladan prilikom vrednovanja različite dobi brojlera pri klanju, istog genotipa.

Zaključak

U radu se vrednuju proizvodno ekonomski pokazatelji četiri različita hranidbena tretmana u tovu Ross 308 brojlera. Tretmani su se razlikovali u vrsti dodanog ulja u finišer smjese (T1 suncokretovo, T2 sojino, T3 repičino i T4 laneno ulje). Istraženi su: Indeks proizvodne efikasnosti (PEI), europski brojlerski indeks (EBI) kao i indeksi ekonomske efikasnosti tova i indeksi troškova hrane (EEI i CIF). Najveći PEI indeks u tovu brojlera ostvaren je kod T4, a najmanji kod T2 hranidbenog tretmana. Indeks ekonomske efikasnosti (EEI) bio je najveći kod T1, a najmanji kod T3 tretmana. Istraživanje je pokazalo kako su prikazani proizvodno ekonomski indeksi prikladni za simultanu usporedbu više hranidbenih tretmana u istraživanju.

Literatura

Aggrey S.E., Karnuah A.B., Sebastian B., Anthony N.B. (2010). Genetic properties of feed efficiency parameters in meat-type chickens. Genetics Selection Evolution. 42(1): 1-5.

Buyarov V., Medanova V., Buyarov A., Andreeva O. (2021). Tehnological and economic aspects of industrial production of broiler meat. Conf. Series: Earth and Environmental Science. 941: 1-9.

Eila N., Lavvaf A.G., Farahvash T., Zarei A. (2012). Incluence of Various Growth Patterns on Preformance of Ross 308 Broilers. Pakistan Veterinary Journal. 32 (3): 372-374.

El-Tahawy A.A.S., Taha A.E., Adel S.A. (2017). Effect of flock size on the productive and economic efficiency of Ross 308 and Cobb 500 broilers. European Poultry Science. 81: 1-10.

Irfan M., Mehmood S., Mahmud A., Hussain,J., Shaheen M.S., Ahmad S., Zia M.W. (2019). Effect of different bedding materials on growth performance, physiological response and economic efficiency in three commercial broiler strains. Indian Journal of Animal Research. 53 (4): 545-550.

Javid I., Asghar A., Tanveer A., Shamsul H., Sohail K. (2012). Comparative performance of different economic traits of four imported broiler strains under local conditions of Pakistan. Pakistan Journal of Agricultural Research. 25 (1): 76-82.

Kralik G. (2007). Omega pilići TP-03/0079. Osijek: Poljoprivredni fakultet u Osijeku, Sveučilište J.J. Strossmayera Osijek. 1-137.

Kralik G., Pavić V., Galović D., Škrtić Z., Kralik Z., Has-Schön E. (2012). Fatty acid composition and lipid and protein oxidation products in muscles of broilers fed with different plant oils. Arch Geflugelkund. 76 (4): 259-69.

Marcu A., Vacaru-Opriş I., Dumitrescu G., Ciochină L. P., Marcu A., Nicula M., Mariş C. (2013). The influence of genetics on economic efficiency of broiler chickens growth. Animal Science and Biotechnologies. 46 (2): 339-346.

Martinez Y., Valdivie M. (2021). Efficiency of Ros 308 broilers under different nutritional requirements. Journal of Applied Poultry Research. 30 (1): 210-223.

Martins J.M.S., Carvalho C.M.C, Litz F.H., Silveira M.M., Moraes C.A., Silva M.C.A., Fagundes N.S., Fernandes E.A. (2016). Productive and economic performance of broiler chickens subjected to different nutritional plans. Brazilian Journal of Poultry Science. 18 (1): 209-216.

Mossavi M., Eslami M., Chaji M., Boujarpour M. (2011). Economic value of diets whit different levels of energy and protein whit constant ratio on broiler chickens. Journal of Animal and Veterinary Advances. 10: 709-711.

Production and economic indicators in broiler fattening

Abstract
The aim of this research was to evaluate the impact of various feeding treatments in broiler fattening on the efficiency of applied feed. Ross 308 chickens (N = 120), which were divided into 4 groups, were used in the research. Feeding treatments differed in finisher mixtures which, in addition to other feeds, also contained 5% oil, namely: T1 sunflower, T2 soybean, T3 rapeseed and T4 flaxseed oil. The mixtures were balanced at the level of 20% crude protein and 13.5 MJ kg/Me. All groups of chickens were housed in the same poultry pen with water and feed provided ad libitum. The paper examines production and economic indicators: Productive efficiency index (PEI), European broiler index (EBI), Economic efficiency index (EEI) and Cost index (CIF). The efficiency of production indices was as follows (PEI and EBI): T1 363 and 351, T2 347 and 335, T3 362 and 351 and T4 367 and 359. PEI indicators depended largely on broiler final weight and feed conversion. EBI values followed PEI values. PEI and EBI indicators were used to compare the productivity of the performance of several treatments (groups) of broilers. Economic efficiency and feed costs can be presented by the values of EEI and CIF indicators.

Key words: broilers, trait efficiency, economic efficiency, Ross 308

IZVORNI ZNANSTVENI RAD

Pregled tržišta ječma u Republici Hrvatskoj primjenom modela parcijalne ravnoteže

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Sažetak

Predmet rada je modelska ocjena, odnosno pregled (eng. *outlook*) tržišta ječma u Republici Hrvatskoj do 2030. godine primjenom AGMEMOD modela parcijalne ravnoteže. Modelom su simulirana buduća kretanja zasijanih površina, prinosa, proizvodnje, uvoza, izvoza i cijena ječma pod *ceteris paribus* tržišnim uvjetima uz postojeće mjere i instrumente Zajedničke poljoprivredne politike. Modelska simulacija ukazuje na nastavak pozitivnih kretanja na tržištu ječma u Hrvatskoj do kraja simuliranog perioda koji se očituju poglavito u rastu prinosa i volumena proizvodnje.

Ključne riječi: pregled tržišta, AGMEMOD, ječam, Republika Hrvatska

Uvod

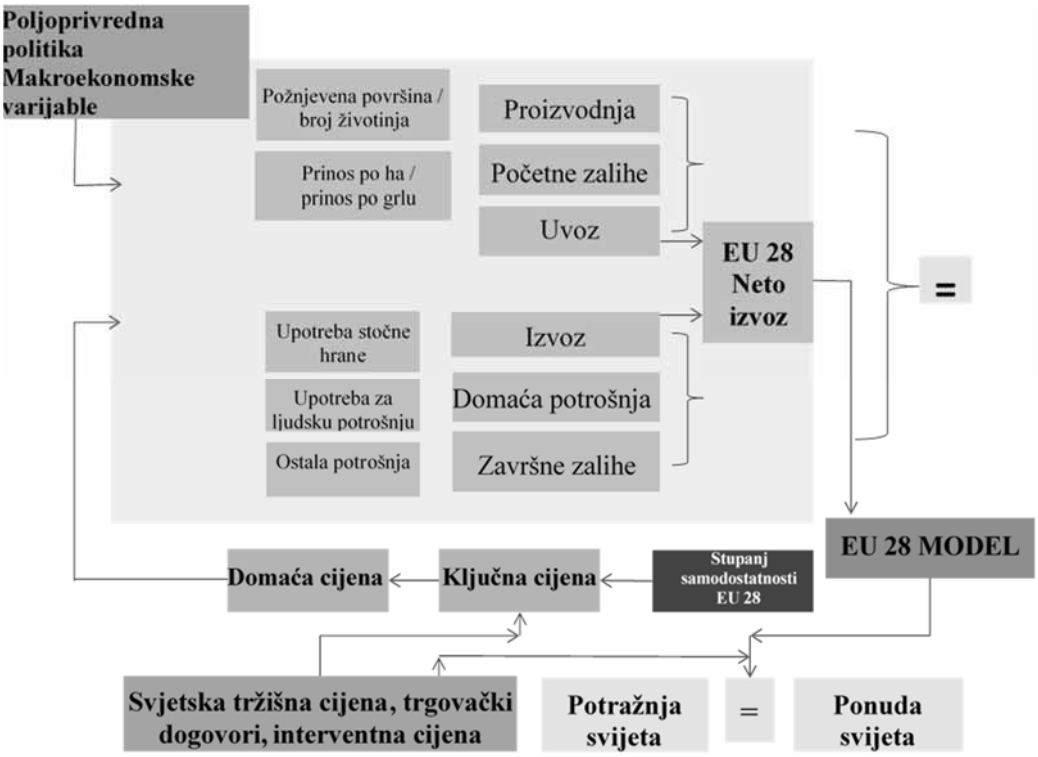
Ječam predstavlja jednu od najstarijih ratarskih kultura, a zbog svoje hranjive vrijednosti i nekih drugih karakteristika prepoznat je u proizvodnji stočne hrane, farmaceutskoj, tekstilnoj i pivarskoj industriji (Hornsey, 2003.; Martinčić i Kolak, 1993.). Od ulaska Hrvatske u Europsku uniju do 2020. godine, zasijane površine ječmom u prosjeku su iznosile 51,8 tisuća ha, a proizvodnja ječma u prosjeku je iznosila 235 tisuća tona uz prosječan prinos od 4,52 t ha^{-1}. U navedenom razdoblju proizvođačka cijena ječma u Hrvatskoj je bila stabilna, te je u prosjeku iznosila 111,78 € t^{-1}. Cilj rada je ekonometrijskim modelom parcijalne ravnoteže izraditi pregled tržišta ječma do 2030. godine uz simulaciju budućih kretanja zasijanih površina, proizvodnje, prinosa, izvoza i uvoza ječma u Republici Hrvatskoj.

Materijal i metode

AGMEMOD (eng. *Agricultural Member State Modelling*) je ekonometrijski, dinamički, višeproizvodni model parcijalne ravnoteže. Osnovni cilj AGMEMOD modela je izrada srednjoročnih pregleda tržišta ključnih odabranih poljoprivrednih proizvoda do 2030. godine. Nacionalni predložak AGMEMOD modela izrađen je prema predlošku autora (Hanrahan, 2001.).

Osnovni podatci koji služe za izradu pojedinih pregleda tržišta poljoprivrednih proizvoda su proizvodno potrošačke bilance odnosno podatci o proizvodnji, potrošnji, uvozu i izvozu promatranog tržišta do 2020. godine. Kao izvor podataka koriste se nacionalne i EU baze podataka za države članice (DZS, MPS, Eurostat). Model uspješno prikazuje odnose ponude i potražnje na promatranom tržištu kroz ekonometrijske bihevioralne jednadžbe s pripadajućim koeficijentima (Chatreuil i sur., 2012.). U odnose ponude i potražnje također su ugrađene egzogene varijable, odnosno podatci o političkim instrumentima, makroekonomskim varijablama i projekcijama svjetskih tržišnih cijena. Prilikom izrade pregleda tržišta ječma u Hrvatskoj bilo je potrebno kalibrirati ekonometrijski procijenjene

jednadžbe, a zatim zajedno s ulaznim podatcima se pristupa izradi rezultata modela. Osnovna struktura AGMEMOD modela prikazana je na Slici 1.



Slika 1. Osnovna struktura AGMEMOD nacionalnog modela
Izvor: Chantreuil i sur., 2010.

Prikazan je opći oblik ekonometrijskih jednadžbi korišten prilikom izrade pregleda tržišta ječma u Republici Hrvatskoj.

Određivanje udjela površine kulture *k* koja pripada grupi *i* ($sh_{i,t}^{k}$) iskazujemo kao:

$$sh_{i,t}^{k} = f\left(p_{i,t-1}^{k}, sh_{i,t-1}^{k}\right) \qquad j,k = 1,\dots,n \tag{1}$$

Jednadžbe prinosa kulture *k* koja se nalazi u grupi kultura *i* računamo prema:

$$r_{i,t}^{k} = f\left(p_{i,t-1}^{j}, r_{i,t-1}^{k}, V\right) \qquad j,k = 1,\dots,n \tag{2}$$

pri čemu je:

$r_{i,t}^{k}$- prinos kulture *k* koja se nalazi u grupi kultura *i*, *V* - vektor, odnosno egzogena varijabla koja može utjecati na prinos kulture *k* (npr. politički instrument).

Jednadžbe uvoza i izvoza generalno u ratarskom modelu imaju sljedeći oblik:

$$Im_{i,t}^{k} = f(PR_{i,t}^{k}, DU_{i,t}^{k}, Im_{i,t-1}^{k}) \tag{3}$$

$$Ex_{i,t}^{k} = f(PR_{i,t}^{k}, DU_{i,t}^{k}, Ex_{i,t-1}^{k}) \tag{4}$$

Pri čemu je:

$Im_{i,t}^{k}$ i $Ex_{i,t}^{k}$- izvoz i uvoz kulture *k* koja se nalazi u grupi kultura *i* u godini *t*; $PR_{i,t}^{k}$ i $DU_{i,t}^{k}$ - označava proizvodnju i ukupnu domaću potrošnja kulture *k* koja se nalazi u grupi kultura *i* u godini *t*; odnosno $Im_{i,t-1}^{k}$i $Ex_{i,t-1}^{k}$- predstavljaju uvoz i izvoz kulture *k* koja se nalazi u grupi kultura *i* u godini *t-1*.

Cijene pojedinih poljoprivrednih proizvoda različito se definiraju s obzirom je li nacionalno tržište proizvoda ključno tržište (*key market*) s ključnom EU cijenom (*key price*) ili nije. Naime, poljoprivredni proizvodi Republike Hrvatske nemaju dovoljne razine proizvodnje

kojom bi utjecali na europsku cijenu, te se ravnotežna cijena na hrvatskom tržištu za sve proizvode iskazuje kao:

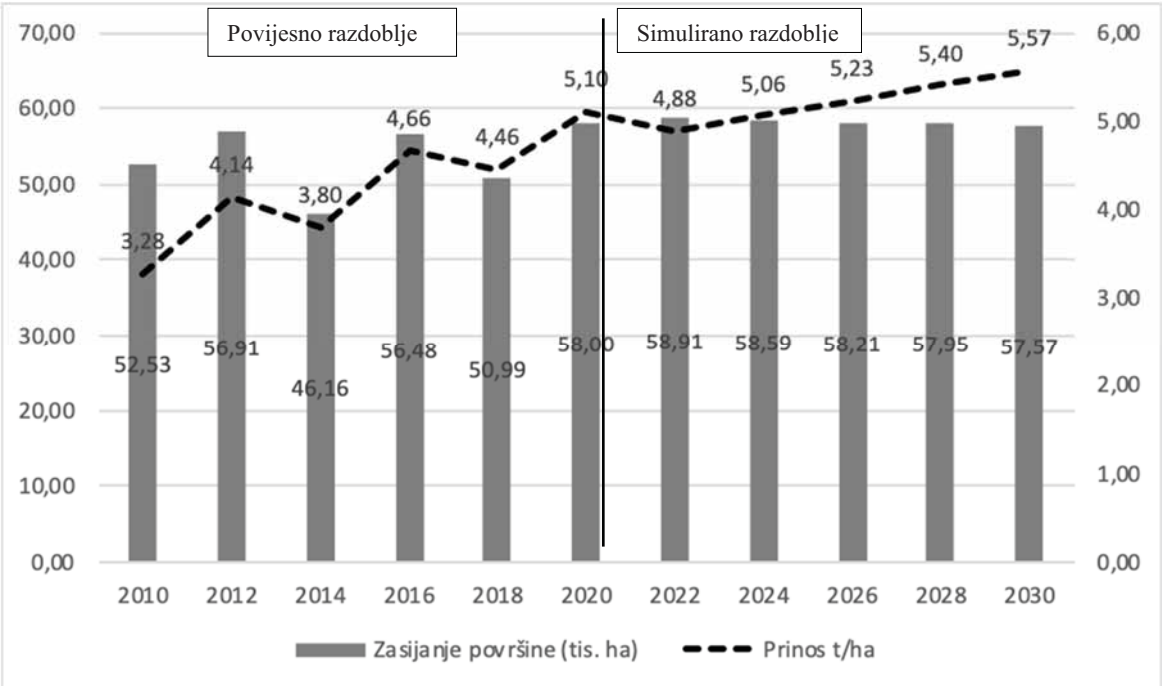
$$p_{i,t} = f\left(Kp_{i,t}, p_{i,t-1}, ssr_{i,t}, Kssr_{i,t}, V\right) \qquad i = 1, \dots, n \qquad (5)$$

Gdje je $p_{i,t}$ domaća cijena kulture *i* u godini *t*, $Kp_{i,t}$ označava ključnu cijenu kulture *i* u istoj godini *t*, $ssr_{i,t}$ predstavlja stupanj samodostatnosti Hrvatske za kulturom *i* u godini *t*, , $Kssr_{i,t}$ je EU stupanj samodostatnosti za kulturu *i* u godini *t*, te *V* predstavlja vektor egzogenih varijabli koji može utjecati na domaću cijenu kulture *i*.

Više detalja o ekonometrijskim bihevioralnim jednadžbama korištenim u AGMEMOD pristupu modeliranja i strukturi uključenih jednadžbi nalazi se u istraživanjima Salamon i sur. (2008.), Erjavec i Donnellan (2005.), te Chantreuil i sur. (2005.). Pregled tržišta ječma do 2030. godine izrađen je pod *ceteris paribus* pretpostavkom i trenutnom strukturom mjera i instrumenata ZPP-a 2015-2022.

Rezultati i rasprava

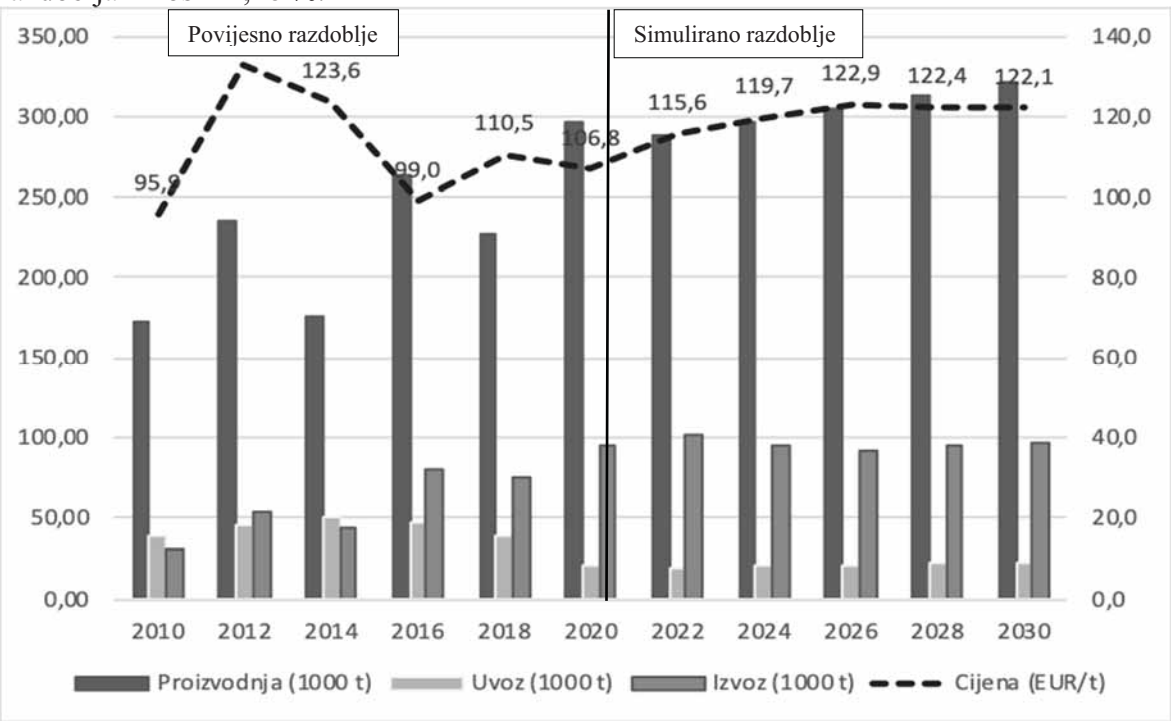
Rezultati modelske simulacije ukazuju na povećanje prinosa ječma u Hrvatskoj do 2030. godine, a očekuje se kako će se površine zasijane ječmom kretati otprilike približno sadašnjim vrijednostima. Do kraja simuliranog razdoblja očekuje se otprilike 5,5 t ha^{-1} prinosa ječma (Grafikon 1).



Grafikon 1. Pregled zasijanih površina i prinosa ječma do 2030. godine
Izvor: AGMEMOD v9.3

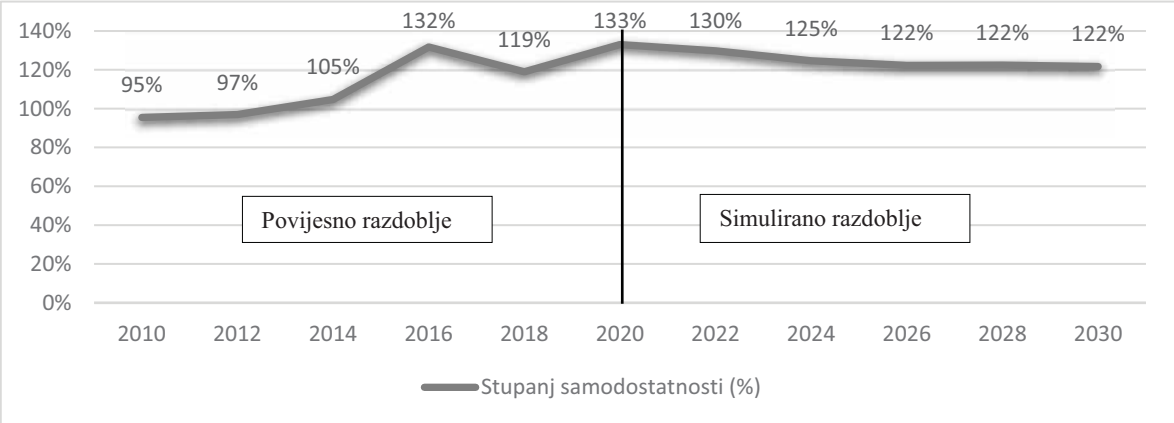
Potražnja ječma na domaćem i EU tržištu je stabilna uz tendenciju blagog rasta potražnje zbog većih potreba prehrambeno-prerađivačke industrije, a s obzirom na očekivani rast prinosa ječma, za očekivati je rast domaće proizvodnje u narednom periodu (Grafikon 2). Proizvodnja ječma sukladno očekivanom rastu prinosa uz nepromijenjene razine zasijanih površina, očekuje rast proizvodnje od 8,29 % do 2030. godine u odnosu na 2020. godinu. S obzirom na pozitivne proizvodne pokazatelja očekuje se blago povećanje izvoza ječma za 1,69 % do kraja simuliranog razdoblja. Uvoz će također rasti, kao posljedica poslovanja na jedinstvenom EU tržištu za otprilike 9 %. Cijena ječma na domaćem tržištu očekuje rast u

narednom razdoblju, a prema simuliranim rezultatima rast cijena ječma do kraja simuliranog razdoblja iznosi 14,28 %.



Grafikon 2. Pregled proizvodnje, izvoza, uvoza i cijene ječma do 2030. godine
Izvor: AGMEMOD v9.3

S obzirom na prethodne rezultate modelske simulacije očekuje se kako će stupanj samodostatnosti ječma u Republici Hrvatskoj iznositi 122 % do 2030. godine (Grafikon 3).



Grafikon 3: Simulirani stupanj samodostatnosti ječmom do 2030. godine
Izvor: AGMEMOD v9.3

Zaključak

AGMEMOD modelom parcijalne ravnoteže simuliran je srednjoročni pregled razvoja tržišta ječma u Hrvatskoj do 2030. godine. Kao pretpostavka modeliranju koriste se *ceteris paribus* tržišni uvjeti uz pretpostavku nastavka postojeće strukture agrarne politike do kraja simuliranog razdoblja, te bez većih tržišnih šokova. Prema navedenim pretpostavkama, rezultati modela ukazuju na stagnaciju zasijanih površina, povećanje prinosa, proizvodnje i izvoza ječma uz osjetan rast domaćih proizvođačkih cijena do 2030. godine. No treba uzeti u obzir kako pristup modeliranja koristi postojeće mjere i instrumente Zajedničke poljoprivredne politike 2015-2022. Nakon 2022. godine doći će do promjene mjera i

instrumenata koji će zasigurno rezultirati promjenama na tržištima poljoprivrednih proizvoda. Nadalje, modeli ovakvog tipa su limitirani, jer nisu u mogućnosti u potpunosti uključiti svu volatilnu prirodu poljoprivrednih tržišta poput naglih tržišnih šokova uzrokovanih klimatskim promjenama, pojavom pandemija, rata i prirodnih katastrofa.

Literatura

Chantreuil F., Levert F., Hanrahan K.F. (2005). The Luxembourg Reform of the CAP: An Analysisusing AGMEMOD Composite Model. In: Modelling agricultural policies: state of the art and new challanges. Proceedings of the 89th EAAE Seminar, Arfini F. (ed.), 632-652. Parma, Italy: University of Parma.

Chantreuil F., Salputra G., Erjavec E. (2010). Impact analysis of direct payments using Agmemod model, PowerPoint prezentacija na Agripolicy meeting. Travnja, Struga, Makedonija, 21-22.

Chantreuil F., Hanrahan K., van Leeuwen M. (eds) (2012). The future of EU agricultural markets by AGMEMOD. Springer: Dordrecht.

Erjavec E., Donnellan T. (2005). Development of the AG-MEMOD Country Level Agricultural Policy Analysis Tool in the New Members States of EU. Paper presented at the 89th EAAE Seminar. Parma, Italy.

Hanrahan K.F. (2001.). The EU Gold Model manual. Mimeo Rural Economy Research Centre, Teagasc. Dublin.

Hornsey I. S. (2003). A history of beer and brewing. Royal Society of Chemistry.

Martinčić J., Kolak, I. (1993). Ječam-Hordeum vulgare L. Conv. Distichum, sirovina za potrebe industrije slada i piva. Sjemenarstvo. 10 (3-4): 163-172.

Salamon P., Chantreuil F., Donnellan T., Erjavec E., Esposti R., Hanrahan K., van Leeuwen M., Bouma F., Dol W. (2008). How to deal with the challenges of linking a large number of individual national models: the case of the AGMEMOD Partnership. Agrarwirtschaft. 57 (8): 373-378.

Outlook on Croatian barley market using a partial equilibrium model

Abstract

Paper presents a medium-term outlook of barley market in Croatia up to 2030. Using the AGMEMOD partial equilibrium model, we simulated future developments of sown areas, yields, production, imports, exports and barley prices under *ceteris paribus* market conditions. The modelling results indicate the continuation of the positive barley market developments in Croatia by the end of the simulated period, which are manifested mainly in the yield growth and increase in production volumes.

Key words: market outlook, AGMEMOD, barley, Republic of Croatia

IZVORNI ZNANSTVENI RAD

Raspolaganje državnim poljoprivrednim zemljištem: zakonska regulativa i primjena

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Sažetak
Ciljevi rada su, temeljem analize sadržaja sekundarnih izvora, opisati glavne elemente zakonske regulative po pitanju raspolaganja poljoprivrednim zemljištem u vlasništvu države i utvrditi čimbenike koji sprječavaju učinkovitu primjenu u praksi. Budući da više od polovice poljoprivrednog zemljišta u državnom vlasništvu u Hrvatskoj nije aktivirano, prioritet države je povećati korištenje i aktivirati neobrađeno poljoprivredno zemljište. Osim nacionalne zemljišne politike rad uzima u obzir i primjere drugih zemalja članica EU u kontekstu zemljišnog zakonodavstva. U odnosu na stare, nove zemlje članice imaju uglavnom strože zakonodavstvo koje regulira tržište poljoprivrednim zemljištem.

Ključne riječi: poljoprivredno zemljište, raspolaganje, zakonodavstvo

Uvod

Poljoprivredno zemljište je važan resurs za proizvodnju hrane i razvoj ruralnih područja, ali i za društveni razvoj općenito. Prepoznajući potrebu da se zaštite i održe neke od gospodarskih, društvenih i ekoloških dobrobiti, načini raspolaganja i tržište poljoprivrednim zemljištem podliježu različitim propisima u zemljama svijeta, kao i diljem EU-a (Zróbek-Rózanska i Zielinska-Szczepkowska, 2019.). Općenito, nacionalni zakoni o zemljištu koji reguliraju transakcije sa zemljištem služe različitim ciljevima, na primjer zadržavanje zemljišta u poljoprivrednoj proizvodnji, zadržavanje ruralnog stanovništva, rješavanje rascjepkanosti zemljišta, jačanje pozicije domaćeg poljoprivrednika u odnosu na stranog investitora te sprječavanje otimanja zemljišta (Godžirov, 2020.).

Povijest razvoja, nasljeđa i zemljišnih reformi u socijalističkom i tranzicijskom razdoblju Hrvatske dovela je do određenih posebno nepovoljnih uvjeta koji se ogledaju u veličini gospodarstva i rascjepkanosti zemljišta. Od Drugoga svjetskog rata privatno vlasništvo nad obiteljskim gospodarstvima bilo je ograničeno raznim agrarnim reformama, poreznom politikom za mala obiteljska gospodarstva, pokušajima kolektivizacije i naknadnom kupnjom zemljišta od privatnih vlasnika kako bi se povećao broj i veličina društvenih gospodarstava (Svržnjak i Franić, 2014.).

Zemljišna politika u Hrvatskoj uglavnom se odnosi na propise o raspolaganju poljoprivrednim zemljištem u državnom vlasništvu. Ostali poslovi sa zemljištem u privatnom vlasništvu podliježu općim propisima koji uređuju raspolaganje nekretninama. To znači da privatni vlasnici i dalje mogu slobodno prodavati i davati u zakup svoje poljoprivredno zemljište drugim hrvatskim fizičkim i pravnim osobama, bez provedbe prava prvenstva kupovine. U radu se nastoje prikazati glavni elementi zakonske regulative po pitanju raspolaganja poljoprivrednim zemljištem u vlasništvu države i utvrditi čimbenici koji sprječavaju učinkovitu primjenu u praksi, zbog čega zakon doživljava česte izmjene. Usporedba sa zemljama članicama služi za uvid koje prakse prevladavaju u zemljišnoj politici članica EU te koje su osnovne razlike u novim i starim zemljama članicama.

Materijal i metode

U radu je korištena analiza sadržaja sekundarnih izvora, prvenstveno nacionalnog zakonskog okvira koji se odnosi na raspolaganje poljoprivrednim zemljištem kao i znanstveni članci te ostali stručni diskurs koji doprinosi razumijevanju primjene zemljišne politike u Hrvatskoj i EU. Također, obavljen je dubinski intervju sa stručnjakom za pitanja poljoprivrednog zemljišta iz Hrvatske poljoprivredne komore (HPK) koji je ujedno i poljoprivredni proizvođač te sam koristi državno poljoprivredno zemljište. Korišteni su i komentari dionika poljoprivrede u sklopu sustava e-savjetovanja u otvorenoj javnoj raspravi o Nacrtu prijedloga zakona o izmjenama i dopunama Zakona o poljoprivrednom zemljištu.

Struktura korištenja poljoprivrednog zemljišta u Hrvatskoj

Prema Popisu poljoprivrede, 2020. u Hrvatskoj postoji ukupno 143.901 poljoprivrednih gospodarstava koja koriste 1.505.294 hektara poljoprivrednog zemljišta. Struktura vlasništva je polarizirana tako da bilježimo nekoliko vrlo velikih gospodarstava s jedne strane i mnogo malih s druge strane. U prosjeku jedno gospodarstvo u svojoj proizvodnji koristi 9,5 ha poljoprivrednog zemljišta, uzgaja 5,5 uvjetnih grla stoke.

Prema podacima DZS-a, 2016., najveći broj hrvatskih poljoprivrednika - njih 93.430, koji čine 69,5 % ukupnog broja poljoprivrednika - koristi u prosjeku manje od 5 ha poljoprivrednog zemljišta. Ovi "mali" poljoprivrednici u svojoj proizvodnji koriste 178.670 ha poljoprivrednog zemljišta, što čini 11,4 % ukupne površine poljoprivrednog zemljišta koje se koristi. U prosjeku, jedan "mali" poljoprivrednik u svojoj proizvodnji koristi 1,9 ha poljoprivrednog zemljišta, uzgaja 2,4 uvjetnih grla stoke i ostvaruje standardni ekonomski rezultat od 5.224 eura.

Veliki poljoprivrednici u svojoj proizvodnji koriste više od 100 ha poljoprivrednog zemljišta. Ima ih 1.620, čine 1,2 % od ukupnog broja poljoprivrednika i koriste ukupno 676.416 ha poljoprivrednog zemljišta (što čini 43,2 % ukupne površine poljoprivrednog zemljišta). Od ukupno korištenih poljoprivrednih površina prevladavaju oranice sa 894.672 hektara. Druge najveće poljoprivredne površine su trajni travnjaci koji zauzimaju 597.279 hektara, dok trajni nasadi zauzimaju 72.372, a vrtovi 72.372 hektara (Ministarstvo poljoprivrede, 2019.).

Nepovoljna i vrlo izražena polarizacija poljoprivrednih gospodarstava stavlja u nepovoljni položaj male proizvođače koji ne postižu konkurentnost na unutarnjem i vanjskom tržištu. Prevladavajuća mala gospodarstva posljedica su sustavnog dijeljenja zemljišta nasljeđivanjem, privatne kupnje i prodaje te kupnje od strane poslovnih subjekata koji se bave poljoprivredom. Takva gospodarstva se smatraju preprekom u optimalnom razvoju poljoprivrede, jer nemaju dobar pristup mehanizaciji, proizvodnja je neučinkovita i uključuje velike troškove za ublažavanje njezinih negativnih učinaka, što rezultira smanjenjem neto prihoda poljoprivrednika (Odak i sur., 2017.). Doduše, prema preglednom radu Bentley (1987.), postoje slučajevi kad rascjepkanost i prateća raspršenost zemljišta imaju određene prednosti: kod upravljanja rizikom, raspoređivanja usjeva, u svrhu izbjegavanja prirodnih ili klimatskih katastrofa, maksimiziranja agroekoloških prednosti određene parcele i sl.

U nekim ruralnim područjima Hrvatske poljoprivreda daje jedini doprinos gospodarstvu i prihodima stoga se ta pozicija nastoji dodatno ojačati mjerama zemljišne politike. Cilj politike je povećati ukupnu površinu obradivog poljoprivrednog zemljišta te osigurati ostanak ljudi, pogotovo mlađe populacije, u ruralnim područjima, okrupnjavanje zemljišta, stavljanje u funkciju zapuštenog privatnog poljoprivrednog zemljišta i državnog poljoprivrednog zemljišta (Ministarstvo poljoprivrede, 2019.).

Tržište i raspolaganje državnim poljoprivrednim zemljištem

Problemi zemljišne politike najveća su prepreka u funkcioniranju politike i cjelokupnog poljoprivrednog sektora prema željenim suvremenim tržišnim standardima. I danas postoje neriješeni vlasnički odnosi, denacionalizacija "državnog" zemljišta nije završena, a mehanizmi otkupa zemljišta još uvijek su previše složeni zbog neuravnoteženog stanja u zemljišnim knjigama. Također, neizvjesnost oko raspolaganja državnim zemljištem očituje se kroz brojne promjene koje su se dogodile od donošenja krovnog Zakona o poljoprivrednom zemljištu (NN 34/1991), a posljednja do danas je objavljena u Narodnim novinama br. 112/2019. Došlo je do 19 manje-više značajnih izmjena prvog Zakona iz 1991. godine i u trenutku ovog pisanja usvojen je novi konačni prijedlog Zakona o izmjenama i dopunama Zakona o poljoprivrednom zemljištu.

Tablica 1. Točke spoticanja zakonske regulative o raspolaganju državnim poljoprivrednim zemljištem

Razdoblje	Kritične točke regulative podložne promjenama
1991.-2008.	Ovlasti raspolaganja poljoprivrednim zemljištem su na razini JLS. Definirani oblici raspolaganja: zakup 25 g. za voćnjake, 50 g. za maslinike, ostalo 10 g. Odluku o raspolaganju zemljištem donosi općina ili grad. Zakupnina je prihod državnog proračuna 25%, proračuna jedinice područne (regionalne) samouprave 25 % i 50 % proračuna jedinice lokalne samouprave na čijem se području poljoprivredno zemljište nalazi. Pravo prvenstva kod zakupa: suvlasnik, OPG, dosadašnji zakupac, PG koje graniči s dotičnim itd.
2008.-2018.	Osnovana Agencija za poljoprivredno zemljište zadužena za raspolaganje poljoprivrednim zemljištem. Zemljište se daje u zakup na rok od 20 g. ili na dugogodišnji zakup od 50 godina. Pravo prvenstva kod zakupa: nositelj OPG-a, pravna osoba, dosadašnji zakupnik ili posjednik, PG stočarske proizvodnje, nositelj OPG-a mlađi od 40 g., PG kojem je odobren projekt u okviru Operativnog programa, prebivalište na području JLS koja provodi natječaj, nositelj OPG obrazovan u području poljoprivrede (SSS, VŠS, VSS), branitelj. Uvedena mogućnost davanja privatnog zapuštenog zemljišta u zakup fizičkoj ili pravnoj osobi na rok od 3 do 10 godina.
2018. do sada	Ukida se Agencija za poljoprivredno zemljište, a dodjela zemlje vraća na JLS. Zakup se regulira na 25 g., umjesto 50 g., uz mogućnost produženja za 25 godina za one koji dobro gospodare zemljom. Pravo prvenstva kod zakupa: OPG, mala i mikro poduzeća, veliki sustavi isključeni, stočari bez zemlje, te mladi poljoprivrednici do 41 g. starosti. Privatna zemlja koja je neobrađena, a vlasnici nedostupni ili nepoznatog boravišta, može se dati u zakup, a novac će vlasnika čekati 5 godina na posebnom računu. Raspodjela prihoda od raspolaganja: državni proračun (25 %), proračun jedinice regionalne samouprave (10 %), proračun jedinice lokalne samouprave, (65 %). Natječaj za zakup raspisuje se za katastarske čestice koje čine proizvodno-tehnološke cjeline površine najviše do 100 hektara.
Novi prijedlog zakona (veljača, 2022.)	Precizira se davanje poljoprivrednog zemljišta u vlasništvu države u zakup putem javnog natječaja na različite rokove, ovisno o vremenu potrebnom za povrat investicije. Predlaže se elektronička provedba natječaja za zakup i prodaju poljoprivrednog zemljišta. Za ostvarivanje prava prvenstva u natječaju za zakup uvodi se bodovanje. Veći broj bodova mogu ostvariti ponuditelji koji na poljoprivrednom zemljištu ostvaruju višu dodanu vrijednost. Predlaže se bodovanje temeljem domicilnosti, mladosti, ekološke poljoprivrede, pridonošenja zelenim ciljevima kao i spremnosti na udruživanje. Maksimalnu površinu za zakup određuju JLS. Prednost pri prodaji imaju gospodarstva koja graniče s česticom.

Izvor: Autori

Glavni razlozi česte izmjene zakona i poteškoća s implementacijom u praksi su prije svega složene i dugotrajne administrativne procedure (Svržnjak i Franić, 2014.). U razdoblju 2008. do 2018. ovlasti raspolaganja državnim poljoprivrednim zemljištem dodijeljene su Agenciji za poljoprivredno zemljište, što je imalo pozitivne učinke u pogledu zemljišnih knjiga, digitalizacije i uspostavljanja nekih jedinstvenih postupaka i obrazaca. No, glavni cilj nije bio postignut: da se što više državnog zemljišta stavi u raspolaganje i da mali poljoprivrednici dobiju pristup poljoprivrednom zemljištu. Natječaji su se sporo raspisivali, a zapušteno poljoprivredno zemljište ostaje neobrađeno. Zakonom iz 2018. (NN 20/2018) ovlasti raspolaganja državnim poljoprivrednim zemljištem vraćene su na razinu jedinica

lokalne samouprave (JLS), što je popraćeno negodovanjima u javnosti uz upozorenje da bi moglo doći do sukoba interesa u sustavu donošenja odluka od strane lokalnih vijećnika u JLS o dodjeli državnog zemljišta.

Nezadovoljstva poljoprivrednika dosadašnjim sustavom raspodjele, što se doznaje putem intervjua i stranice e-savjetovanje, odnose se na prekratak rok zakupa od 25 godina za trajne nasade, izjednačavanje tvrtki, mikro i malih poduzeća s OPG-om kod prava prvenstva zakupa, neredovitost ili manjak kontrole izvršavanja Gospodarskog programa. U prilog nezadovoljstvu ide i nerješavanje pitanja zapuštenog zemljišta koje predstavlja dobar potencijal za povećanje proizvodnje. To su uglavnom površine nekadašnjih zajedničkih pašnjaka, livade ili površine čije privođenje poljoprivrednoj proizvodnji nije dovršeno i sad se utjecajem prirode vraćaju u prvobitno stanje (šumu). Budući da se izravna plaćanja dijelom temelje na broju hektara po gospodarstvu, u nekim slučajevima interesi za zakup postoje samo u svrhu povećanja prihoda.

Trenutno se državno poljoprivredno zemljište pretežito i dalje koristi na temelju ugovora o privremenom korištenju i izvansudskih nagodbi na rok od 2 godine uz moguće produženje, što je privremeno i kratkoročno rješenje koje ne daje sigurnost poljoprivrednim proizvođačima za dugoročno planiranje i dodatna ulaganja u proizvodnju (Vlada RH, 2022.).

Rasprava: usporedba sa zemljama članicama

Prema istraživanjima autora Cianan (2018.) i Vranken i sur. (2021.) zemljišne politike zemalja članica se razlikuju. Dok neke države imaju složena i stroga pravila kao Hrvatska (Mađarska, Poljska i Rumunjska) druge članice prakticiraju vrlo liberalan pristup u tržištu zemljištem (Češka, Danska, Irska, i Finska). U Mađarskoj i Poljskoj pravne osobe ne mogu biti vlasnici zemljišta. Bugarska, Španjolska i Slovačka su članice s najvećim brojem propisa koji prvenstveno sprječavaju fragmentaciju zemljišta. Stroge procedure oko prodaje državnog zemljišta u novim članicama kao što su Bugarska, Češka, Estonija, Španjolska, Hrvatska, Latvija, Litva, Mađarska, Rumunjska, Slovenija i Slovačka, često se uvode kako bi se osigurali jednaki uvjeti za sve zainteresirane kupce te kako bi se izbjeglo da neki kupci iskoriste svoj privilegirani položaj. Od starih članica Francuska se ističe po vrlo striktnim propisima oko tržišta zemljištem kako bi se izbjegle špekulacije, dala prednost domaćim, štoviše mladim poljoprivrednicima, aktivnostima okrupnjavanja zemljišta i zaštiti okoliša.

Države sa strogom regulativom različito reguliraju pravo prvenstva zakupa ili kupnje zemljišta, dok one s liberalnim pristupom uglavnom nemaju regulirano pravo prvenstva. Većina zemalja daje prednost kupnje redom suvlasnicima, poljoprivrednicima koji graniče s parcelom koja se prodaje, dotadašnjem zakupcu, mladim ili malim poljoprivrednicima itd. U većini članica država ili neko državno tijelo ima pravo prvenstva otkupa kako bi se provodile politike i projekti koji su u javnom interesu.

Zaključak

Hrvatska zemljišna politika svojim zakonskim mjerama nastoji povećati ukupnu površinu obradivog poljoprivrednog zemljišta te osigurati ostanak ljudi, pogotovo mlađe populacije, u ruralnim područjima, okrupniti zemljište, te staviti u funkciju zapušteno državno i privatno poljoprivredno zemljište. Dosad se pokazalo da regulativa ima poteškoće pri primjeni u praksi stoga je od 1991. učestalo mijenjana, međutim još uvijek velik dio državnog poljoprivrednog zemljišta nije stavljen u funkciju. U odnosu na stare, nove zemlje članice imaju uglavnom strože zakonodavstvo koje regulira tržište poljoprivrednim zemljištem.

Napomena

Rad je nastao temeljem završnog rada studenta Mihaela Hercega koji je obranjen dana 14. rujna 2021. na Agronomskom fakultetu Sveučilišta u Zagrebu.

Literatura

Bentley J. W. (1987). Economic and Ecological Approaches to Land Fragmantation: In Defense of A Much-Maligned Phenomenon. Annual Review of Anthropology. (16): 31-67.

Ciaian P., Kancs D. and Espinosa M. (2018). The impact of the 2013 CAP reform on the decoupled payments' capitalization into land values, JRC Technical Report EUR 27940, Publications Office of the European Union, Luxembourgh.

E-savjetovanja: Savjetovanje o Prijedlogu Zakona o izmjenama i dopunama Zakona o poljoprivrednom zemljištu. Raspoloživo: https://esavjetovanja.gov.hr/ECon/MainScreen?entityId=15531

Državni zavod za statistiku – DZS (2016). Struktura poljoprivrednih gospodarstava – konačni podaci, stanje 1. lipnja 2016. Priopćenje: 1.1.29. od 29. 12. 2017.

Godžirov I. (2020). Restrictions on the acquisition of certain categories of real estate in relation to the basic market freedoms in the European Union. EU and Comparative Law Issues and Challenges Series (ECLIC). (4): 844–868.

Ministarstvo poljoprivrede – MP (2019). Zahtjev za produljenje prijelaznog razdoblja tijekom kojega će Republika Hrvatska zadržati postojeća ograničenja za stjecanje vlasništva nad poljoprivrednim zemljištem za dodatne tri godine, odnosno do 01. srpnja 2023. godine.

Narodne novine: Zakoni o poljoprivrednom zemljištu (34/1991, 54/1994, 66/2001, 39/2013, 20/2018)

Odak I., Tomić H., Mastelić Ivić S. (2017). Vrednovanje fragmentacije poljoprivrednog zemljišta. Geodetski list. (3): 215–232.

Svržnjak K., Franić R. (2014). Developing agrarian structure through the disposal of state-owned agricultural land in Croatia. Agroeconomia Croatica. 4 (1): 50-57.

Vlada Republike Hrvatske (2022). Konačni prijedlog Zakona o izmjenama i dopunama Zakona o poljoprivrednom zemljištu.

Vranken L., Tabeau E., Roebeling P., Ciaian P. (2021). Agricultural land market regulations in the EU Member States, JRC Technical Report EUR 30838, Publications Office of the European Union, Luxembourgh.

Zróbek-Rózanska A., Zielinska-Szczepkowska,J. (2019). National Land Use Policy against the Misuse of the Agricultural Land-Causes and Effects. Sustainability. 11 (6403): 1-18.

Disposal of state agricultural land: legislation and application

Abstract

The goals of the paper are: to describe the main elements of legislation on the disposal of state-owned agricultural land and to identify factors that prevent effective implementation in practice. Since more than half of the state-owned agricultural land in Croatia has not been activated, the state's priority is to increase the use and activate uncultivated agricultural land. In addition to national land policy, the paper takes into account the examples of other EU member states in the context of land legislation. Compared to the old ones, the new member states have generally stricter legislation regulating the agricultural land market.

Key words: agricultural land, disposal, legislation

IZVORNI ZNANSTVENI RAD

Proizvodnja kulena kao dio tradicije u Baranji

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Sažetak
Baranjski kulen je jedan od hrvatskih autohtonih prehrambenih proizvoda koji na tržištu EU nosi zaštićenu oznaku zemljopisnog podrijetla (ZOZP). Cilj ovoga rada je bio putem ankete doći do podataka o čuvanju tradicije u proizvodnji i potrošnji kulena na području Baranje i kod onog dijela stanovnika koji ne pripadaju udruzi proizvođača „Baranjskog kulena“ – ZOZP. Iz rezultata je vidljivo da više od 50 % ispitanika drži do tradicionalnog načina proizvodnje kulena, uporabi mesa i začinske paprike iz vlastitog uzgoja ili iz Baranje, te pozitivnog stava o zaštiti imena Baranjskog kulena ZOZP koja je sveobuhvatno pridonijela većoj prodaji, višoj cijeni, te brendiranju Baranje kao poželjne turističke destinacije.

Ključne riječi: Baranjski kulen, tradicija, ZOZP

Uvod

Prehrambene navike stanovništva nekog kraja oblikovali su ekološko okruženje, civilizacijski razvoj, kao i utjecaj određenih vjersko-kulturoloških navika određenih populacijskih grupa (Jašić, 2009.). Hrvatska Baranja obiluje vodom, šumama, pašnjacima i plodnom zemljom što je preduvjet razvoja ratarske i stočarske proizvodnje. Tijekom povijesti razvoj stočarsko-ratarskog sustava, život u istoj državi i doseljavanje Nijemaca i Mađara u ove prostore ostavlja veliki trag u prehrambenim navikama stanovnika, te nastanak izvornih domaćih proizvoda od mesa, a neke od proizvoda učinio je autohtonima (Petričević i sur., 2010.). Autohtoni prehrambeni proizvodi karakteristični su po svojim tehnološkim, prehrambenim i organoleptičkim specifičnostima koje ih čine posebnijim od istih ili sličnih proizvoda iz istog ili drugih područja. Oni svojom kvalitetom i posebnošću konkuriraju na globalnom tržištu ostalim prehrambenim proizvodima, a štite se od zlouporabe imena određenim vrstama oznaka stvarajući prepoznatljiv identitet. Zaštita autohtonih proizvoda na EU tržištu zakonski je definirano područje, a oznake mogu biti zaštićena oznaka zemljopisnog podrijetla (ZOZP), zaštićena oznaka izvornosti (ZOI) i zaštićena oznaka tradicionalnog specijaliteta (ZOTS) (Svijet kvalitete, 2013.). Među hrvatskim registriranim proizvodima od mesa je „Baranjski kulen“ – ZOZP (Europa Commission, 2015.).

Na prostoru Baranje proizvodnja kulena ima dugu tradiciju, i danas se proizvodi u velikom broju domaćinstava za vlastite potrebe ili prodaju, neovisno o pripadnosti Udruzi proizvođača kulena – baranjski kulen. Kulen koji se proizvodi na ovim prostorima spada u kategoriju trajnih fermentiranih kobasica dobivenih od usitnjenog svinjskog mesa i čvrstog masnog tkiva, soli, crvene začinske paprike i češnjaka uz mogućnost dodatka drugih začina i šećera, napunjen u svinjsko slijepo crijevo. Ovi proizvodi dobivaju se postupcima fermentacije, sušenja i zrenja uz postupke hladnog dimljenja (Pravilnik o mesnim proizvodima, NN br. 62/18; Specifikacija proizvoda, 2014.). Sušenje ili dehidriranje hrane je najstariji poznati postupaka čuvanja hrane. To je i danas jedan od glavnih postupaka čuvanja namirnica za duži vremenski period (Clark, 2009.). Osim tradicionalnog načina

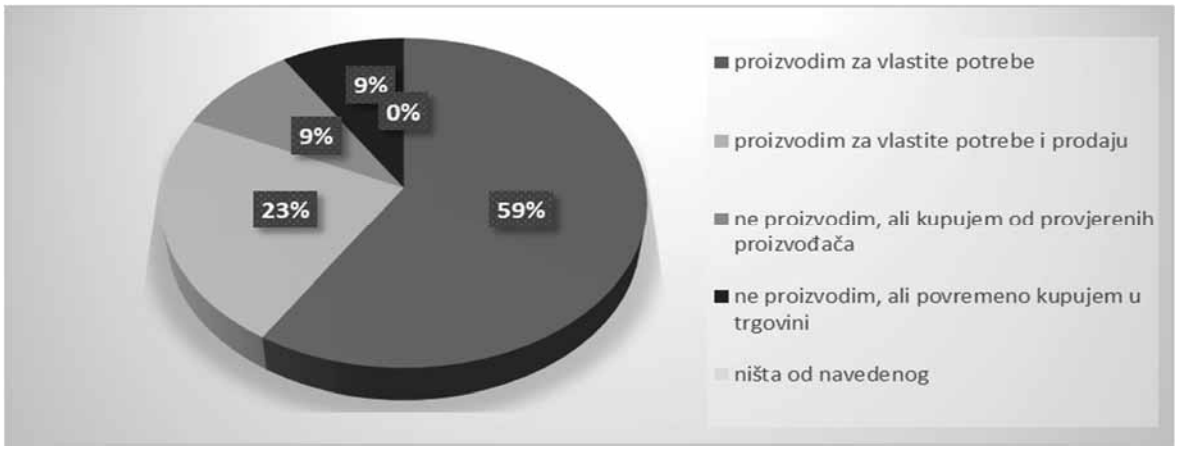
proizvodnje kvaliteta kulena određena je i korištenjem minimalno 80 % „zrelog“ svinjskog mesa prve kategorije odabranih pasmina: crne slavonske svinje, velikog jorkšira i švedskog landrasa, te njihovih križanaca s pasminom durok (Kovačević, 2014.).

Materijal i metode

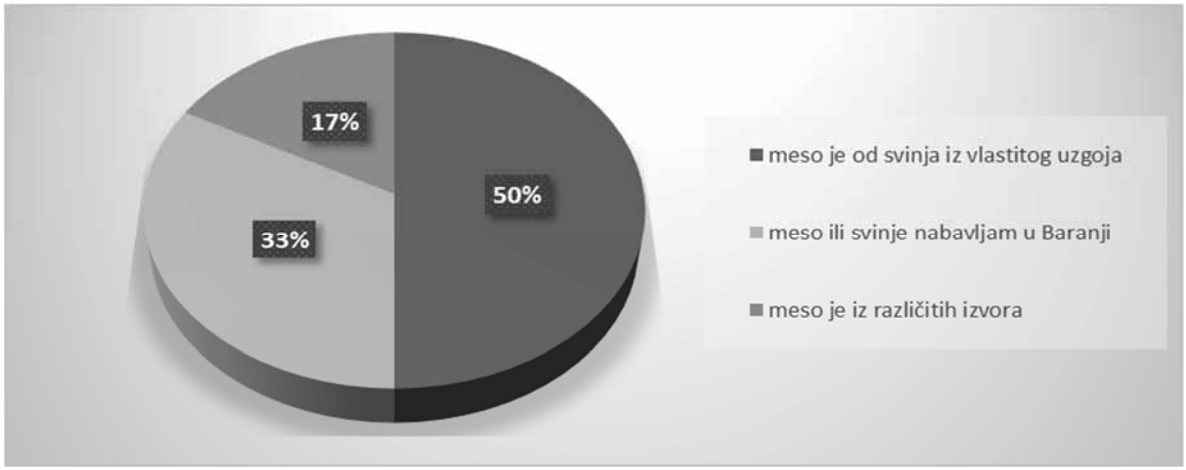
Za potrebe rada provedena je anketa putem programa *Microsoft forms*. Anketom je obuhvaćeno 50 domaćinstava koja se bave seoskim turizmom (OPG) u naseljima: Beli Manastir, Bilje, Draž, Batina, Karanac, Vardarac, Darda, Kneževi Vinogradi, Švajcarnica, Kamenac, Čeminac i Kopačevo. Cilj istraživanja je bio doći do podataka o čuvanju tradicije u načinu proizvodnje kulena i uzgoja svinja na području Baranje i kod onog dijela proizvođača koji nisu nužno članovi Udruge proizvođača kulena – baranjski kulen. Pitanja u anketi su se odnosila na čuvanje tradicionalne recepture, podrijetlo osnovnih sirovina za proizvodnju kulena, da li su iz vlastitog uzgoja, s područja Baranje ili drugdje. Istraživanjm se prikupilo podatake o proizvodnji kulena za vlastite potrebe, za prodaju ili potrošnju u vlastitom turističkom objektu, te mišljenje o dobrobiti zaštite imena „Baranjskog kulena“ – ZOPZ.

Rezultati i rasprava

Iz rezultata prikazanih na Grafikonu 1 je vidljivo da 59 % ispitanika proizvodi kulen za vlastite potrebe, a 23 % za vlastite potrebe i prodaju, što je ukupno 82 % ispitanika koji proizvode kulen kako za vlastite potrebe ili i prodaju. Tek 18 % ispitanika kupuje kulen od provjerenih proizvođača ili u trgovinama. Svi ispitanici su se izjasnili da proizvode, prodaju ili kupuju kulen, što potvrđuje da je konzumacija kulena neizostavna kod svih ispitanika.

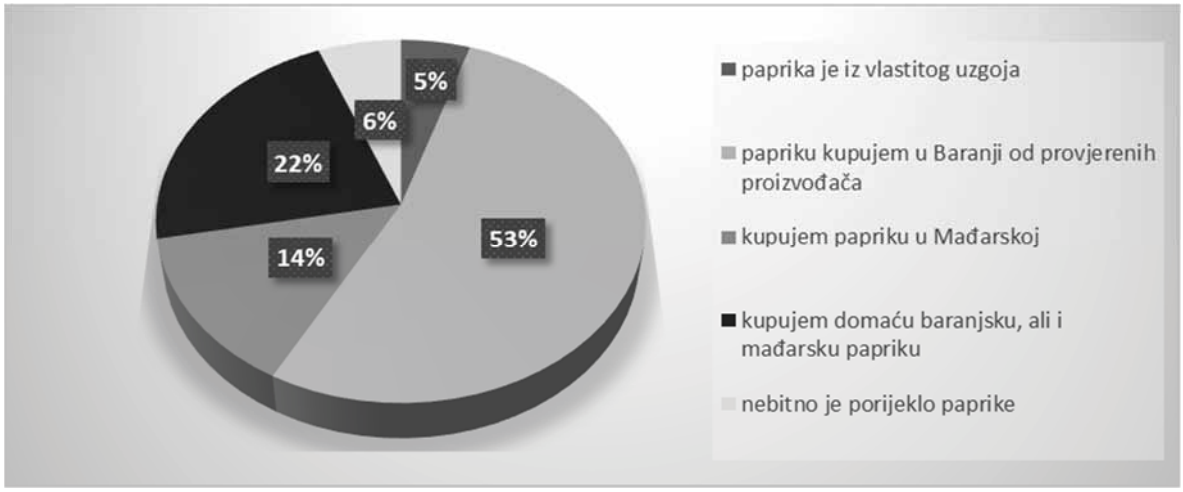


Grafikon 1. Proizvodnja Baranjskog kulena za vlastite potrebe i prodaju



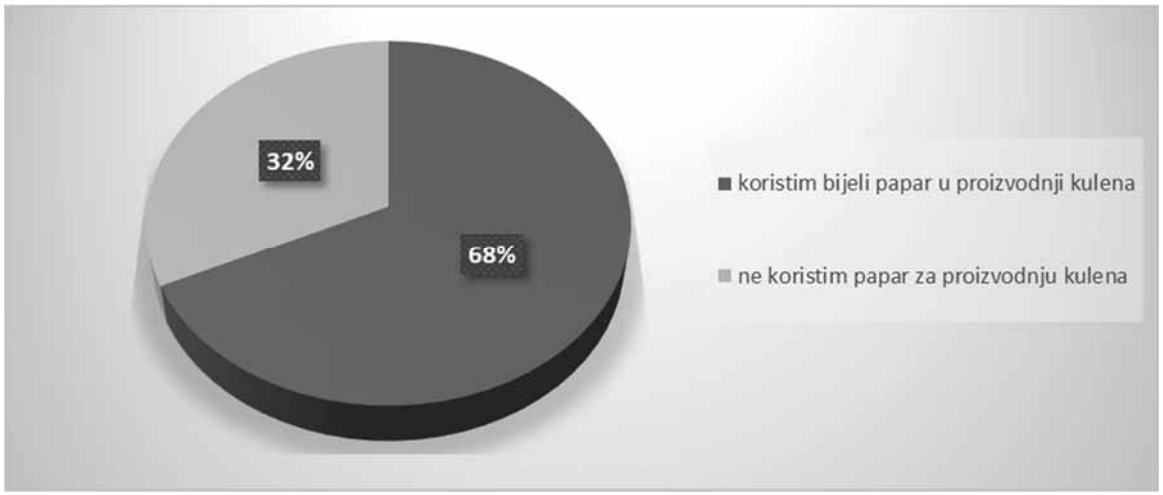
Grafikon 2. Porijeklo mesa za proizvodnju Baranjskog kulena

Na pitanje o porijeklu mesa za proizvodnju kulena, 50 % ispitanika koji proizvode kulen izjasnilo se da sami uzgajaju svinje, 33 % nabavlja svinje ili meso u Baranji, što je ukupno 83 %. Ovaj podatak potvrđuje da još uvijek veliki broj domaćinstava uzgaja svinje za vlastite potrebe ili i prodaju. Preostalih 17 % proizvođača nabavlja meso iz različitih izvora.



Grafikon 3. Porijeklo začinske paprike za proizvodnju Baranjskog kulena

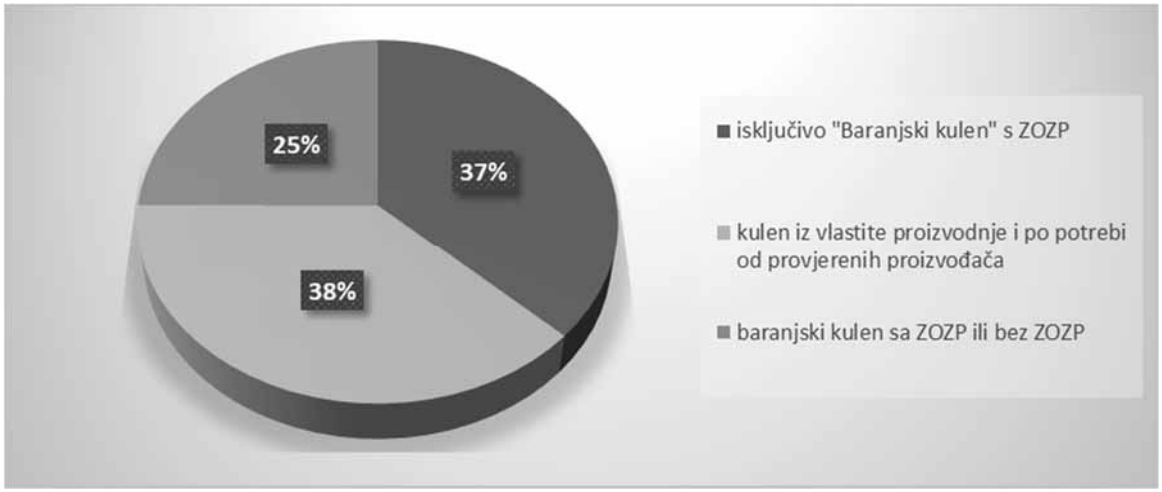
Rezultati prikazani na Grafikonu 3 prikazuju odgovore ispitanika vezano za porijeklo začinske paprike, kao jednog od osnovnih začina o kojem uvelike ovisi kvaliteta kulena. Najveći broj ispitanika se izjasnio da papriku nabavlja u Baranji od provjerenih proizvođača njih 53 %, što je i očekivano jer Baranja je hrvatska pokrajina koja je poznata po proizvodnji začinske paprike. Ukupno 5 % ispitanika koristi vlastito proizvedenu papriku, a 14 % kupuje mađarsku, 22 % kupuje ili baranjsku ili mađarsku. Tek 6 % proizvođača se izjasnilo da im nije bitno porijeklo paprike.



Grafikon 4. Uporaba bijelog papra u proizvodnji Baranjskog kulena

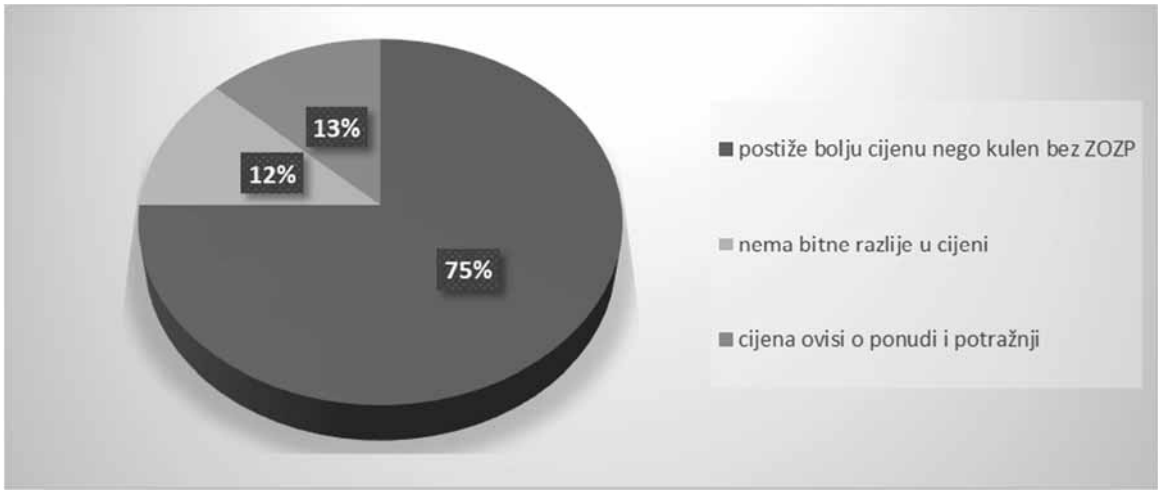
Korištenje bijelog papra je specifičnost u proizvodnji kulena na području Baranje. Za proizvodnju „Slavonskog kulena“ čije je ime također zaštićeno ZOZP na EU tržištu, ne koristi se papar, a ljutina i pikantnost potječe od ljute začinske paprike. Tradicija korištenja papra u Baranji povezuje se s proizvodnjom „Panonskog kulena“ koji je preteča „Baranjskog kulena“. Za proizvodnju „Panonskog kulena“ koristio se bijeli papar kao i za ostale salame na prostoru tadašnje južne Ugarske odnosno Baranje (Specifikacija proizvoda, 2014.).

Ukupno 68 % ispitanika i dalje koristi bijeli papar u proizvodnji kulena, a 32 % ispitanika uopće ne koristi papar, što govori o razlici u recepturi u pojedinim naseljima, ali i odstupanju od recepture koja je određena Specifikacijom proizvoda "Baranjskog kulena" – ZOZP.



Grafikon 5. Porijeklo kulena koji se poslužujete gostima u vlastitom objektu

Grafikon 5 pokazuje da 38 % ispitanika u vlastitom objektu poslužuje kulen iz vlastite proizvodnje ili od provjerenih proizvođača, dok 37 % poslužuju isključivo „Baranjski kulen“ – ZOZP, a 25 % poslužuje „Baranjski kulen“ sa ili bez ZOZP. Ovi odgovori mogu biti rezultat nedovoljne proizvodnje „Baranskog kulena“ – ZOZP, ali i proizvodnje kulena visoke kakvoće i kod proizvođača koji nisu članovi udruge proizvođača „Baranjskog kulena“ – ZOZP.



Grafikon 6. Odnos cijena Baranjskog kulena sa i bez ZOZP

Grafikon 6 pokazuju rezultate ispitanika vezano za stav oko cijene kulena sa i bez ZOZP. Ukupno 75 % ispitanika potvrđuje da „Baranjski kulen“ – ZOZP postiže višu cijenu u odnosu na kulen bez ZOZP, dok 12 % je stava da nema bitne razlike u cijeni. Ovisnost cijene kulena o ponudi i potražnji potvrđuje 13 % ispitanika.

U anketi se iznio i stav vezano za zaštitu imena „Baranjskog kulena“ – ZOZP. Veliki dio ispitanika čak 83 % zaštitu imena „Baranjskog kulena“ – ZOZP, ocijenjuje pozitivnim utjecajem na povećanje proizvodnje i prodaje, prepoznatljivost kulena kao regionalne delicije, kao i utjecaj na brendiranje Baranje kao poželjne turističke destinacije.

Zaključak

Rezultati istraživanja pokazuju da na prostoru Baranje veliki dio domaćinstava čuva tradicionalne navike proizvodnje kulena za vlastite potrebe, ali i za prodaju. Za proizvodnju kulena meso je iz vlastitog uzgoja ili s područja Baranje, tek manji dio ispitanika nabavlja meso iz drugih izvora. Ovo potvrđuje da se još uvijek veliki dio stanovnika Baranje bavi svinjogojstvom. Začinska paprika je ili iz Baranje ili mađarska, koja se također odlikuje visokom kvalitetom. Bijeli papar kao specifičan začin u proizvodnji „Baranjskog kulena“ i dalje je prisutan u proizvodnji kulena kod 68 % ispitanika, što upućuje na čuvanje tradicionalne recepture, ali i postojanje razlike u recepturi između pojedinih naselja. Kulen koji se poslužuje gostima u vlastitom objektu, podjednako je zastupljen iz vlastite proizvodnje ili kupljen od provjerenih proizvođača sa ili bez ZOZP. Zaštita imena „Baranjskog kulena“ – ZOZP pozitivno je utjecala na proizvodnju, plasman i prepoznatljivost Baranje, a time i veći materijalni dohodak stanovnika Baranje.

Literatura

Clark J. P. (2009). *Practical Desing, Construction and Operation of Food Facilities*. Elsevier, Academic Press is an imprint of Elsevier, USA. 242.

Kovačević D. (2014). Tehnologija kulena i drugih fermentiranih proizvoda. Prehrambeno-tehnološki fakultet Osijek. 34.

Europan Commission (2015). Raspoloživo: https://ec.europa.eu/info/food-farming-fisheries/food-safety-and-quality/certification/quality-labels/geographical-indications-register/

Jašić M. (2009). Povijest hemije prehrane. Tehnologija hrane. Raspoloživo: https://www.tehnologijahrane.com/enciklopedija/povijest-hemije-prehrane

Petričević A., Benčević K., Kušec G. (2010). Slavonski domaći kulen i kobasice. II., dopunjeno i izmjenjeno izdanje. EU Agro Hrvatska, Osijek. 24-25.

Pravilnik o mesnim proizvodima (NN br. 62/2018).

Specifikacija proizvoda (2014). Baranjski kulen. Raspoloživo: https://poljoprivreda.gov.hr/istaknute-teme/hrana-111/111

Svijet kvalitete (2013). Autohtoni prehrambeni proizvodi. Raspoloživo: https://www.svijet-kvalitete.com/index.php/norme-i-hrana/autohtoni-proizvodi

Production of *Kulen* as the part of Baranja tradition

Abstract

"Baranjski kulen" is one of the Croatian autochthonous food products that bears a protected geographical indication (PGI) on the EU market. The aim of this paper was to obtain data on the preservation of traditions in the production and consumption of „kulen“ in the Baranja region in the part of the population that are not member of the association of producers of „Baranjski kulen“ – PGI. The obtained results show that more than 50% of respondents adhere to the traditional way of producing „kulen“, using meat and spices from their own production or from Baranja. Furthermore, the results show the positive attitude towards the protection of name of „Baranjski kulen“– PGI, all of which has comprehensively contributed to increased sales, higher prices and branding Baranja as desirable tourist destination.

Key words: Baranjski kulen, tradition, PGI

IZVORNI ZNANSTVENI RAD

Utjecaj učestalosti praćenja vremenske prognoze na prepoznavanje klimatskih promjena

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Sažetak
Klimatske promjene sve su češća tema u medijima. Uslijed velike količine takvih informacija može se pretpostaviti da one imaju utjecaja na mišljenje poljoprivrednika o promjeni klime. S ovim ciljem provedeno je terensko istraživanje putem ankete na uzorku nositelja poljoprivrednih gospodarstava na području Jadranske Hrvatske. Utvrđeno je da nositelji koji jednom ili više puta dnevno prate vremensku prognozu intenzivnije doživljavaju opću promjenu klime u odnosu na one koji to čine nekoliko puta tjedno ili rijeđe od toga. Dakle, putem redovitih informacija o vremenskoj prognozi moguće je utjecati na mišljenje nositelja poljoprivrednih gospodarstava o klimatskim promjenama.

Ključne riječi: vremenska prognoza, nositelji poljoprivrednih gospodarstava, klimatske promjene, Jadranska Hrvatska

Uvod

Veliki broj istraživanja pokazuje kako su poljoprivrednici svjesni klimatskih promjena, te su iste i percipirali (Vedwan i Rhoades 2001.; Hageback i sur., 2005.; Thomas i sur., 2007.; Ishaya i Abaje, 2008.; Gbetibouo, 2009.; Mertz i sur., 2009.; Diggs, 1991.; Leiserowitz, 2006.; Semenza i sur., 2008.; Akter i Bennett 2009.; preuzeto iz Deressa i sur., 2010.). Klimatskim promjenama ili promjeni klime prethodi promjena vremenskih varijabli (primjerice, temperatura, oborine) u dužem vremenskom razdoblju, te posljedično pojava prirodnih nepogoda. Poljoprivrednici ih pretežno doživljavaju kroz povećanje prosječne temeperature zraka i smanjenje količine oborina, te češću pojavu suše, tuče i mraza. Upravo promjena vremenskih prilika u poljoprivredi utječe s na produktivnost i stabilnost biljne proizvodnje u svim fazama uzgoja i na pravovremeno i kvalitetno obavljanje svih agrotehničkih zahvata. Istraživanja pokazuju kako upravo informacije o vremenskoj prognozi i klimatskim promjenama utječu na donošenje odluka poljoprivrednika (Reyes i sur., 2009.) o rokovima sjetve, navodnjavanja, gnojidbe, berbe plodove i sl. Osim navedenog, važnost praćenja vremenske prognoze ogleda se i u uspješnijem upravljanju poljoprivredne proizvodnje u cjelosti, ali i poboljšanju života u ruralnim područjima (Feleke, 2015.).

Vremensku prognozu moguće je pratiti putem radija, televizije, ali i pomoću raznih aplikacija i interentskih stranica. Danas, u poslovnom odlučivanju postoji potreba za brzim, jednostavnim, pravovremenim i korisnim informacijama. Upravo inovativni alati koji omogućuju, između ostalog, uvid u vremensku prognozu, ekstremne vremenske nepogode i njena predviđanja („tailor weather forecasts“), te pružaju pravovremena upozorenja („early warnings“) na lokalnoj razini, pomažu pri upravljanju rizikom klimatskih promjena. Dostupnost informacija o vremenu i promjeni vremena pomaže poljoprivrednicima u prilagodbi klimatskim promjenama (Bryan i sur., 2009.). Arimi (2020.) navodi kako pristup pravovremenim informacijama ima manji udio ispitanika (49,2 %). Upravo oni pojedinici

koji imaju pristup pravovremenim informacijama mogu lakše prilagoditi svoje poslovanje neizvjesnim događajima (Arimi, 2020.).

Cilj ovog rada je istražiti utjecaj učestalosti praćenja vremenske prognoze na opće mišljenje nositelja poljoprivrednih gospodarstava o promjeni klime u zadnjih 30–ak godina na njihovom području.

Materijal i metode

Anketno ispitivanje provedeno je na uzorku nositelja poljoprivrednih gospodarstava na području Jadranske Hrvatske (uključujući 6 županija: Istarska, Primorsko-goranska, Zadarska, Šibensko-kninska, Splitsko-dalmatinska, Dubrovačko-neretvanska županija). Odabir gospodarstava u uzorak proveden je uvažavajući kriterije ravnomjerne teritorijalne i proizvodne distribucije, a kao vodič korišteni su podaci iz Upisnika poljoprivrednih gospodarstava (APPRRR, 2019.). Dodatno, popis gospodarstava formiran je i temeljem kontakata s poljoprivrednim udrugama. Anketiranje je provedeno putem neposrednog kontakta s ispitanicima i putem online upitnika koji je dizajniran u platformi Microsoft Forms. Upitnik se sastojao od nekoliko seta pitanja među kojima su bila pitanja o učestalosti praćenja vremenske prognoze, mišljenju o općoj promjeni klime u zadnjih 30-ak godina, kao i pitanja o socio-demografskim i ekonomskim osobinama ispitanika. Izjave o općoj promjeni klime preuzete su iz istaživanja Li i sur., 2013., te su ocjenjivane na skali od 1 (potpuno neslaganje s izjavom) do 5 (potpuno slaganje s izjavom). Ukupno su prikupljena 275 ispravno ispunjena upitnika. Uzorak je opisan pomoću frekvencije, dok provjera utjecaja učestalosti praćenja vremenske prognoze na opću promjenu klime analizirana je putem jednosmjerne analize varijance i post hoc Tukey testa na razini značajnosti $p \leq 0,05$. Navedeni statistički postupci provedeni su u statističkom programu SPSS ver. 26.

Rezultati i rasprava

U anketiranom uzorku prevladavaju osobe muškog spola (75,8 %), u dobi od 52 do 65 godina 44,4 %), sa završenom srednjom školom (58,2 %). Većina ispitanika nema formalno obrazovanje iz područja poljoprivrede (81,1 %). Za većinu nositelja poljoprivreda je dopunski izvor prihoda u kućanstvu (59,6 %), te su većinom s područja Splitsko-dalmatinske (32 %) i Zadarske županije (23,3 %) i (Tablica 1).

Tablica 1. Socio–demografska obilježja ispitanika (N=275)

Varijable	N	%
Spol		
Ženski	50	18,2
Muški	216	75,8
Ne želim odgovoriti	9	3,3
Godine starosti		
0 – 40	45	16,4
41 – 51	52	18,9
52 – 65	122	44,4
66 i više	56	20,4
Razina obrazovanja		
Osnovna škola	45	16,4
Srednja škola	160	58,2
Preddiplomski studij	35	12,7
Diplomski studij	28	10,2

Poslijediplomski studij	7	2,5
Obrazovanje u području poljoprivrede		
Da	52	18,9
Ne	223	81,1
Udio poljoprivrede u prihodima kućanstva	41	
Jedini izvor prihoda	70	14,9
Dominantni izvor prihoda (>50 % od ukupnog prihoda kućanstva)		25,5
Dopunski izvor prihoda (<50 % od ukupnog prihoda kućanstva)	164	59,6
Lokacija gospodarstva		
Istarska županija	40	14,5
Primorsko-goranska županija	19	6,9
Zadarska županija	64	23,3
Šibensko-kninska županija	26	9,5
Splitsko-dalmatinska županija	88	32,0
Dubrovačko-neretvanska županija	35	12,7

Većina nositelja poljoprivrednih gospodarstava prati vremensku prognozu jednom ili nekoliko puta dnevno (48,4 %), 4 – 6 puta tjedno vremensku prognozu prati 30,2 % njih, dok 1 – 3 puta tjedno prati 19,3 %, tek mali postotak ispitanika prati vremensku prognozu rjeđe od jednom tjedno (2,2 %) (Tablica 2).

Tablica 2. Učestalost praćenja vremenske prognoze

Učestalost praćenja vremenske prognoze	N	(%)
Jednom ili nekoliko puta dnevno	133	48,4
4 – 6 puta tjedno	83	30,2
1 – 3 puta tjedno	53	19,3
Rjeđe od jednom tjedno	6	2,2

Mišljenje ispitanika o općoj promjeni klime u zadnjih 30-ak godina na njihovom području relativno je ujednačeno. S navedenim izjavama o promjeni klime ispitanici se uglavnom slažu. Najveći stupanj slaganja ima izjava da su oborine sve neravnomjernije raspoređene (4,24), te da su ljeta sve toplija (4,00), dok izjava da su zime sve hladnije (3,39) ima najniži stupanj slaganja od strane nositelja poljoprivrednih gospodarstava (Tablica 3).

Tablica 3. Mišljenje o općoj promjeni klime na vašem području u zadnjih 30-ak godina

Izjava	M	SD	MOD
Oborine su sve ne ravnomjernije raspoređene	4,24	0,774	4
Ljeta su sve toplija	4,00	0,928	4
Suše su sve češće i intenzivnije	3,88	1,001	4
Kasni proljetni i rani jesenski mrazevi su sve češći	3,75	1,057	4
Oborine su sve rjeđe	3,67	1,038	4
Oluje su sve češće	3,61	1,017	4
Tuče su sve češće	3,55	1,047	4
Zime su sve hladnije	3,39	1,152	4

Provedena jednosmjerna analiza varijance i post hoc Tukey test, pokazali su da učestalost praćenja vremenske prognoze utječe na mišljenje o promjeni klime u zadnjih 30-ak godina (Tablica 4). Prije provođenja jednosmjerne analize varijance izjave praćenja vremenske prognoze 4 – 6 puta jedno i 1 – 3 puta jedno su spojene radi jednostavnijeg pregleda podataka.

Tablica 4. Rezultati ANOVE (utjecaj učestalosti praćenja vremenske prognoze na prepoznavanje promjena klima)

Izjava	Učestalost praćenja vremenske prognoze	M	F	p vrijednost ($p \leq 0,05$)
Ljeta su sve toplija	1 ili nekoliko puta dnevno	$4,24^{a}$	9,978	0,000
	Nekoliko puta tjedno	$3,79^{b}$		
	1 tjedno ili rjeđe	$3,33^{b}$		
Zime su sve hladnije	1 ili nekoliko puta dnevno	3,44	0,233	0,792
	Nekoliko puta tjedno	3,35		
	1 tjedno ili rjeđe	3,50		
Oborine su sve rjeđe	1 ili nekoliko puta dnevno	$3,85^{a}$	4,474	0,012
	Nekoliko puta tjedno	$3,51^{b}$		
	1 tjedno ili rjeđe	$3,17^{ab}$		
Oborine su sve ne ravnomjernije raspoređene	1 ili nekoliko puta dnevno	$4,43^{a}$	11,207	0,000
	Nekoliko puta tjedno	$4,10^{b}$		
	1 tjedno ili rjeđe	$3,33^{c}$		
Suše su sve češće i intenzivnije	1 ili nekoliko puta dnevno	$4,07^{a}$	5,457	0,005
	Nekoliko puta tjedno	$3,74^{b}$		
	1 tjedno ili rjeđe	$3,17^{b}$		
Oluje su sve češće	1 ili nekoliko puta dnevno	$3,77^{a}$	3,886	0,022
	Nekoliko puta tjedno	$3,48^{b}$		
	1 tjedno ili rjeđe	$3,00^{b}$		
Tuče su sve češće	1 ili nekoliko puta dnevno	$3,73^{a}$	4,068	0,018
	Nekoliko puta tjedno	$3,38^{b}$		
	1 tjedno ili rjeđe	$3,33^{b}$		
Kasni proljetni i rani jesenski mrazevi su sve češći	1 ili nekoliko puta dnevno	3,83	0,915	0,402
	Nekoliko puta tjedno	3,66		
	1 tjedno ili rjeđe	3,67		

*slova a, b i c označavaju postojanje statistički značajnih razlika, Tukeyev test, $p \leq 0,05$

Šest od osam izjava o općoj promjeni klime pokazale su se značajnim obzirom na učestalost praćenja vremenske prognoze. Ispitanici koji prate vremensku prognoze jednom ili više puta dnevno pridaju veće ocjene pojedinoj izjavi o promjeni klime u odnosu na one koji to čine tjedno ili rjeđe. Dakle vidljiv je utjecaj medija na mišljenje nositelja poljoprivrednih gospodarstava o klimatskim promjenama.

Zaključak

Klimatske promjene dio su izazova s kojim se poljoprivrednici svakodnevno susreću i čijeg su postojanja sve svjesniji. Pravovremeno dobivanje korisnih informacija značajno može doprinijeti prilagođavanju vremenskim uvjetima, a za to su prvenstveno zaslužni mediji koji redovito obavještavaju o vremenskim prilikama. Redovitim praćenjem vremenske prognoze jednom ili nekoliko puta dnevno moguće je utjecati na mišljenje poljoprivrednika i na taj način uputiti ih na pravovremenu prilagodbu klimatskim promjenama.

Napomena
Istraživanje neophodno za ovaj rad dio je projekta „Agrobioraznolikost – osnova za prilagodbu i ublažavanje posljedica klimatskih promjena u poljoprivredi“ KK.05.1.1.02.0005 financiranog iz Europskog fonda za regionalni razvoj i Fonda za zaštitu okoliša i energetsku učinkovitost u sklopu poziva Shema za jačanje primijenjenih istraživanja za mjere prilagodbe klimatskih promjena KK.05.1.1.02

Literatura

Agencija za plaćanje u poljoprivredi, ribarstvu i ruralnom razvoju RH (2019.). Upisnik poljoprivrednih gospodarstava.

Arimi K. S. (2021). Climate change adaptation and resilience among vegetable farmers. International Journal of Vegetable Science. 27 (5): 496-504.

Bryan E., Deressa T. T., Gbetibouo G. A., Ringler C. (2009). Adaptation to climate change in Ethiopia and South Africa: options and constraints. Environmental science & policy. 12 (4): 413-426.

Deressa T. T., Hassan R. M., Ringler C. (2011). Perception of and adaptation to climate change by farmers in the Nile basin of Ethiopia. The Journal of Agricultural Science. 149 (1): 23-31.

Feleke H. G. (2015). Assessing weather forecasting needs of smallholder farmers for climate change adaptation in the Central Rift Valley of Ethiopia. Journal of Earth Science and Climate Change. 6 (10): 1-8.

Li C., Tang Y., Luo H., Di B., Zhang L. (2013). Local farmers’ perceptions of climate change and local adaptive strategies: a case study from the Middle Yarlung Zangbo River Valley, Tibet, China. Environmental management. 52 (4): 894-906.

Reyes C. M., Domingo S. N., Mina C. D., Gonzales K. G. (2009). Climate variability, SCF, and corn farming in Isabela, Philippines: a farm and household level analysis. Philippine Institute for Development Studies.

Influence of frequency of weather forecast following on climate change recognition

Abstract
Climate change is an increasingly common topic in the media. Due to the large amount of such information, it is likely to have an impact on farmers' views on climate change. For this purpose, a field study was conducted, interviewing a sample of farmers in Adriatic Croatia. It was found that farmers who follow weather forecasts one or more times a day are more likely to perceive general climate change than those who do so several times a week or less frequently. Thus, by providing regular information about the weather forecast, it is possible to influence farmers' opinions about climate change.

Key words: weather forecast, farmers, climate change, Adriatic Croatia

PRETHODNO PRIOPĆENJE

Jačanje održivosti poljoprivrednog sektora kroz bioekonomiju

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Sažetak
Prema procjenama Europske komisije, bioekonomija u postizanju ciljeva Zelenog plana može pridonijeti rastu BDP-a EU 27 te otvaranju novih radnih mjesta u ruralnim područjima. Cilj je ovog rada istražiti koje su mogućnosti investiranja poljoprivrednih proizvođača u svrhu razvoja bioekonomije, a koje mogu pridonijeti jačanju održivosti poljoprivrednog sektora. Istraživanje je provedeno u dvije faze. U prvoj fazi ispitani su poljoprivredni proizvođači kroz šest fokus grupa kako bi se utvrdile njihove mogućnosti i spremnost na financiranje inovacija u okviru bioekonomije. U drugoj fazi analizirali su se dostupni podaci o mogućnostima financiranja bioekonomije na nacionalnom nivou i europskom nivou. Rezultati su pokazali kako su proizvođači otvoreni za inovacije i bioekonomiju, nepremni za veća ulaganja iz vlastitih sredstava te da postoji potreba bolje integracije fondova i infomacija o istima.

Ključne riječi: bioekonomija, financiranje, fondovi, poljoprivreda.

Uvod

Bioekonomija pretvara biološke resurse i tokove otpada u proizvode s dodanom vrijednošću kao što su hrana, održivi materijali i energija (EK, 2012.). Strategija o bioekonomiji pod nazivom «Inovacija za održivi rast: Bioekonomija za Europu» nastala je 2012. godine (EK, 2012.). Strategija je imala za cilj otvoriti put inovativnijoj i održivijoj proizvodnji koja može konkurirati na tržištu, ali istovremeno očuvati okoliš. Strategija je revidirana 2018. godine (EK, 2018.) te je prilagođena ciljevima održivog razvoja Ujedinjenih naroda (UN, 2015.) i Pariškom sporazumu (UN, 2015b.). Bioekonomija mora doprinijeti razvoju vrijednosnih lanaca, kao što su obnovljiva energija, novo biogospodarstvo, kružno gospodarstvo i ekoturizam, pogodno su okruženje za dobar rast i stvaranje radnih mjesta u ruralnim područjima, uz istodobno očuvanje prirodnih resursa (SLEU, 2021.). Također, bioekonomija doprinosi ciljevima Zelenog plana (EK, 2020.). Svaka strategija kako bi bila uspješna mora imati financijsku potporu. Prva strategija se temeljila na Sedmom okvirnom programu za istraživanje u tehnološkom razvoju (FP7) i Okvirnom programu EU-a za istraživanje i inovacije (Obzor, 2020.) iz kojeg se kasnije u okviru prioriteta Društveni izazovi - Sigurnost hrane, održiva poljoprivreda i šumarstvo, istraživanje mora, pomorja i unutarnjih voda i bioekonomija izdvojilo 4,1 milijardu eura. Također, bioekonomija je mogla naći prostor za financiranje i u ostalim prioritetima, ali navedeni prioritet je davao najviše prostora za financiranje. Ukupan proračun za Obzor 2014.-2020. iznosio je 78,6 milijardi eura. (Obzor, 2020.) Osim Obzora, investicije u razvoj bioekonomije osigurale su se i u brojnom fondovima poput strukturnih i kohezijskih fondova, te dodatnih financiranja Europske

komisije (EK) u okviru Life i Interreg programa. U ovom radu istražit će se spremnost i mogućnosti proizvođača na investicije u bioekonomiju te potencijalni izvori financiranja bioekonomije u novom programskom razdoblju. Analizirat će se ključni fondovi koji mogu pomoći uključivanju proizvođača u postizanju visoko ambicioznih održivih ciljeva proizvodnje i razvoju bioekonomije u poljoprivrednom sektoru.

Materijal i metode

U okviru istraživanja napravljena je analiza u dvije faze. U prvoj fazi u ispitivanju je sudjelovalo šest fokus skupine prosječno oko 10 ispitanika iz sektora ratarstva, povrtlarstva, ekološke poljoprivrede i ljekovitog bilja, voćarstva, maslinarstva, vinogradarstva i vinarstva, šumarstva, pčelarstva, svinjogojstva, mljekarstva, peradarstva, ovčarstva i kozarstva, tovnog govedarstva, sustava krava-tele. Idealna veličina fokus grupa je od 6 do 10 ispitanika, iako broj može varirati. (Rabiee, 2004.). Dionici fokus grupa su vodeći poljoprivrednici i predstavnici poljoprivrednika koji participiraju u radu sektorskih odbora Hrvatske poljoprivredne komore. Zastupljeni su bili različiti oblici gospodarstava (OPG, obrt, tvrtke, zadruge, proizvođačke organizacije, udruge). Ispitivanje je provedeno on-line putem aplikacije Zoom koja omogućava snimanje uz prethodno odobrenje ispitanika. Svaka fokus grupa prosječno je trajala 120 minuta, a ispitivanje je provedeno delfi metodom koja se provodi u dva ili više krugova. Prema navedenoj metodi, ispitanici su mogli komentirati rezultate od prvog kruga ispitivanja te odlučiti žele li ga zadržati (Blind i sur., 2001.). Analizirala se prosječna spremnost financiranja inovacija u okviru bioekonomije. U prvom krugu ispitivanja prezentirana su istraživanja o održivoj kružnoj bioekonomiji te su ispitanici razmijenili mišljenja. U drugom krugu ispitancima su se postavljala pitanja na temelju njihovih zaključaka iz prvog kruga. U drugoj fazi analizirali su se dostupni podaci o mogućnostima financiranja bioekonomije na nacionalnom nivou i europskom nivou. Podaci za analizu mogućnosti financiranja projekata u okviru bioekonomije za potrebe ovog rada, prikupljani su iz strateških dokumenata, uredbi, službenih stranica dostupnih programa, službenih stranica EK i međunarodnih mreža. Tijekom analize fokus je bio stavljen na fondove koji mogu pomoći razvoju bioekonomije, ali u agrarnom sektoru. Podaci dobiveni istraživanjem mogu pružiti informacije o financijskim mogućnostima razvoja bioekonomije.

Rezultati i rasprava

U prvoj fazi, u fokus grupama ispitanici su kroz raspravu najčešće spominjali kako bi se upustili u inovacije ukoliko bi im bio osiguran siguran izvor prihoda. Poljoprivredni proizvođači koji su ispitani tijekom fokus grupa, razvoj bioekonomije i investicija gledaju u okviru profitabilnosti svog gospodarstva. Međutim, bioekonomija ima suprasektorski karakter (Adamowicz, 2017.) te mora održivo povezati profitabilnost i okoliš.

Tablica 1. Najčešće spominjane prepreke za investiranje u bioekonomiju

1.	Loša financijska situacija gospodarstva
2.	Nedostižnost projekata u okviru bioekonomije
3.	Administrativno opterećenje
4.	Niska razina mogućnosti sufinanciranja (100.000 kn)
5.	Nisu sigurni u financijsku isplativost

Izvor: Sinteza autora

Iznos za financiranje koji su poljoprivredni proizvođači spremni samostalno izdvojiti je nizak. Stav može biti povezan s nemogućnostima stavljanja vlastitog kapitala u nešto novo i njima još uvijek nepoznato te s određenom dozom straha od neizvjesnosti. Sličan problem skepticizma uočen je u istraživanju (Rossi i Hinrichs, 2011.) osobito vezano za korist od

bioekonomije na lokalnoj razini. Proizvođači bi se upustili u takve investicije uz maksimalno sufinanciranje iz nacionalnih fondova koji su im poznati.

Tablica 2. Najčešće spominjani potencijali za investiranje u bioekonomiju

1.	Otvorenost za sudjelovanje u istraživanjima i pilot projektima
2.	Interes za inovacije u području obnovljivih izvora energije i zbrinjavanje ostataka iz proizvodnje
3.	Mogu ih pokrenuti informacije o isplativosti investicije

Izvor: Sinteza autora

Fondovi koji se financiraju na razini cijele Europe i u kojima tijekom prijava konkuriraju drugim državama članicama ispitanicima nisu poznati. Proizvođači su otvoreni za inovacije i bioekonomiju, ali mogućnosti financiranja im se čine nedostižne i administrativno komplicirane. Također, nedostatak povezanosti informacija o mogućnostima financiranja u okviru fondova i znanja poljoprivrednih proizvođača kako iste koristiti ukazuje na potrebu bolje integracije fondova i infomacija. U drugoj fazi, napravljena je analiza mogućnosti financiranja u uključivanja poljoprivrednih proizvođača kroz fondove koji pomažu razvoju bioekonomije. Nisu obuhvaćene sve mogućnosti financiranja bioekonomije već one koji su ključne. Rezultati su pokazali kako postoje znatna sredstva koja se ulažu u razvoj bioekonomije, ali kao što su rezultati fokus grupa pokazali, postoji potreba za umrežavanjem informacija, znanja i proizvođača kako bi se ista maksimalno iskoristila. Slično su ukazali i Dupont-Inglis i Borg, 2018. godine, na potrebu bolje integracije fondova koji će financirati bioekonomiju te su u Europskom kružnom bioekonomskom fondu (ECBF) vidjeli mogućnost okupljanja sredstava iz različitih mehanizama financiranja.

Tablica 3. Ključni fondovi za razvoj bioekonomije u sektoru poljoprivrede

Naziv programa/fonda	Glavne mjere i aktivnosti iz kojih će se moći financirati razvoj bioekonomije	Ukupno planirana sredstva
Europski poljoprivredni fond za ruralni razvoj (EPFRR) – (MP) (2023.-2027.). (MP) (2021).	Program kroz niz mjera pomaže modernizaciji i razvoju ruralnih područja te razvoju bioekonomije. U nastavku izdvajamo nekoliko najvažnijih. 1. Ulaganja u obnovljive izvore energije 2. Ulaganja u inovacije (Europsko inovacijsko partnerstvo EIP-Operativne skupine)	1. 20 milijuna eura 2. 4.3 milijuna eura
Europski fond za jamstva u poljoprivredi (EFJP) – mjere izravnih plaćanja i zajedničke organizacije tržišta (2021).	Eko shema - Intenzivirano održavanje ekološki značajnih površina obvezom održavanja ekološko značajne površine na minimalno 10% poljoprivrednih površina koje obuhvaćaju i površine pod kulturama kratke ophodnje.	41.4 milijuna eura
Industrijska tranzicija (MRR, 2021).	Proces industrijske tranzicije u Republici Hrvatskoj započeo je sa izradom planova za tri regije u industrijskoj tranziciji: Panonska Hrvatska, Jadranska Hrvatska, Sjeverna Hrvatska. • Horizontalni transformacijski projekt za jačanje regionalnog eko sustava za poduzetnike • Podrška inovacijskim klasterima • Strateška partnerstva za inovacije • Podrška rastu i razvoju inovativnih start-up tvrtki i MSP-ova	1.5 milijuna eura 2.1 milijuna eura 3.3 milijuna eura 4.7 milijuna eura

	• Regionalni lanci vrijednosti, Zeleni rast, AGRI-FOOD, Zdrava i funkcionalna hrana	
LIFE 2021.-2027. (Life, 2021).	Kružno gospodarstvo i kvaliteta života "Prijelaz na čistu energiju"	2.3 milijuna eura.
INTERREG (IRE, 2021).	Interreg Europe pomaže regionalnim i lokalnim vlastima diljem Europe da razviju i provedu bolju politiku. Istraživanje i inovacije Konkurentnost malih i srednjih poduzeća (Cirkularna ekonomija u poljoprivredno-prehrambenom sektoru) Niskougljična ekonomija Okoliš i učinkovitost resursa	379.5 milijuna eura.
Fond za inovacije (EK, 2019).	Programi za demonstraciju inovativnih nisko ugljičnih tehnologija.	Veliki projekti 7.5 milijuna. Mali projekti 7.5 milijuna
Horizon Europe Klaster 6 – hrana, bioekonomija, prirodni resursi, poljoprivreda i okoliš (EK, 2021).	Klima, energija i mobilnost Hrana, biogospodarstvo, prirodni resursi, poljoprivreda i okoliš 8952	Obzor Europa raspolaže proračunom od 95.5 milijardi eura za razdoblje 2021.–2027. To uključuje 5,4 milijarde eura iz instrumenta Next Generation EU. 15.1 milijarda eura 8. 9 milijardi eura.
Circular Bio-based Europe Partnership (CBE) zajedno). (EP, 2020).	Zelena tranzicija prema otpornom i ekološki održivom gospodarstvu	2.0 milijarde eura (EU i privatnih fondova)
Europski kružni bioekonomski fond (ECBF)	ECBF je prvi rizični fond koji je isključivo fokusiran na bioekonomiju i kružnu bioekonomiju u Europi u postizanju ciljeva Europskog zelenog dogovora da Europa postane klimatski neutralna do 2050. godine.	Cilj je investicija od 250.0 miljuna eura. Europska investicijska banka (EIB) će sudjelovati sa 100.0 milijuna eura.

Izvor: Sinteza autora

Zaključak

Poljoprivredni proizvođači tijekom ispitivanja pokazali su želju za sudjelovanjem u bioekonomiji. Međutim, vidljivo je kako nisu upoznati s opcijama kroz razne fondove koji im mogu pomoći u većoj uključenosti i transformiranju gospodarstava. Vraćali su se na tekuću problematiku poput komplicirane administracije, dostupnosti informacija, isplativosti investicija, loše financijske situacije gospodarstava. Nemaju širu sliku koja uključuje povezivanje kroz međunarodne projekte u kojima oni mogu sudjelovati kao pregledno gospodarstvo i u okviru istraživanja. Iako su otvoreni za nove metode kroz bioekonomiju i dalje postoji skepticizam može li određena investicija polučiti željene rezultate. Iznos 100.000,00 kn koji su proizvođači spremni uložiti u razvoj bioekonomije ukazuje na njihovo nepovjerenje u ulaganja u inovacije kao i trenutno slabe financijske mogućnosti koja može biti povezana s veličinom gospodarstava i njihovom kreditnom sposobnosti. Istraživanje u drugoj fazi pokazuje koje su mogućnosti za investicije iz različitih fondova. Dio fondova nije povezan s izravnim financiranjem poljoprivrednih proizvođača, ali im omogućavaju sudjelovanje u projektima povezanim s inovacijama u

okviru bioekonomije putem udruženja, instituta, fakulteta. Mogu se financirati aktivnosti poput kreiranja vrijednosnih lanaca, razvoja novih tehnologija, razvoja novih materijala, testiranja energetskih potencijala ostataka proizvodnje, smanjenja ugljičnog otiska itd. Tijekom istraživanja uočena je potreba za jedinstvenom platformom na kojoj bi proizvođači imali priliku za razmjenu informacija o inovacijama u okviru bioekonomije i financiranja iste. Uočena je i potreba za specijalizirnim savjetnicima koji će pomoći poljoprivrednim prozvođačima oko prijenosa znanja, kao i informiranja o mogućnostima razvoja poljoprivrednog gospodarstva kroz bioekonomiju, izračuna isplativosti ulaganja, informiranja o dostupnim mogućnostima financiranja.

Napomena

Dio istraživanja neophodno za ovaj rad dio su projekta „Istraživanje o kapacitetima i sklonostima korištenja inovacija u poljoprivredi, proizvodnji hrane, šumarstvu i ruralnim područjima unutar održive kružne bioekonomije“, ID 24 u okviru godišnjeg provedbenog akcijskog plana Mreže za 2020. godinu, Mjere 20 »Tehnička pomoć« iz Programa ruralnog razvoja Republike Hrvatske za razdoblje 2014. - 2020., KLASA: 910-05/20-01/02 kojeg financira Ministarstvo poljoprivrede.

Literatura

Blind K., Cuhls K., Grupp (2001). Personal attitudes in the assessment of the future of science and technology: A factor analysis approach. Technological Forecasting & Social Change. 68: 131-149.

Dupont-Inglis J., Borg A. (2018). Destination bioeconomy – The path towards a smarter, more sustainable future. New Biotechnology. 40:140-143.

Europska komisija (EK) (2012). Innovating for Sustainable Growth. A bioeconomy for Europe. Bruxelles.

Europska komisija (EK) (2018). A sustainable bioeconomy for Europe - strengthening the connection between economy, society and the environment : updated bioeconomy strategy. Bruxelles.

Europska komisija (EK) (2019). Innovation Fund.

Europska komisija (EK) (2020). How the bioeconomy contributes to the European Green Deal. Raspoloživo: https://op.europa.eu/en/web/eu-law-and-publications/publication-detail/-/publication/66722c8d-2e03-11eb-b27b-01aa75ed71a1

Europska komisija (EK) (2021). Horizon Europe. Work Programme 2021-2022. Food, Bioeconomy, Natural Resources, Agriculture and Environment. Raspoloživo: https://ec.europa.eu/info/funding-tenders/opportunities/docs/2021-2027/horizon/wp-call/2021-2022/wp-9-food-bioeconomy-natural-resources-agriculture-and-environment_horizon-2021-2022_en.pdf

Europski kružni bioekonomski fond (ECBF) Raspoloživo: https://www.ecbf.vc/team

Europsko partnerstvo u okviru Horizon Europe (EP) (2020). Raspoloživo: https://ec.europa.eu/info/sites/default/files/research_and_innovation/funding/documents/ec_rtd_he-partnerships-circular-biobased-europe.pdf

Interreg Europe 2021-2027 (IRE, 2021). Raspoloživo: Interreg_Europe_2021-2027_CP_final_version__September_2021_.pdf (interregeurope.eu

Life Hrvatska 2021–2027 (Life, 2021.) Raspoloživo: https://lifeprogramhrvatska.hr/hr/life-program/o-programu-life/

Ministarstvo poljoprivrede (2022). Strategija poljoprivrede do 2030.

Ministarstvo znanosti i obrazovanja (MZO) (2020). Agencija za mobilnost i programe EU. Obzor 2020. Što je Obzor 2020.? Raspoloživo: https://www.obzor2020.hr/obzor2020/sto-je-obzor-2020

Ministarstvo regionalnog razvoja (MRR) (2021). Plan za industrijsku tranziciju Jadranske Hrvatske u okviru Integriranog teritorijalnog programa 2021. – 2027. Raspoloživo: https://hgk.hr/documents/uvodna-prezentacija-jadranska-hrvatska6226167d088d8.pdf

Ministarstvo poljoprivrede (MP) (2021). Strateški plan Zajedničke poljoprivredne politike Republike Hrvatske 2023. - 2027. (SP, 2021). Raspoloživo https://www.mrr.hr/files/1.-Nacrt-Strateskog-plana-Zajednicke-poljoprivredne-politike-Republike-Hrvatske-2023.-%E2%80%93-2027..pdf

Rabiee F. (2004). Focus-Group Interview and Data Analysis. Proceedings of the Nutrition Society. 63: 655-660.

Rossi A.M., Hinrichs C.C. (2011). Hope and skepticism: farmer and local community views on the socio-economic benefits of agricultural bioenergy. Biomass Bioenergy. 35: 1418-1428.

Službeni list Europske unije (SLEU) (2021). Uredba (EU) 2021/2115 Europskog parlamenta i Vijeća od 2. prosinca 2021. o utvrđivanju pravila o potpori za strateške planove koje izrađuju države članice u okviru zajedničke poljoprivredne politike (strateški planovi u okviru ZPP-a) i koji se financiraju iz Europskog fonda za jamstva u poljoprivredi (EFJP) i Europskog poljoprivrednog fonda za ruralni razvoj (EPFRR)

Ujedinjeni narodi (UN) (2015a). Department of Economic and Social Affairs. Sustainable Development. The 17 goals. Raspoloživo: https://sdgs.un.org/goals

Ujedinjeni narodi (UN, 2015b). The Paris Agreement. Raspoloživo: https://unfccc.int/process-and-meetings/the-paris-agreement/the-paris-agreement

Strengthening the sustainability of the agricultural sector through bioeconomy

Abstract

According to European Commission estimates, in achieving the goals of the Green Deal, bioeconomy can contribute to the GDP growth of the EU 27 and to the creation of new jobs. The aim of this paper is to explore the possibilities for investment for the purpose of bioeconomy development, which can contribute to strengthening the sustainability of the agricultural sector. In the first phase, farmers were examined through six focus groups to determine their capabilities and willingness to finance innovation within the bioeconomy. In the second phase, the available data on the possibilities of financing the bioeconomy at the national and European level were analyzed. The results showed that producers are open to innovation and the bioeconomy, unready for greater investments from their own resources and that there is a need for a better integration of funds and information about them.

Key words: bioeconomy, financing, funds, agriculture

PREGLEDNI RAD

Smjernice i potencijali zdravstvenog turizma u Republici Hrvatskoj

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Sažetak
Cilj rada je analizirati i istražiti potencijale zdravstvenog turizma, međuodnos pojedinih oblika zdravstvenog turizma kao i potencijale i smjernice daljnjeg razvitka. Zdravstveni turizam je vrsta turizma gdje je glavna motivacija turistima poboljšanje ili očuvanje njihovog zdravstvenog stanja, odnosno liječenje ili prevencija raznih oblika bolesti, ali i umno opuštanje od svakodnevnice. Naime, radi se o kompleksnom turističkom proizvodu te se razlikuju tri oblika: wellness, lječilišni i medicinski turizam.

Ključne riječi: zdravstveni turizam, oblici zdravstvenog turizma, pružatelji zdravstveno-turističkih usluga, ruralni turizam, Republika Hrvatska

Uvod

Republika Hrvatska, kao zemlja u kojoj je turizam najvažnija gospodarska grana, posjeduje sve prirodne blagodati koje su potrebne za razvoj zdravstvenog turizma. Uz sva prirodna bogatstva: more, obala, plaže i baština, Republika Hrvatska je bogata i prirodnim ljekovitim činiteljima koje čine: morski, toplički i klimatski (Geić, 2007.). U Republici Hrvatskoj zdravstveni turizam donosi prihod od 300 milijuna eura, bez pratećih usluga, što čini manje od 3 % posto ukupnog prihoda u turizmu u 2013. godini (Poslovni dnevnik, 2015.). Danas, zdravstveni turizam označava vrstu turizma kada pojedinci putuju radi ozdravljenja ili za vlastitu dobrobit, zbog medicinskih ili zdravstvenih usluga. Ugodna klima, visoka kvaliteta zraka, bujna vegetacija, Jadransko more, termalne vode, ljekovito blato, ekološki proizvedena hrana i seoska imanja samo neki od njih. Lječilišta u Republici Hrvatskoj nude sve potrebne prirodne i ljekovite uvjete, stručno medicinsko osoblje, visoke standarde zdravstvenih usluga, najmoderniju opremu i pristupačne cijene Hrvatska turistička zajednica. Broj bolnica, poliklinika, lječilišta i wellness centara neprestano raste, a postojeći se objekti obnavljaju i usklađuju s najvišim međunarodnim standardima kvalitete. Ruralna Hrvatska čini veliku ulogu u zdravstvenom turizmu odražavajući se kroz ekonomski turizam (proizvodnja hrane) i neekonomski turizam (količina aktivnosti baštine i tradicije).

Materijali i metode

U radu su se koristile sekundarne baze podataka te su na temelju pregleda literature i istraživanja različitih istraživača provedene metode analize, sinteze i komparacije različitih oblika zdravstvenog turizma u Republici Hrvatskoj. Primijenjene su teorijske i aplikativne metode prilikom istraživanja potencijala zdravstvenog turizma. SWOT analiza daje pregled koji ukazuje da je ključan pravac za razvoj zdravstvenog turizma u Republici Hrvatskoj kroz razvijanje zdravstveno-turističke ponude.

Rezultati i rasprava

Republika Hrvatska ima bogatu tradiciju zdravstvenog turizma još od rimskog doba zbog mnogobrojnih prirodnih ljekovitih činitelja koji predstavljaju veliki nacionalni kapital i

potencijal za daljnji razvoj svih oblika zdravstvenog turizma, ali i turizma općenito (Vukonić i sur., 2001.). Glavne nositelje zdravstvenog turizma predstavljaju turističke destinacije koje su zbog svojih prirodnih pogodnosti (klimatskih, morskih, ljekovita blata, naftalana, termomineralnih voda) bazirane na razvoju zdravstvenog turizma. To su specijalne bolnice za medicinsku rehabilitaciju i lječilišta u koje je nužno ulagati kako bi se unaprijedila medicinska rehabilitacija i lječilišta, specifična edukacija zdravstvenog i nezdravstvenog osoblja, sustavna promocija i slično. Osvrćući se na potražnju za pojedinim segmentima zdravstvenog turizma u Republici Hrvatskoj, ističe se činjenica da raspoloživi podaci postoje samo za segment lječilišnog turizma. Upravo to logična je preslika činjenice da usluga (hotelskog i/ili ne-hotelskog) wellnessa gotovo nigdje nije prerasla u prepoznatljiv i specifičan motiv turističkog dolaska. Zaključuje se da je o volumenu wellness potražnje moguće posredno prosuđivati samo na temelju 11 kretanja popunjenosti hrvatske hotelske ponude viših kategorija. Uzimajući u obzir koncentraciju ljudi i sezonska razdoblja od lipnja do rujna, dolazi se do zaključka kako zdravljem motivirani gosti nisu do sada značajnije prepoznali potencijal hrvatske wellness ponude (Ivandić i sur., 2015.). Presudan faktor za prepoznatljivost turizma kroz cijelu godinu je područje ruralne Hrvatske, značaj ekološki proizvedene hrane kao komplementarne grane zdravstvenog turizma.

Tablica 1. SWOT analiza zdravstvenog turizma u Republici Hrvatskoj

Snage	Slabosti
Blizina velikim emitivnim tržištima i dostupnost Hrvatske Atraktivnost i ekološka očuvanost Hrvatske Raspoloživost, kvaliteta i tradicija korištenja prirodnih ljekovitih činitelja Kvaliteta medicinskog kadra i dobra reputacija zdravstvenih usluga Konkurentne cijene Rastući broj hotela s kvalitetnom wellness ponudom Rastući broj kvalitetnih privatnih zdravstvenih ustanova zainteresiranih za zdravstveni/medicinski turizam Početak samoorganiziranja privatnog sektora Rast broja privatnih zdravstvenih osiguravatelja	Nedostatak razvojnog modela Nedostatak razvojne vizije Neusklađenost zakona s područja zdravlja i turizma Zastarjeli/potkapitalizirani objekti lječilišta i specijalnih bolnica (SB) Ograničen razvojni potencijal lječilišta i SB zbog postojeće vlasničke strukture Nedostatak tržišne usmjerenosti lječilišta i SB (prevelika ovisnost o HZZO sustavu) Nedovoljna snaga specijaliziranih posrednika Manjkav destinacijski lanac vrijednosti Nedovoljna multidisciplinarnost obrazovanja Ograničena nacionalna promocija Koncesijska politika za termalne izvore Nepostojanje akreditacije i nedostatno certificiranje Nedostatak kontrole kvalitete usluge Nedovoljna snaga klastera i udruga
Prilike	**Prijetnje**
Rizik profesionalnih bolesti Starenje populacije Svijest o potrebi očuvanja zdravlja Komplementarnost EU sustava zdravstvenog osiguranja Proširenje zdravstvenog osiguranja na usluge preventive u EU Diverzifikacija potrošačkih segmenata i proizvoda zdravstvenog turizma Rastuća uloga kompetencija u medicini Međunarodna prepoznatljivost Hrvatske kao turističke destinacije Prepoznatljivost Srednje Europe kao zdravstveno-turističke destinacije Razvoj specijaliziranih facilitatora Raspoloživost EU fondova/programa	Urušavanje nacionalnih sustava zdravstvenog osiguranja Sve veći broj konkurentskih destinacija Brzi razvoj tehnologije koji nameće potrebu za stalnim visokim investicijama Sve veći zahtjevi/očekivanja (međunarodnih) potrošača

Izvor: Ivandić i sur., 2014.

SWOT analiza daje pregled jakih i slabih strana zdravstvenog turizma u Republici Hrvatskoj, kao i prilika koje bi trebalo kapitalizirati, odnosno prijetnji koje bi trebalo neutralizirati u narednom razdoblju. U pogledu snaga, ističe se raspoloživost i kvaliteta prirodnih ljekovitih činitelja, kvaliteta medicinskog kadra te dugogodišnja tradicija u pružanju zdravstveno turističkih usluga. Gledajući na globalnoj razini, ipak dominiraju one slabije strane. Najznačajniju slabost hrvatskog zdravstvenog turizma predstavlja činjenica da još uvijek ne postoji njegova konkretna razvojna vizija, kao ni razvojni model koji bi omogućio priljev novog razvojnog kapitala. Također, ističe se i neusklađenost zakona u ingerenciji Ministarstva turizma i Ministarstva zdravstva kojima se regulira poslovanje u sferi zdravstvenog turizma. No, unatoč svemu prethodno navedenom, bitno je istaknuti da smjernice i potencijali u zdravstvenom turizmu pružaju brojne prilike na kojima bi Republika Hrvatska ipak mogla kapitalizirati. Upravo zbog sve većeg trenda o potrebi očuvanja zdravlja, kao pojačan rizik od bolesti što se u vrijeme pandemije i života u izolaciji pokazalo kao ključan čimbenik, zaključuje se korištenje sve većeg broja turističkih destinacija koje pružaju usluge zdravstvenog turizma. Rastući broj hotela, zdravstvenih ustanova, kvalitetno korištenje prirodnih ljekovitih činitelja i kroz usavršavanje medicinskog kadra Republika Hrvatska bi mogla u relativno kratkom roku svoje razvojne potencijale istaknuti na globalno-turističko tržište.

Oblici zdravstvenog turizma

Zdravstveni turizam je kompleksan turistički proizvod te se razlikuju tri oblika: wellness, lječilišni i medicinski turizam. Međuodnos pojedinih oblika zdravstvenog turizma prikazuje Tablica 2.

Tablica 2. Oblici zdravstvenog turizma i pružatelji zdravstveno-turističkih usluga

OBJEKTI UGOSTITELJSKE PONUDE		ZDRAVSTVENE USTANOVE	
Wellness ponuda (hoteli, toplice, centri)	Prirodna lječilišta		Klinike/bolnice
	Lječilišta	Specijalne bolnice	
Holistički wellness Medicinski wellness	Holistički wellness Medicinski wellness Lječilišni turizam	Medicinski wellness Lječilišni turizam	Medicinski turizam
Wellness usluge			
	Lječilišne usluge		
		Medicinske usluge	

Izvor: Ministarstvo turizma, 2014.

Wellness podrazumijeva zdrav način života, ali uz to u svoj proces uključuje izobrazbu, iskustva, osobni rast i razvoj, a prije svega samoodgovornost, kao i odricanje (Gojčić, 2005.). Ova vrsta turizma podrazumijeva postizanje tjelesne i duhovne ravnoteže, pri čemu treba razlikovati medicinski od holističkog wellnessa. Medicinski wellness organizirano je provođenje zdravstveno-preventivnih i kurativnih programa u svrhu prevencije bolesti te očuvanja i unaprjeđenja zdravlja uz multidisciplinarni tim koji nužno uključuje liječnika, kao i drugo stručno osoblje, poput fizioterapeuta, kineziologa, nutricionista. S druge strane, holistički wellness obuhvaća ostalu, vrlo šaroliku nemedicinsku wellness ponudu (Trbojević, 2016.). Pojam wellness je nastao kao odgovor na suvremeni način življenja pojedinca. Čovjeku daje do znanja kako sve više bolesti nastaje kao posljedica stresa i nastoji

ljude usmjeriti ka prirodnom načinu života i prehrane. Za zdravlje se smatra kako je ono stanje potpune fizičke, mentalne i socijalne ravnoteže pa stoga wellness upravo svojom raznolikom ponudom nastoji kvalitetno zadovoljiti spomenute potrebe ljudi (Rašić, 2017.).

Drugi oblik zdravstvenog turizma je lječilišni turizam. Korisnici zdravstvenog turizma osobe su dobrog zdravlja i u toplice odlaze na vrstu relaksacije, obrane od stresa, ali i kako je na samom početku navedeno, preventivno. S druge strane, korisnici lječilišnog turizma su osobe koje su bolesne i odlaze na liječenje i terapije u specijalne bolnice, lječilišta ili toplice uz stručni nadzor (Gregorić i sur., 2015.). Iako lječilišni aspekt ima sve važniju ulogu u zdravstvenom turizmu što je prepoznato i od samih stručnjaka, on zahtijeva velika ulaganja u postojeću infrastrukturu. Ova grana turizma ima prilično važnu ulogu kroz cijelu godinu i nije vezana uz sezonalnost te je bitan faktor uključivanja stručnjaka kako bi se na moderan i kvalitetan način napravio iskorak u tom obliku. Lječilišni je turizam prilično popularan u hrvatskom turizmu jer na ovim prostorima postoji veliko bogatstvo prirodnih činitelja, kao i duge tradicije nekih lječilišnih centara poput onih u Opatiji, Crikvenici, Varaždinskim Toplicama, Daruvarskim toplicama, Stubičkim Toplicama i ostalim lječilišnim mjestima (Gregorić i sur., 2015.). Ono što Republiku Hrvatsku čini atraktivnom stranim gostima je bogatstvo prirodnih resursa u njezinom priobalnom, otočnom i kontinentalnom dijelu. Uz to, Republika Hrvatska obiluje dugom tradicijom lječilišnih destinacija koje vuku korijene još od prije par tisuća godina, iz Rimskog doba kada je i otkrivena termomineralna voda i ostali prirodni ljekoviti čimbenici važni za ljudsko zdravlje.

Medicinski turizam je jedinstvena kombinacija medicinskih i turističkih usluga. Riječ je o uspješnom kombiniranju zdravstvenih tretmana i hotelskih i drugih usluga u turističkoj destinaciji. Danas se pod tim pojmom podrazumijeva pružanje široke palete sofisticiranih i visokokvalitetnih medicinskih usluga. Ova vrsta turizma postaje lukrativni biznis i ubrzano se razvija u brojnim turističkim zemljama svijeta. Vrijednost industrije medicinskog turizma neprestano raste, a trenutno je vrijedna oko 60 milijardi dolara godišnje na svjetskoj razini (Favis-Villafuerte, 2009.). Najveći nedostatak korištenja medicinskih usluga u drugoj zemlji za korisnika je moguća nesigurnost, odnosno mogućnost dobivanja neadekvatne zdravstvene usluge u odnosu na očekivanu, odnosno u odnosu na onu medicinsku uslugu koju bi korisnik dobio u svojoj zemlji. No istraživanja pokazuju da taj čimbenik ne uzrokuje pad ni stagnaciju medicinskog turizma, zbog sve veće standardizacije kvalitete medicinskih usluga na globalnoj razini, uz korištenje najnovijih znanja iz područja medicine te najsuvremenije tehnologije. Zdravstvena turistička ponuda može se kombinirati i s brojnim drugim oblicima turističke ponude, primjerice, kulturnim turizmom ili sportsko-rekreacijskim turizmom. Broj i širina usluga koje se pružaju u medicinskom turizmu u razvoju je te se intenzivno proširuje i na druga područja.

Zaključak

Popularnost zdravstvenog turizma u konstantnom je porastu kako u svijetu, tako i u Hrvatskoj. Motivirani poboljšanjem vlastitog zdravlja i promjene načina života, suočavanje sa svakodnevnim stresom, ljudi se sve više okreću ovom profitabilnom obliku turizma. Zdravstveni turizam je vrsta turizma gdje je glavna motivacija turistima poboljšanje ili očuvanje njihovog zdravstvenog stanja, odnosno liječenje ili prevencija raznih oblika bolesti. Nerijetko se definira kao putovanje osobe izvan svog mjesta boravka kako bi se dobila najbolja zdravstvena usluga. Pametno organizirana kombinacija odmora, razonode i medicinskih usluga, jest ono što privlači ljude da se odluče uložiti u svoje zdravlje i izvan svoje zemlje. Zdravstveni turizam u Hrvatskoj ima dugu tradiciju, prirodne predispozicije (prirodni ljekoviti činitelji), respektabilne ustanove (bolnice, poliklinike, lječilišta i wellness centre), stručan kadar no još uvijek nedovoljno iskorišten. U Hrvatskoj zdravstveni turizam čine tri segmenta (oblika): wellness, lječilišni i medicinski turizam. Rezultati sekundarnog

istraživanja i temeljem sagledavanja obilježja zdravstvenog turizma kroz SWOT analizu, upućuju na zaključak kako Hrvatska ima dobar geostrateški položaj, prirodne ljekovite činitelje, ekološku očuvanost, zadovoljavajući broj hotela, lječilišta, toplica i wellness centara, kvalitetu medicinskog i stručnog kadra te konkurentne cijene. Nadalje, trendovi u smislu orijentacije na zdrav život (povećana briga o zdravlju) te starenje populacije ukazuju da činjenicu da zdravstveni turizam u Republici Hrvatskoj ima potencijal. Budućnost treba biti usmjerena na turizam kroz cijelu godinu, implementirati ruralni turizam, trenutno neiskorišteni potencijal kojem se ne pridodaje dosta važnosti.

Literatura

Favis-Villafuerte N. (2009). Medical Tourism – a Flourishing Business. Manila Bulletin.

Geić S. (2007). Organizacija i politika turizma. Split: Sveučilište u Splitu.

Gojčić S. (2005). Wellness: zdrav način življenja: Nova zvrst turizma. Ljubljana: GV Založba.

Gregorić M., Musliu T. (2015). Lječilišni aspekt zdravstvenog turizma u Republici Hrvatskoj. Zbornik radova Međimurskog veleučilišta u Čakovcu. 6 (2): 59-66.

Hrvatska turistička zajednica (2022). Zdravstveni turizam u Hrvatskoj. Raspoloživo: https://www.htz.hr/sites/default/files/2018-02/HTZ%202017%20Zdravstvena%20brosura%20HR.pdf

Poslovni dnevnik. (2015). Hrvatska obiluje neograničenim resursima za daljnji rast zdravstvenog turizma. Raspoloživo: http://www.poslovni.hr/hrvatska/hrvatskaobiluje-neogranicenimresursima-za-daljnji-rast-zdravstvenog-turizma-291936

Ivandić N. Kunst I., Telišman-Košuta N. (2015). Pretpostavke održivosti zdravstvenog turizma u Republici Hrvatskoj – Načela razvoja i ključni činitelji uspjeha. Varaždin: HAZU Varaždin.

Ivandić N., Kunst I., Telišman-Košuta N., Marković, I. (2014). Nacionalni program – akcijski plan razvoja zdravstvenog turizma. Zagreb: Institut za turizam.

Milas Lj. (2015). Potencijali za razvoj zdravstvenog turizma. Zadar: Sektor za turizam Hrvatska gospodarska komora.

Ministarstvo turizma (2014). Akcijski plan razvoja zdravstvenog turizma. Zagreb: Institut za turizam.

Rašić D. (2014). Wellness turizam. Neodoljiva Hrvatska. Zagreb: Lux.

Turner L. (2007). First World Healthcare at Third World Prices, Globalization, Biosocieties.

Trbojević A. (2016.) Razvoj zdravstvenog turizma u Hrvatskoj. Pula: Sveučilište Jurja Dobrile u Puli, Fakultet ekonomije i turizma Dr. Mijo Mirković.

Vukonić B., Keča K. (2001.). Turizam i razvoj - pojam, načela, postupci. Zagreb: Mikrorad, Ekonomski fakultet u Zagrebu.

Guidelines and potentials of health tourism in the Republic of Croatia

Abstract

The aim of this paper is to analyze and explore the potentials of health tourism, the relationship between individual forms of health tourism as well as the potentials and guidelines for further development. Health tourism is a type of tourism where the main motivation for tourists is to improve or preserve their health, or treatment or prevention of various forms of disease, but also mental relaxation from everyday life. Namely, it is a complex tourist product with three forms: wellness, spa and medical tourism.

Key words: health tourism, forms of health tourism, providers of health tourism services, rural tourism, Republic of Croatia

ORIGINAL SCIENTIFIC PAPER

Attitudes on biodiversity and willingnes to support local breed conservation - the case of the Istrian donkey

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Abstract
The preservation of indigenous breeds that provide value to local culture, history and increases biodiversity. The paper aims to determine the association between attitudes on biodiversity and willingness to participate in the conservation of the Istrian donkey in order to preserve local biodiversity. Study presents the results of a survey conducted with Croatian students. Results show that the importance of the Istrian donkey for biodiversity conservation in Istria is well known among the students, but they are not too willing to participate in its conservation. They believe that the state should continue to play a significant role in this protection.

Key words: indigenous breeds, biodiversity, Istrian donkey

Introduction

Globalisation and the reckless use of natural resources have led to a decline in the biodiversity of many ecosystems. Therefore, this problem has become the focus of politics and human awareness in recent decades. Biodiversity is defined as the totality of genes, species and ecosystems that make up life on Earth and provide essential services to society (Ovaska and Soini, 2016). The indigenous breeds are an important part of biodiversity, and their conservation and breeding guarantee the preservation of cultural and historical values (Soini et al., 2019). Indigenous (syn. local, domestic, native, autochthonous) breeds are livestock breeds that originate from and are adapted to specific geographical regions and are used in regions for a long time where they are of economic, scientific and cultural importance (Rands et al., 2010). However, it has long been common practice to replace local breeds in favour of highly productive commercial breeds (Schäler et al., 2019), thus putting many indigenous breeds at risk of extinction (Biscarini et al., 2015). In order to preserve biodiversity, it is therefore necessary to involve policy makers and other stakeholders in the process of conserving native breeds (Ovaska et al., 2021).

Consumers can also play an important role in local breeds conservation, for example, through the concept of "conservation by eating" – a concept that explains that the meat of a local breed must be eaten to be able to conserve it (Menger and Hamm, 2021). In addition, consumers can financially support the preservation of local breeds or participate in promoting local breeds and their importance. Thus, the European Union (EU) supports farmers to continue breeding local breeds with the purpose to preserve their social, cultural, and environmental values (Bojkovski et al., 2015).

The Istrian donkey is an European donkey breed and one of the three Croatian autochthonous donkey breeds with Littoral Dinaric donkey and North Adriatic donkey. It is well adapted to the microclimatic conditions in Istria. The Istrian donkey preserves the local habitat by grazing the lower vegetation, contributing to its comparative advantage in this geographical

region. Nevertheless, the Istrian donkey is an endangered breed; in 2021, only 863 animals were registered (HAPIH, 2022).

This paper aims to determine the association between attitudes on biodiversity and willingness to participate in the conservation of the Istrian donkey in order to preserve local biodiversity. This preliminary research was conducted on a convienience sample of Croatian students.

Materials and methods

An online questionnaire has been developed. It included closed-ended questions on the socio-demographics of the respondents, their familiarity with the term biodiversity and attitudes towards biodiversity (Huang and Lin, 2014) and towards the Istrian donkey, as well as their willingness to participate in the Istrian donkey conservation. Before the attitude questions, the biodiversity definition was presented to familiarise all respondents with the concept. The survey was conducted from January to March 2022 on a convenience sample of students from various universities and polytechnics in Croatia recruited through social networks and email. A total of 193 respondents completed the questionnaire fully and correctly.

The collected responses were processed and analysed using the IBM SPSS Statistics 21. Descriptive statistics were used to describe the sample and respondents' attitudes, knowledge and the willingness to support Istrian donkey conservation to preserve the local biodiversity.

Factor and cluster analysis were used to explore differences between respondents in terms of their attitudes towards biodiversity and to create distinct segments. The Chi-square test was used to assess the possible association between segment membership and willingness to participate in the Istrian donkey conservation.

Results and discussion

Sample description

The sample was dominated by female respondents (71.5%) due to a self-selection bias. The respondents' age ranged between 18 and 33 years. More than half of the respondents (56.0%) grew up in cities, and 21.8% of respondents stated that they currently live in a rural area of Croatia. Only 8.3% of respondents come from Istria.

Attitude towards biodiversity

A large majority of respondents (90.7%) are familiar with the biodiversity concept, and they believe that each individual can contribute to solving the problem of biodiversity loss. Even 26.9% of respondents believe that the solution to the biodiversity problem should be left to experts, unlike in the study of Huang and Lin (2014) where only 2.0% of American students think that way. They do not agree with the principle that biodiversity should be sacrificed for economic growth, and they do not think that biodiversity concerns are exaggerated (Table 1). As many as 92.7% of respondents believe that biodiversity loss is a serious problem, with one third (32.1%) considering it a very serious problem. Furthermore, 4.7% of respondents state that they are committed to biodiversity protection and another 40.4% state that they would like to be even more committed to biodiversity protection. However, 40.9% of respondents say they are not involved in biodiversity protection because they do not know how they can contribute to solving the problem. Other studies also showed a positive students' attitude towards biodiversity but also their low willingness to get involved in environmental protection (Nisiforou and Charalambides, 2012).

In order to identify significantly different groups of students based on their attitudes towards biodiversity, a factor and cluster analysis were conducted. Responses to the statements listed

in Table 1 were used as input variables for a principal factor analysis with varimax rotation. The statement *Science and technology can solve all the problems of biodiversity* was excluded due to low factor loading. The analysis extracted 3 orthogonal factors explaining 52.5% of the variance. The first factor includes four variables associated with the oppinion that *development of society is more important than biodiversity conservation* and explains 22.6% of the variance. The second factor, that we can label "*Not my business"*, consists of two variables that explain 17.4% of the variance. Finally, the third factor, *prone to biodiversity conservation,* consists of three variables explaining 12.6% of the variance (Table 1).

Table 1. Factor analysis on attitudes towards biodiversity

Statements	Mean*	Std. Deviation	Factor 1	Factor 2	Factor 3
I think that each of us can make a significant contribution to addressing the issue of biodiversity loss.	4.16	0.889			(-)0,695
Nature should be left alone.	3.52	1.031		0.694	
I think it is necessary to maintain biodiversity even if it means sacrificing many goods.	3.26	0.980		0.708	
Almost all human activities disrupt biodiversity.	3.03	1.021		0.568	
Biodiversity conservation goals are a threat to further economic prosperity.	3.02	1.237	0.575		
Solving the problem of biodiversity should be left to experts.	2.98	1.011			0.729
Science and technology can solve all the problems of biodiversity.	2.87	0.904	NA	NA	NA
In order to increase economic growth, it is justified to ease the constraints that exist due to the conservation of biodiversity.	2.41	0.941	0,794		
The exploitation of natural resources for basic human needs must be developed even if it results in the loss of wildlife and wildlife populations.	1.99	0.951	0.722		
People worry too much about the problem of biodiversity.	1.85	0.848	0.554		

* 1- do not agree at all, … 5 – fully agree

The factor scores were used as input variables for the hierarchical cluster analysis. In the first step, the nearest neighbour method was used to exclude outliers. After excluding 5 respondents, the Ward method was performed, resulting in 3 clusters: **Conservationist** (50.5% of respondents): They are willing to sacrifice many things to preserve biodiversity and believe that each individual can preserve biodiversity through their actions. **Hesitant** (30.3% of respondents): Respondents in this cluster are committed to biodiversity conservation but are unwilling to sacrifice their comfort to achieve its goals. They tend to leave problem-solving to science but feel that people do not care enough about the problem of biodiversity. **Sceptical** (19.1% of respondents): They believe that an individual cannot contribute to solving the problem of biodiversity conservation and that people care too much about that problem. For them human needs are more important than biodiversity conservation, i.e. it is justified to ease the current limitations to humant activities due to biodiversity conservation purposes.

Willingness to preserve Istrian donkey

Most of the surveyed students (64.2%) have heard of the indigenous Istrian donkey, and 25.4% have seen it, while 36.8% are not sure if they have ever seen an Istrian donkey. The respondents value the role of the Istrian donkey in preserving biodiversity and tradition in Istria (Table 2).

Table 2. Respondents' attitude towards the Istrian donkey

Statements	Mean	Std. Deviation
It is necessary to work on the preservation of the Istrian donkey.	4.32	0.929
The Istrian donkey is the guardian of the Istrian tradition.	4.22	0.882
The Istrian donkey enriches the tourist offer of Istria.	4.18	0.968
The Istrian donkey is important for the preservation of Istria's biodiversity.	4.14	0.944
Istrian donkey meat products contribute to the attractiveness of Istria's gastronomic offer.	3.37	1.183
Istrian donkey meat products are easily available on the market.	2.49	0.913

Only 14.5% of the respondents are willing to financially support the conservation of the Istrian donkey, while the others are either not willing (44.1%) or not sure (41.5%). The surveyed students showed a slightly higher willingness to promote the Istrian donkey by spreading information about the donkey on social networks (32.6%) or by eating meat (24.9%). However, most respondents (almost 70.0%) believe that it is necessary to continue making state payments to farmers.

Table 3. Willingness of the respondents (%) to participate in the conservation of the Istrian donkey

Question	Yes	No	I am unsure
Would you financially help protect and preserve the Istrian donkey once a year?	14.5	44.0	41.5
Would you buy meat and meat products from the Istrian donkey and thus contribute to preserving the breed?	24.9	43.5	31.6
Would you promote the Istrian donkey and thus contribute to preserving the breed (e.g., share content about the Istrian donkey on social networks)?	32.6	37.3	30.1
Do you think that state payments for preserving the Istrian donkey should continue to be paid?	69.9	7.8	22.3

Chi-square tests were performed to explore the association between the students' assignment to the biodiversity attitude segments and their willingness to partticipate in the preservation of the Istrian donkey. The results revealed no significant association. This outcome suggests that despite having positive attitudes towards biodiversity and the Istrian donkey, students still do not perceive the importance of personal engagement in preserving the indigenous breeds. Additionaly, most of the respondents are not from Istria, and it is known from the literature (Cheng et al., 2021) that the willingness to participate in the conservation decreases with distance. It is therefore important to actively promote and educate the importance of local breeds and ways to preserve them among students and young people in general, and to involve them in activities that contribute to the preservation of local breeds as also suggested by Franzolin et al. (2021).

Conclusion

The results of the survey show that many students have a positive attitude towards biodiversity and its conservation, and that they can be split into three groups in terms of their attitude and behaviour: Conservationist, Hesitant and Sceptical, with the last group being the

least represented. Although the importance of the Istrian donkey for biodiversity conservation in Istria is well known among the students, they are not too willing to participate in its conservation, especially financially (less than one-fifth). However, they believe that the state should continue to play a role in this protection (more than three-fifths).The link between attitudes towards biodiversity and willingness to participate in the conservation of the Istrian donkey could not be demonstrated, implying that positive attitudes do not lead to proactive behaviour. These results should be tested on a larger, representative sample.

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References

Biscarini F., Nicolazzin E. L., Stella A., Boettcher P. J., Gandini G. (2015). Challenges and opportunities in genetic improvement of local livestock breeds. FrontiersinGenetics/ LivestockGenomics. Vol. Article 33.

Bojkovski D., Simči M., Kompan D. (2015). Supports for local breeds in the European region – an overview. Poljoprivreda. 21: 2015 (1) Supplement, 7-10.

Cheng P., Tang H., SiyangZhu S., Jiang P., Wang J., Kong X., Liu K. (2021). Distance to river basin affects residents' willingness to pay for ecosystem services: Evidence from the Xijiang river basin in China. Ecological Indicators. Vol. 126, 107691.

Franzolin F., Carvalho G.S., Santana C.M.B., Calegari A.d.S., Almeida E.A.E.d., Soares J.P.R., Jorge J., Neves F.D.d., Lemos E.R.S. Students' Interests in Biodiversity: Links with Health and Sustainability. Sustainability. 2021, 13, 13767.

HAPIH (2020). Godišnje izvješće – Kopitari. Hrvatska agencija za poljoprivredu i hranu. Available from: https://www.hapih.hr/

Huang H.-J., Lin Y.-T.K. (2014). Undergraduate Students' Attitudes toward Biodiversity. Universal Journal of Educational Research. 2 (4): 379-386.

Menger A.K., Hamm U. (2021). Consumers' knowledge and perceptions of endangered livestock breeds: How wording influences conservation efforts. Ecological Economics. Volume 188, 107117.

Nisiforou O., Charalambides A. G. (2012). Assessing Undergraduate University Students' Level of Knowledge, Attitudes and Behaviour Towards Biodiversity: A case study in Cyprus. International Journal of Science Education. 34 (7): 1027–1051.

Ovaska U., Bläuer A., Kroløkke C., Kjetså M., Kantanen J., Honkatukia M. (2021). The Conservation of Native Domestic Animal Breeds in Nordic Countries: From Genetic Resources to Cultural Heritage and Good Governance. Animals. Vol. 11, 2730.

Ovaska U., Soini K. (2016). Local Breeds – Rural Heritage or New Market Opportunities? Colliding Views on the Conservation and Sustainable Use of Landraces. Sociologia Ruralis. 57 (S1): 709-729.

Rands M. R. W., Adams W. M., Bennun L., Butchart S. H. M., Clements A., Coomes D., Entwistle A., Hodge I., Kapos V., Scharlemann J. P. W., Sutherland W. J., Vira B. (2010). Biodiversity Conservation: Challenges Beyond 2010. Science. 329 (5997): 1298-1303.

Schäler J., Addo S., Thaller G., Hinrichs D. (2019). Exploration of conservation and development strategies with a limited stakeholder approach for local cattle breeds. Animal. 13 (12): 2922-2931.

Soini K., Pouta E., Latvala T., Lilja T. (2019). Agrobiodiversity Products in Alternative Food System: Case of Finnish Native Cattle Breeds. Sustainability. 2019, 11, 3408.

REVIEW ARTICLE

Trends in eggs production and consumption

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Abstract
The objective of this study was to identify the main trends in the production and consumption of eggs and omega-3 fatty acid-enriched eggs. Egg producers are turning to the production of eggs with different intrinsic and extrinsic characteristics to meet the growing demands of consumers. Consumers are showing a growing interest in eggs from alternative production systems, but price, egg size and origin are also very important to them. There will be an increase in the share of alternative eggs production systems, as well as a greater emphasis on animal welfare. The obtained results are useful for egg producers for production planning, for consumers who will be additionally informed about egg market trends and the benefits of consuming omega-3 enriched eggs, and for marketing experts to develop strategies to increase eggs demand.

Key words: eggs, omega-3 enriched eggs, trends

Global trends in eggs production and consumption

Eggs are a very important source of protein, fat, and microelements (Kralik and Lovreković, 2018) and represent a food that is affordable and frequently consumed all over the world (Lesnierowski and Stangierski, 2018). According to Martinez-Michel et et al. (2011) consumers prefer eggs because they are safe to eat, easy to prepare, versatile, and cheap comparee with other sources of animal protein.

However, in the last 10 years, egg production has experienced a number of challenges that have had a major impact on the cost-effectiveness of egg production. Consumer demand for healthier, more environmentally friendly, and animal welfare-friendly foods is steadily increasing (Rahmani et al., 2019), which has also led to increased demand for alternative production systems (organic eggs, free-range eggs, and floor rearing eggs). Organic eggs come from free range laying hens raised according to organic production standards. Free-range eggs are produced in a rearing system where the laying hens have constant access to outdoor run during the day. Eggs from floor-raised hens are produced in a rearing system where the laying hens are housed in a barn on the floor (litter). It is worth mentioning the growing world population, which points to the challenge of increasing egg production while respecting the principles of sustainability (FAO, 2017). Moreover, egg production generates high greenhouse gas emissions (Abín et al., 2018) because it relies on a large number of natural resources, such as land, water, and energy, as well as cereals for animal feed, while modern consumers show a growing interest in sustainable products. In addition, in the last 10 years, we have frequently witnessed food scandals related to salmonella or the contamination of eggs with pesticides (Li et al., 2017, Li et al., 2019), which negatively affects the demand for eggs. It is also worth mentioning numerous diseases of modern society related to the nutritional properties of eggs, such as allergies (Loh and Tang, 2018) and high cholesterol (Zhu et al., 2018), which also affects consumer preferences. For these reasons, egg producers are forced to turn to egg production with different improved intrinsic and extrinsic characteristics (organic eggs, free-range eggs, eggs enriched with functional

ingredients such as omega-3 fatty acids, lutein, vitamins A and E, etc.). Knowing egg consumers' preferences is critical to market success. According to the authors Rondoni et al. (2020.), the most important characteristics for consumers when buying eggs are the so-called sensory characteristics (size, eggshell color, yolk appearance and color) and nutritional characteristics (omega-3 enriched), while for Baba et al. (2017) the most important characteristics are price, egg size, origin and production method. Consumers prefer larger eggs (Baba et al., 2017) and eggs of local origin (Gracia et al., 2014). Berkhoff et al. (2020) noted that consumers prefer to consume farm eggs rather than industrial eggs. The proportion of white and brown shell eggs consumed is about 50:50 worldwide, but significant differences have been found between continents. In Europe, Africa, and the Far East, consumers prefer eggs with brown shells, while in the Americas and the Middle East, they prefer eggs with white shells (Cavero et al., 2012). Consumers prefer deep yellow color of the egg yolk (Ayim-Akonor and Akonor, 2014). According to previous research, price is the most important determinant in eggs purchase (Baba et al., 2017) and consumers are not willing to pay a higher price for enriched eggs (Güney and Giraldo, 2019). However, consumers are willing to pay a higher price for cage-free eggs because they associate such production with greater animal welfare (Doyon et al., 2016) and higher food safety standards (Yang, 2018). Moreover, modern consumers demand sustainable products. Nevertheless, Rahmani et al. (2019) concluded that consumers in Spain are not willing to pay a premium price for eggs produced with lower greenhouse gas emissions and reduced water consumption.

Global trends in production and consumption of omega-3-enriched eggs

Functional foods are foods that have been shown to have a beneficial effect on human health in addition to their basic nutritional function (Alongi and Anese, 2021). One of the functional products for which there is a growing demand are eggs enriched with essential nutrients such as omega-3 fatty acids, vitamins or selenium. Omega-3 enriched eggs help reduce the risk of heart disease and maintain normal blood cholesterol levels (Baba et al., 2017) and consumers associated omega-3 enriched eggs with health benefits (Sass et al., 2021). To produce omega-3 enriched eggs, producers need to modify the hens diet. Typically, they feed laying hens with fish and/or flaxseed oil and flaxseed, but they also use antioxidants in the animal feed. According to Transparency Market Research's 2017-2025 forecasts, demand for omega-3 products, including omega-3 enriched eggs, will increase in developed economies, especially among consumers who care about health and believe that food has a significant impact on health. One potential problem for the omega-3-enriched egg market is the perception that consumers are unwilling to pay a higher price for omega-3 enriched eggs. The main reason for this is limited knowledge about the benefits of consuming such eggs (Sass et al., 2018). This is supported by the results of a study conducted in Croatia, which showed that only half of the study participants were aware of omega-3 enriched eggs (Kralik et al., 2020). However, research in Italy shows that unmarried women and consumers with higher economic status are more willing to pay a higher price for omega-3 enriched eggs, but also that willingness to pay a higher price for omega-3 enriched eggs is higher among consumers who place more value on the size of the eggs, the rearing conditions and feeding of the hens, the origin and brand of the eggs (Palmieri et al., 2022). According to Sass et al. (2021) omega-3 eggs are categorized as expensive, so their buyer is described as a “person with high purchasing power”.

Conclusion

According to previous research ,it can be concluded that several factors influence the demand for eggs, which producers should consider when planning their production policies.

In addition to intrinsic characteristics such as the appearance and color of the yolk, extrinsic characteristics such as price, egg size, origin, and production method are also extremely important when purchasing eggs. Socioeconomic characteristics of consumers and cultural factors play an important role in egg consumption behavior, which is reflected in different preferences for brown and white eggshells, egg size, and willingness to pay. It is therefore important to consider different segmentation variables (e.g., place of residence, lifestyle, income level) to help producers find their target consumer group.

Previous research results can help marketers to develop better communication policies to final consumers, for example, by highlighting the production method or egg size in promotions. Since consumers are not willing to pay a higher price for enriched eggs or eggs with lower GHG emissions, primarily due to their limited knowledge, it is necessary to educate consumers about the benefits of purchasing and consuming such eggs.

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References

Abín R., Laca A., Laca A., Díaz M. (2018). Environmental assesment of intensive egg production: A Spanish case study. Journal of Cleaner Production. 179: 160–168.

Alongi M., Anese M. (2021). Re-thinking functional food development through a holistic approach. Journal of Functional Foods. 81: 104466.

Ayim-Akonor M., Akonor P. T. (2014). Egg consumption: Patterns, preferences and perceptions among consumers in Accra metropolitan area. International Food Research Journal. 21 (4): 1457-1463.

Baba Y., Kallas Z., Realini C. (2017). Application of the analytical hierarchy process to evaluate consumer acceptance and preferences for omega-3 enriched eggs. British Food Journal. 119 (7): 1459-1472.

Berkhoff J., Alvarado-Gilis C., Keim J. P., Alcalde J. A., Vargas-Bello-Pérez E., Gandarillas M. (2020). Consumer preferences and sensory characteristics of eggs from family farms. Poultry Science. 99 (11): 6239-6246.

Cavero D., Schmutz M., Icken W., Preisinger R. (2012). Attractive eggshell color as a breeding goal. Lohmann Information. 47 (2): 15-21.

Doyon M., Bergeron S., Cranfield J., Tamini L., Criner G. (2016). Consumer Preferences for Improved Hen Housing: Is a Cage a Cage? Canadian Journal of Agricultural Economics. 64 (4): 739-751.

FAO (2017). Gateway to poultry production and products. Available from: http://www.fao.org/poultry-production-products/production/en/

Gracia A., Barreiro-Hurlé J., López-Galán B. (2014). Are Local and Organic Claims Complements or Substitutes? A Consumer Preferences Study for Eggs. Journal of Agricultural Economics. 65 (1): 49-67.

Güney O. I., Giraldo L. (2019). Consumers' attitudes and willingness to pay for organic eggs: A discrete choice experiment study in Turkey. British Food Journal. 122 (2): 678-692.

Kralik Z., Lovreković M. (2018). Utjecaj hranidbe na kvalitetu i obogaćivanje jaja funkcionalnim sastojcima. Meso, 20 (1): 58-65.

Kralik Z., Kralik G., Hanžek D. (2020). Mišljenje potrošača u Hrvatskoj o konzumaciji omega-3 obogaćenih jaja. In Zbornik radova 55. hrvatskog i 15. međunarodnog simpozija agronoma. Mioč B., Širić, I. (ed.). 440–443. Zagreb, Hrvatska: Sveučilište u Zagrebu, Agronomski fakultet.

Lesnierowski G., Stangierski J. (2018). What's new in chicken egg research and technology for human health promotion? - A review. Trends in Food Science and Technology. 71: 46-51.

Li T., Bernard J. C., Johnston Z. A., Messer K. D., Kaiser H. M. (2017). Consumer preferences before and after a food safety scare: An experimental analysis of the egg recall. Food Policy. 66: 25-34.

Li X., Li H., Ma W., Guo Z., Li X., Song S., Tang, H., Li X., Zhang Q. (2019). Development of precise GC-EI-MS method to determine the residual fipronil and its metabolites in chicken egg. Food Chemistry. 281: 85-90.

Loh W., Tang M. L. K. (2018). The epidemiology of food allergy in the global context. International Journal of Environmental Research and Public Health. 2043 (15): 1-8.

Martinez-Michel L., Punter P., Wismer W (2011). Perceptual attributes of poultry and other meat products: a repertory grid application. Meat Science. 87 (4): 349-355.

Palmieri N., Stefanoni W., Latterini F., Pari L. (2022). Factors Influencing Italian Consumers' Willingness to Pay for Eggs Enriched with Omega-3-Fatty Acids. Foods. 11: 545.

Rahmani D., Kallas Z., Pappa M., Gil J. M. (2019). Are Consumers' Egg Preferences Influenced by Animal-Welfare Conditions and Environmental Impacts? Sustainability. 11 (22): 6218

Rondoni A., Asioli D., Millan E. (2020). Consumer behaviour, perceptions, and preferences towards eggs: A review of the literature and discussion of industry implications. Trends in Food Science & Technology. 106: 391-401.

Sass C. A. B., Kuriya S., Da Silva G. V., Silva H. L. A., Da Cruz A. G., Esmerino E. A., Freitas M. Q. (2018). Completion task to uncover consumer's perception: A case study using distinct types of hen's eggs. Poultry Science. 97 (5): 2591-2598.

Sass C. A., Pimentel T. C., Guimarães J. T., Silva R., Pagani M. M., Silva M. C., Queiroz M. F., Cruz A. G., Esmerino, E. A. (2021). How buyer-focused projective techniques can help to gain insights into consumer perceptions about different types of eggs. Food Research International. 144, 110320.

Transparency Market Research. Omega-3 Eggs Market - Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2017-2025. Available from: Omega-3 Eggs Market | Global Industry Report, 2025 (transparencymarketresearch.com)

Yang Y. C. (2018). Factors affecting consumers' willingness to pay for animal welfare eggs in Taiwan. International Food and Agribusiness Management Review. 21 (6): 741-754.

Zhu Y., Vanga S. K., Wang J., Raghavan V. (2018). Impact of food processing on the structural and allergenic properties of egg white. Trends in Food Science and Technology. 78: 188-196.

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Genetika, oplemenjivanje bilja i sjemenarstvo

03

Genetics, Plant Breeding and Seed Production

ORIGINAL SCIENTIFIC PAPER

Studies on the inheritance of agronomic traits in hybrids of different types of tobacco

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Abstract

The aim of this paper was to study the inheritance of: green and dry leaf and seed yield per plant, as well as to detect heterosis in four F_1 hybrids obtained by crossing five tobacco varieties, four of which were Oriental in the role of mother and a Burley as a father, in 2019 and 2020.

The most common mode of inheritance was the intermediate, and then the partial-dominant. There was no heterosis in the inheritance of green and dry leaves yield per plant, while for the yield of seeds in all combinations there was positive heterosis. The highest heterotic effect had MS 8/1 x B-9/91.

The mode of inheritance of the agronomic traits per plant indicates a shorter and more reliable successive selection when creating superior varieties. The paper indicates further research into hybrids with strong heterosis for seed yield, in line with the new trends in alternative tobacco use.

Keywords: tobacco (*Nicotiana tabacum* L), leaf-yield, seed-yield, inheritance, heterosis.

Introduction

Tobacco is a plant used by humans for centuries in various ways, most often for smoking. Today there is a strong propaganda about its harmful effect on human health. Therefore, efforts are being made for alternative use of this crop in the direction of seed production, extraction of oil from it and providing renewable resources of biomass and biofuel. However, there is a huge number of smokers who, despite the anti-advertising, do not give up the pleasure offered by this ancient culture.

The aim of this investigations was to study the mode of inheritance and to discover a possible heteroteric effect on the yield of green and dry leaves and seed yield per plant in the F_1 generation obtained by crossing varieties from different types, which will give important guidelines for future selection programs for tobacco breeding in the direction of increasing leaf yield and seed yield.

Material and methods

For genetic studies on inheritance of green and dry leaf yield and seed yield, five varieties of tobacco were selected, four of which in the role of mother: three of the type Prilep (P-23, P 18-50/4 and P 76/86), one of the Basmak type (MS 8/1), and the Burley variety B-9/91 in the role of a father.

In 2018, in field conditions, with manual castration and pollination, four crosses were made: P-23 x B-9/91, P 18-50/4 x B-9/91, P 76/86 x B-9/91 and MS 8/1 x B-9/91.

In 2019, at the experimental field at the Scientific Tobacco Institute - Prilep, we set up an experiment with nine genotypes (five parents and four F_1 hybrids), according to a randomized block design in four replications. Each plot had 8.1 m^2, one repetition had 72.9 m^2, and the whole experiment covered a working area of 291.6 m^2. Oriental genotypes were

planted at 45 x 15 cm, large-leaf variety B-9/91 at 80 x 50 cm, while F_1 hybrids at 60 x 30 cm. In the same year we collected seeds for the second generation and again made the same crosses from which we collected seeds for the first generation. In 2020, an experiment was set up using the same method, in which the same set of nine genotypes was planted. The paper presents two-year results from the first generation.
The yield of green leaves was measured after each harvest, the weight of all harvests from each plot was valuated and divided by the number of plants from which the tobacco was harvested, giving us the weight of the green leaf per plant. Leaf yield was expressed as an average of 20 plants per repetition or 80 plants out of four repetitions. Dry leaf yield was measured and calculated after tobacco manipulation. Seed yield was expressed as an average of 10 plants per repetition or 40 plants out of four repetitions. The mode of inheritance of traits was determined on the basis of test significance from the mean value of the F_1 generation relative to the average of both parents, according to Borojevic (1981).

Results and discussion

Yield is a priority and its increase is an eternal aspiration for every breeder. Usually yield and quality do not go in the same direction. Therefore, the duty of the breeder is to increase the yield in his creations, without compromising the quality. This paper covers genetic studies on the inheritance of leaf and seed yields in tobacco.
The highest yield of green leaf mass per plant among the parent genotypes in 2019 and 2020 had the large-leaf air-cured variety B-9/91, and among the oriental parents the variety P 76/86. The most productive F_1 hybrid was P 76/86 x B-9/91, and the least productive was P-23 x B-9/91. The same rank was obtained for the yield of dry leaf mass per plant in the two years of investigations (Table 1).
MS 8/1 is characterized by the highest yield of seed per plant, and with the lowest P 18-50/4. Among the F_1 hybrids MS 8/1 x B-9/91 had the highest and P-23 x B-9/91 the lowest seed yield per plant (Table 1). Inheritance of green leaf mass yield per plant in 2019 and 2020 in F_1 offspring was intermediate. Only P-23 x B-9/91 had partial dominance mode. The mode of inheritance points to fast and reliable selection in the process of creating new superior varieties. An identical inheritance occured for the dry leaf mass yield per plant in the two years of investigation. The same mode of inheritance in leaf yield was obtained by the following scientists: Gixhari & Sulovari (2010) conducted three-year research at two locations, in a genetically diverse population of eight oriental tobaccos and their one-way diallel hybrids and obtained heterosis. Aleksoski & Korubin – Aleksoska (2011) conducted three-year studies in a one-way diallel on three oriental and one large-leaf variety and their six F1crosses. Both, positive and negative heterosis was obtained.
In an experiment in Khan Gari, Mardan, Pakistan, with seven Virginia flue-cured genotypes and their 42 two-way diallel crosses, Imtiaz et al. (2014) found heterosis with high heterotic effect and possibility its utilization. Kinay & Yilmaz (2016), in Tokat Province, Turkey found an average heterosis of 28.4%, in the investigation with hybrids obtained with semidiallel hybridisations of Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa. The heterotic effect on dry leaf yield was 4%. Ganachari et al. (2018) examined six flue-cured tobaccos and their 30 crosses at ZAHRS (Zonal Agricultural and Horticultural Research Station) College of Agriculture - Shivamogga, Karnataka - India. The authors identified five hybrids that showed a highly significant heterotic effect. In a one-way diallel of four small-leaf aromatic and one large-leaf flue-cured variety and their 10 F1 crosses, Aleksoski (2019) found positive heterosis. In eight Burley newly created hybrid combinations from the first generation, Dyulgerski (2019) found that all of them are better than the standard variety Pliska 2002, which indicates the possibility of exploiting heterosis in Burley tobacco.

Qaizar et al. (2019) did research on seven flue-cured varieties and lines and their complete diallel crosses and found positive and negative heterosis. The best hybrids with favorable significant heterotic effect on yield were: KHG24 x Spt G 28; KHG21 x NC606 and Spt G 126 x KHG24. In a semi-diallel of seven genotypes (Xanthi-2A, Nail, Gümüşhacıköy, Taşova, Katerini, Canik and Erbaa) and 21 F1 hybrids, tested in two locations in the Black Sea region of Turkey, Kinay et al. (2020) obtained positive heterosis in yield.

The trait seed yield was previously analized in several studies: Bucciarelli et al. (2012), in two genotypes found that Line 1917 gave a seed yield of 1.8 t ha^{-1} and a dry leaf yield of 1.93 t/ha, while the G105 variety gave a seed yield of 1.47 t ha^{-1} and dry leaf yield of 1.51 t ha^{-1}, and correspondingly the weight of 1000 seeds in Line 1917 is 73 mg, and in G105 69 mg; Grisa et al. (2016), in the Solaris variety for alternative tobacco production in Italy, carried by the idea that tobacco could indeed become a new industrial crop that offers opportunities for its use to obtain biomass and biofuel, recorded 31 to 34 g of seeds per plant, or 1.1-1.8 t ha^{-1} from one harvest, with oil yield up to 0.59 t ha^{-1} and from two seed harvests they determined a total seed yield of 4.5 t ha^{-1}, from which 1.48 t of oil per ha can be obtained.

In our investigations, the seed yield per plant in the parent genotypes ranges from 10.30 g $plant^{-1}$ (2020) in P 18-50/4 to 11.38 g per plant (2020) in MS 8/1. Compared with the results obtained from Grisa et al. (2016) - 34 g per plant, our varieties gave much lower seed yields. However, F_1 hybrids had significantly higher yield ranging from 16.27 g per plant (2019) in P-23 x B-9/91 to 23.52 g per plant (2020) in MS 8/1 x B-9/91 (Table 1).

The biennial analysis of inheritance in seed yield per plant in all crosses obtained in present study informs of positive heterosis. This knowledge gives an opportunity for further studies in the field of hybridization and evaluation of the heterotic effect.

Table 1. shows the mean values for the yield of green leaves, dry leaves and seed per plant in the parent genotypes and their diallel F_1 offspring, as well as the mode of inheritance for the traits in 2019 and 2020.

Table 1. Mode of inheritance for the yield of green leaves, dry leaves and seeds per plant of F_1 tobacco hybrids in 2019 and 2020

No.	Genotypes and F_1 hybrids		Yield per plant (g) 2019			Yield per plant (g) 2020		
			Green leaves	Dry leaves	Seeds	Green leaves	Dry leaves	Seeds
			$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
1.	P-23	P1 (♀)	113.14	19.13	11.32	127.66	20.86	10.55
2.	P 18-50/4	P1 (♀)	123.11	20.14	10.41	125.59	20.48	10.30
3.	P 76/86	P1 (♀)	142.10	22.17	10.88	144.82	22.20	11.00
4.	MS 8/1	P1 (♀)	102.71	16.52	11.36	104.19	16.39	11.38
5.	B-9/91	P2 (♂)	1296.53	179.69	10.78	1302.07	184.29	10.98
6.	P-23 x B-9/91	F_1	502.82 ^{pd}	75.18 ^{pd}	16.27 $^{+h}$	513.82 ^{pd}	75.58 ^{pd}	16.55 $^{+h}$
7.	P 18-50/4 x B-9/91	F_1	594.56 ^{i}	88.82 ^{i}	16.48 $^{+h}$	603.07 ^{i}	89.41 ^{i}	17.11 $^{+h}$
8.	P 76/86 x B-9/91	F_1	726.84 ^{i}	103.86 ^{i}	17.05 $^{+h}$	737.15 ^{i}	106.04 ^{i}	16.86 $^{+h}$
9.	MS 8/1 x B-9/91	F_1	558.53 ^{i}	82.93 ^{i}	23.24 $^{+h}$	555.75 ^{i}	80.53 ^{i}	23.52 $^{+h}$

i=intermediate, pd=partial dominance, +h=positive heterosis

Conclusions

Inheritance of green leaf mass yield per plant in F1 hybrids in 2019 and 2020 was intermediate, with the exception of P-23 x B-9/91 where partial dominance was observed. Detected mode of inheritance indicates rapid and reliable successive selection in the process

of creating new superior varieties. A completely identical mode of inheritance occurs for the yield of dry leaf mass per plant. The biennial analysis of the inheritance of seed per plant in all crosses informs about positive heterosis. This knowledge provides an opportunity for further studies in the field of hybridization and evaluation of heterotic effect. The mode of inheritance of the yield was identical in both years of investigations, which maens that the environmental factors have insignificant impact. The results of this paper are an excellent original source material for further successive selection activity in the direction of obtaining varieties with higher yield of leaves, and varieties with higher seed yield. The paper reveals the possibility of seed production, in line with new trends for alternative use of tobacco in providing renewable resources of biomass and biofuel.

Refferences

Aleksoski J., and Korubin – Aleksoska A. (2011). Degree of inheritance and heritability of yield in parental genotypes and F_1 hybrids of tobacco. Journal of Agricultural Sciences. 56(3): 165–172. Available from: http://www.doiserbia.nb.rs/img/doi/1450-8109/2011/1450-81091103165A.pdf

Aleksoski J. (2019). Genetic studies and combining abilities of varieties and their diallel F_1 hybrids in tobacco (*Nicotiana tabacum* L.). Doctoral dissertation: 230, Prilep, Republic of North Macedonia, Scientific Tobacco Institute – Prilep.

Borojevic S. (1981). Principi i metode oplemenjivanja bilja. Book: 386, Novi Sad, Republic of Serbia, Cirpanov.

Bucciarelli S., Civitella D., Fecondo G., Ghianni G., Ntino C., del Piano L., Abet M., Stanisci V., dell' Arciprete R. (2012). Tabacco, olio da semi per energia in alternativa all'uso della foglia. Terra e Vita. 53:42–44. Available from: https://studylibit.com/doc/976553/tabacco--olio-da-semi-per-energia-in-alternativa-all-uso-...

Dyulgerski Y. (2019). Estimation of new hybrids in first generation of Burley tobacco. Bulgarian Journal of Crop Science. 56 (2): 20–25. Available from: https://www.cabdirect.org

Ganachari M., Mohan Kumar H.D., Dushyantha Kumar B.M., Nataraju S.P., Ravindra H. (2018). Yield in FCV (flue-cured Virginia) tobacco (*Nicotiana tabacum* L.). International Journal of Current Microbiology and Applied Sciences. 7(8): 2726-2733. Available from: https://www.ijcmas.com/abstractview.php?ID=9251&vol =7-8-2018&SNo=285

Gixhari B., and Sulovari H. (2010). Nature of inheritance and heterosis estimated on some morphological quantitative characters that influence the tobacco yield. Studii şi Cercetări (SCSB), Universitatea "Vasile Alecsandri" din Bacău, Romania, XVIII: 46-50. Available from: https://scholar.google.com

Grisa S., Polizzotto R., Raiola P., Cristiani S., Ventura F., di Lucia F., Zuin M., Tommasini S., Morbidelli R., Damiani F., Pupilli F., Bellucci M. (2016). Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. Agronomy for Sustainable Development. 36: 55. Available from: https://www.researchgate.net/publication/308534538_Alternative_use_of_tobacco_as_a_sustainable_crop_for_seed_oil_biofuel_and_biomass

Imtiaz A., Ashiq M., Haneef R., Aziz Ur R., Ihsan Ul K., Muhammad I., Anas I., Muhammad A. (2014). Performance of flue cured Virginia tobacco. International Journal of Basic & Applied Sciences IJBAS-IJENS. 14(02): 1-3. Available from:

https://www.researchgate.net/publication/337811676_Performance_of_Flue_Cured_Virginia_Tobacco

Kinay A. and Yilmaz G. (2016). Effects of heterosis on agronomically important traits of oriental tobacco (*Nicotiana tabacum* L.) hybrids. Süleyman Demirel Üniversitesi Ziraat Fakültesi Dergisi. 11 (1): 89-94. Available from: https://dergipark.org.tr/tr/download/article-file/308601

Kinay A.,Yilmaz G., Kandemir N. (2020). Yield and quality properties of some Oriental tobacco (Nicotiana tabacum L.) hybrids. GENETIKA. 52 (2): 735-750. Available from: http://doiserbia.nb.rs/Article.aspx?id=0534-00122002735K#.Yd1TNMnMKUk

Qaizar A., Fida M., Sheraz A., Sultan Akbar J., Imtiaz A., Ajmalud D. (2019). Heterotic studies in flue-cured tobacco across environments. Sarhad Journal of Agriculture. 32(2): 112-120. Available from: http://researcherslinks.com/current-issues/Heterotic-Studies-Flue-Cured-Tobacco-Environments/14/1/198/htmll

IZVORNI ZNANSTVENI RAD

Utjecaj svjetla na mineralni sastav te sadržaj klorofila i karotenoida kod mladih izdanaka heljde

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Sažetak
Posljednjih godina sve više raste interes za konzumacijom mladih izdanaka. Mladi izdanci izuzetno su bogati mineralnim tvarima, klorofilom te vitaminima. Način proizvodnje značajno utječe na njihov nutritivni sastav. Vrsta svjetla smatra se vrlo važnim okolinskim čimbenikom koji utječe na fotokemijske procese kod biljaka, koji utječu na koncentraciju mineralnih tvari, klorofila i karotenoida. Cilj istraživanja bio je utvrditi mineralni sastav te sadržaj klorofila i karotenoida kod mladih izdanaka heljde uzgojenih pod FLUO i LED osvjetljenjem. Mladi izdanci uzgajani pod FLUO osvjetljenjem imali su veći sadržaj P, K, Mg i klorofila *b*, dok je kod mladih izdanaka uzgajanih pod LED osvjetljenjem utvrđen veći sadržaj Fe i klorofila *a*.

Ključne riječi: heljda, mladi izdanci, osvjetljenje, minerali, biljni pigmenti

Uvod

U posljednje vrijeme klijanci, mladi izdanci i "baby leaf" povrće predstavljaju rastući segment u sektoru poljoprivredne proizvodnje (Di Gioia i sur., 2017.). Zbog visokog sadržaja klorofila i bioaktivnih spojeva (minerala, vitamina, fenola, karotenoida) ubrajaju se u funkcionalnu hranu. Mladi izdanci konzumiraju se svježi, bez termičke obrade stoga ne dolazi do gubitka nutrijenata kojima su vrlo bogati. Osim hranjivih sastojaka, sadrže bioaktivne spojeve koji mogu pozitivno utjecati na fiziološke procese u organizmu i/ili smanjiti rizik od bolesti (Di Gioia i Santamaria, 2015.).

Način proizvodnje mladih izdanaka značajno utječe na njihov nutritivni sastav. Vrlo važan okolinski čimbenik kod proizvodnje mladih izdanaka je vrsta svjetla. Naime, svjetlost utječe na fotokemijske procese, a time i na sadržaj mineralnih tvari, klorofila i karotenoida u mladim izdancima. Svjetlost na različite načine djeluje na fiziološke reakcije u biljci, a ovisno o vrsti, spektralni sastav i jačina svjetlosti mogu izazvati stresne ili pozitivne reakcije biljke u prirodi ili u proizvodnim uvjetima (Ouzounis i sur., 2015.).

Crveni spektar je osnovni dio vidljive svjetlosti kojeg biljka apsorbira te omogućuje normalan rast biljke i odvijanje fotosinteze. Međutim, dokazani su pozitivni učinci korištenja samo plavog LED spektra svjetlosti (440 – 476 nm) ili u kombinaciji s crvenim LED osvjetljenjem na veći udio klorofila u kineskom kupusu (*Brassica rapa* subsp. *pekinensis*) (Olle i Viršilė 2013.). Rajan i sur. (2019.) također su utvrdili veći sadržaj klorofila i antocijana kod mladih izdanaka crvenog i zelenog bosiljka (*Ocimum basilicum*) uzgajanih pod plavim i crvenim LED svjetlom. Korištenje kombinacije plavog (20 %) i crvenog LED svjetla (80 %) doprinjelo je povećanju sadržaja ukupnog klorofila te klorofila *a* i *b* u mladim izdancima brokule (Zhang i sur., 2020.). Istraživanje koje su proveli Vaštakaitė i sur. (2017.) pokazalo je da „microgreens“ uzgojeni pod utjecajem LED osvjetljenja različitih valnih duljina akumuliraju više makro- i mikroelemenata u odnosu na biljke uzgojene pod

osvjetljenjem *High pressure sodium* (HPS) svjetiljki. Nam i sur. (2018.) analizirali su sadržaj klorofila u kotiledonama kod heljdinih klijanaca koji su bili izloženi raznim izvorima svjetlosti. Utvrđen je veći sadržaj klorofila kod mladih izdanaka izloženih crvenom (RL) i fluorescentom (FL) svjetlu u odnosu na plavo svjetlo (BL). Spektri svijetla FL i RL inducirali su veliko povećanje u akumulaciji klorofila od prvog do devetog dana nakon proklijavanja koje je doseglo razinu od 14,88 odnosno 14,74 mg g^{-1} svježe tvari, što je bilo znatno više u odnosu na heljdine klijance uzgajane pod BL svijetlom (6,51 i 1,09 mg g^{-1} svježe tvari). Cilj ovog istraživanja bio je utvrditi mineralni sastav te sadržaj klorofila i karotenoida kod mladih izdanaka heljde uzgojenih pod FLUO i LED osvjetljenjem.

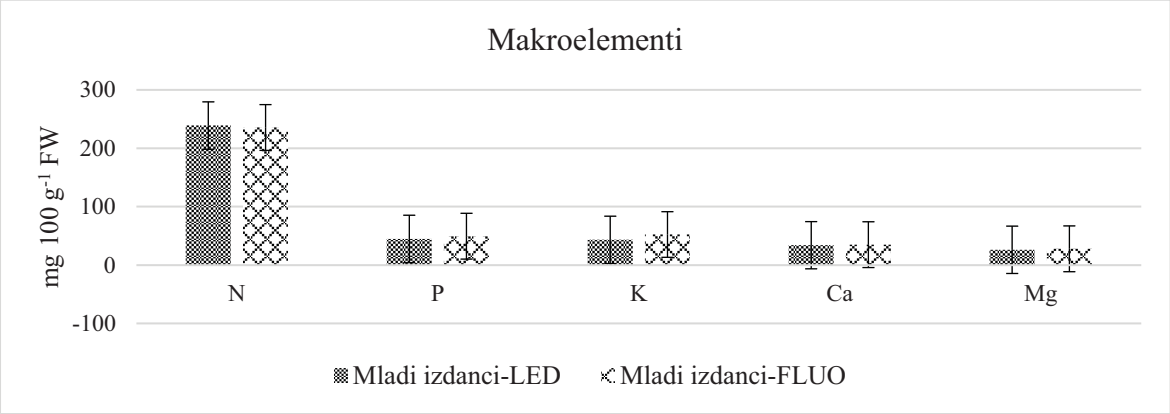
Materijal i metode

Uzgoj i evaluacija mladih izdanaka heljde *Fagopirum Esculentom Moench* ('Varaždinska heljda') pod LED i FLUO osvjetljenjem provedena je u komorama rasta i laboratoriju Zavoda za oplemenjivanje bilja, genetiku i biometriku te u Laboratoriju za ishranu bilja Sveučilišta u Zagrebu Agronomskog fakulteta. Sjeme heljde prije sjetve močeno je 4 sata u destiliranoj vodi. Zatim je po 400 sjemenki posijano u posudice dimenzija 10 x 11 cm napunjenih supstratom (prirodni treset). Svakih dva dana posudice su zalijevane s 50 ml vode. U komorama rasta gdje su se uzgajali mladi izdanci fotoperiod je iznosio 16/8 sati (svjetlo/tama), pri konstanoj temeperaturi od 22 °C i konstantnoj vlažnosti zraka od 48 %. Izvori umjetnog osvjetljenja bile su fluorescentne lampe Osram L 36W/77 FLUORA ili LED diode proizvođača Philips, model GP LED DR/B 120 HB LO s omjerom crvenog i plavog svjetla 52 % : 48 %. Kod oba tipa osvjetljenja intenzitet je bio jednak, 40 µE $m^{-2}s^{-1}$.
Pokus je postavljen prema slučajnom bloknom rasporedu u tri ponavljanja. Uzgoj mladih izdanaka trajao je 11 dana. Uzorci su nakon toga osušeni na 105°C do konstantne mase i analizirani s ciljem utvrđivanja sastava mikro- i makroelemenata te klorofila i karotenoida. Analiza makro- i mikroelemenata osušenih uzoraka provedena je u analitičkom laboratoriju Zavoda za ishranu bilja sljedećim metodama: ukupni dušik (% N/ST) - Kjeldahlova metoda nakon razgradnje uzoraka (digestije) koncentriranom HNO_3 i $HClO_4$ (ETHOS 1 MICROWAVE), ukupni fosfor (% P/ST) - spektrofotometrija (EVOLUTION 60S UV-VISIBLE), ukupni kalij (% K/ST) - plamenfotometrija (JANWEY PFP 7), ukupni kalcij i magnezij (% Ca/ST, % Mg/ST) - atomska apsorpcijska spektrometrija (AAS SOLAR THERMO SCIENTIFIC), ukupni mikroelementi (mg kg^{-1} na ST) - atomska apsorpcijska spektrometrija (AAS SOLAR THERMO SCIENTIFIC), AOAC (2015.).Određivanje klorofila i karotenoida provedeno je prema protokolu opisanom u radu Niroula i sur. (2019.) te izračunato na temelju sljedećih formula: Chl *a* = 12.21 * A 663 – 2.81 * A 646; Chl *b* = 20.13 * A 646 – 5.03 * A 663; Car = 4.69 * A 440.5 – 0.268 * (Chl a + Chl b).
Vrijednosti prikazane u rezultatima preračunate su na svježu tvar. Za statističku analizu korištena je jednofaktorijalna analiza varijance i Bonferroni test razine značajnosti 5 % . Analize su provedne pomoću SAS verzije 9.1. (SAS/STAT, 2010.).

Rezultati i rasprava

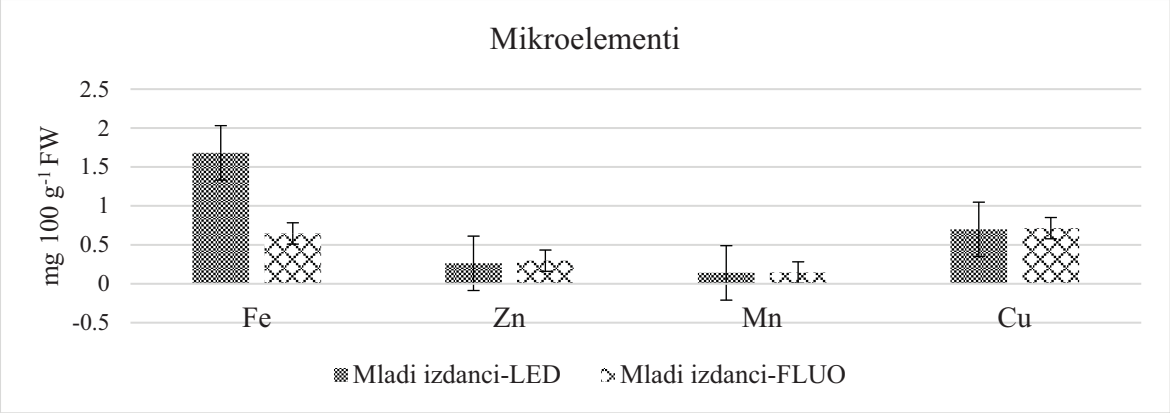
Tip osvjetljenja značajno je utjecao na sadržaj fosfora, kalija i magnezija u mladim izdancima heljde (Grafikon 1). Značajno veći sadržaj prethodno navedenih makroelemenata utvrđen je kod mladih izdanaka uzgajanih pod FLUO osvjetljenjem. Iako je najzastupljeniji makroelement kod mladih izdanaka heljde bio dušik (239 mg 100 g^{-1} svježe tvari (LED) i 236 mg 100 g^{-1} svježe tvari (FLUO), značajne razlike u sadržaju dušika, kao i u sadržaju kalcija, s obzirom na tip osvjetljenja nije bilo. Najmanje zastupljen makroelement u mladim izdancima pod utjecajem obiju vrsta osvjetljenja je bio magnezij, čiji je sadržaj pri LED osvjetljenju iznosio 26 mg 100 g^{-1} svježe tvari i bio je značajno manji u odnosu na sadržaj pri FLUO osvjetljenju od 28 mg 100 g^{-1} svježe tvari. Dobiveni rezultati u suprotnosti su s

rezultatima Kopsell i sur. (2014.) koji su utvrdili kod mladih izdanaka brokule značajno povećanje svih makroelemenata pod LED osvjetljenjem kod omjera crvenog i plavog svjetla 80 % : 20 % u odnosu na FLUO osvjetljenje. Međutim Toscano i sur. (2021.) utvrdili su da različite biljne vrste različito reagiraju na nakupljanje mikro- i makroelemenata s obzirom na tip osvjetljenja, što je potvrđeno i ovim istraživanjem. Dobiveni rezultati navode nas na zaključak da kod mladih izdanaka heljde spektralni sastav FLUO osvjetljenja povoljnije utječe na nakupljanje fosfora, kalija i magnezija.



Grafikon 1. Vrijednosti makroelemenata izmjerene u mladim izdancima heljde pod LED i FLUO osvjetljenjem.Vrijednosti označene istim slovom ne razlikuju se pri P<0,01

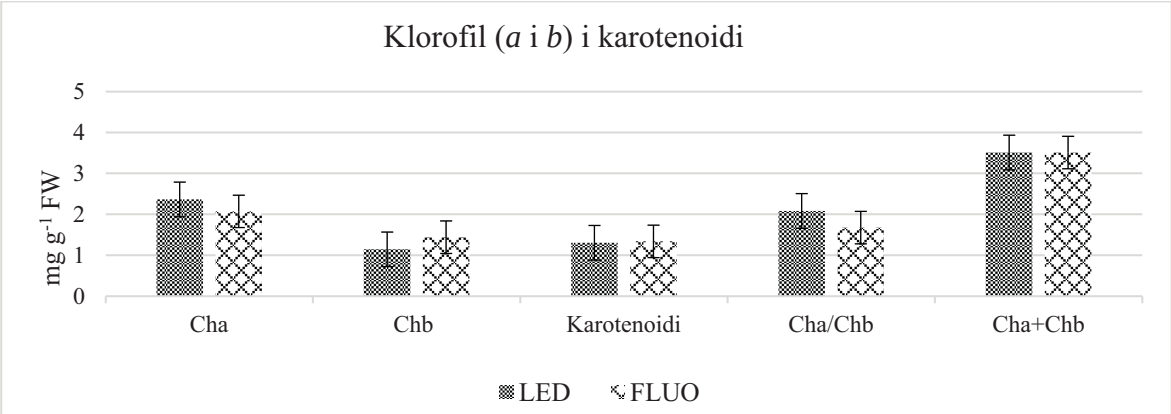
Tip osvjetljenja značajno je utjecao na sadržaj željeza i cinka u mladim izdancima heljde, a nije utjecao na sadržaj mangana i bakra (Grafikon 2). Sadržaj željeza pri LED osvjetljenju iznosio je 1,68 mg 100 g^{-1} svježe tvari i značajno se razlikovao od sadržaja željeza kod mladih izdanaka uzgojenih pod FLUO osvjetljenjem (0,65 mg Fe 100 g^{-1} svježe tvari). Značajno povećanje željeza pod LED osvjetljenjem utvrdili su i Kopsell i sur. (2014.) u mladim izdancima brokule. Za razliku od željeza, na povećan sadržaj cinka značajno je utjecalo FLUO osvjetljenje. Brazaityte i sur. (2019.) su utvrdili pozitivan utjecaj UV-a svjetla od 402 nm valne dužine na povećan sadržaj fosfora, magnezija, mangana, cinka i bakra u odnosu na LED osvjetljenje.



Grafikon 2. Vrijednosti mikroelemenata izmjerene kod mladih izdanaka heljde pod LED i FLUO osvjetljenjem. Vrijednosti označene istim slovom ne razlikuju se pri P<0,001.

U Grafikonu 3. prikazan je sadržaj klorofila (*a* i *b*) te karotenoida u mladim izdancima heljde uzgajanim pod LED i FLUO osvjetljenjem. Značajno veći sadržaj klorofila *a* te veći udio klorofila *a* u odnosu na klorofil *b* utvrđen je u mladim izdancima uzgajanim pod LED osvjetljenjem te je iznosio 2,36 mg g^{-1} svježe tvari. Sadržaj klorofila *b* bio je veći pri FLUO

osvjetljenju te je iznosio 1,44 mg g^{-1} svježe tvari. Ukupni sadržaj klorofila *a*+*b* se nije razlikovao s obzirom na tip osvjetljenja. Brojni autori utvrdili su povećan sadržaj klorofila kod mladih izdanaka uzgojenih pod LED osvjteljenjem (Lobiuc i sur., 2017.; Kopsell i sur., 2014.; Vaštakaitė i sur., 2017.; Rajan i sur., 2019.), iako se povećanje odnosilo na obje vrste klorofila, a ne samo na klorofil *a* kao u ovom istraživanju. Sadržaj karotenoida kod mladih izdanka kod obje vrste osvjetljenja bio je gotovo jednak te je iznosi oko 1,3 mg g^{-1} svježe tvari. Rezultati drugih istraživanja vezanih uz varijaciju sadržaja karotenoida s obzirom na tip osvjetljenja oprečni su. Lobiuc i sur. (2017.) utvrdili su gotovo jednak sadržaj karotenoida pod LED i FLUO osvjetljenjem kod mladih izdanaka crvenog i zelenog bosiljka što je u suglasnosti s rezultatima ovog istraživanja. Nasuprot tome Kapsell i sur. (2014.) utvrdili su značajno veći sadržaj karotenoida pod LED osvjetljenjem kod mladih izdanaka brokule pri kombinaciji crvenog i plavog svjetla u omjeru 80 % : 20 %. Stoga možemo zaključiti da svaka biljna vrsta preferira određenu vrstu svijetla koja utječe na pojedine biljne pigmente.



Grafikon 3. Sadržaj klorofila (*a* i *b*) i karotenoida kod mladih izdanka heljde pod LED i FLUO osvjetljenjem. Vrijednosti označene istim slovom ne razlikuju se pri P<0,05.

Zaključak

Tip osvjetljenja kod uzgoja mladih izdanaka ima značajan utjecaj na sadržaj pojedinih makro- i mikroelemenata kao i na sadržaj biljnih pigmenata. Značajan utjecaj na povećanje sadržaja fosfora, kalija, magnezija i cinka imalo je FLUO osvjetljenje. LED osvjetljenje je imalo značajan utjecaj na povećanje željeza. Ovim istraživanjem nisu potvrđeni rezultati drugih autora, koji su utvrdili značajan utjecaj LED osvjetljenja na povećanje sadržaja makro- i mikroelemenata kod mladih izdanaka. Stoga bi u sljedećim istraživanjima trebalo testirati veći broj LED svjetala s različitim spektrom kako bi se mogao donjeti precizniji zaključak o utjecaju LED svjetala na sadržaj nutrijenata kod mladih izdanaka heljde. Na koncentraciju ukupnog klorofila kao niti karotenoida nije značajno utjecao tip osvjetljenja.

Literatura

AOAC (2015). Officinal Method of Analysis of AOAC International, Gaithersburg, Maryland, USA.

Brazaityte A., Viršile A., Samuoliene G., Vaštakaite-Kairiene V., Jankauskiene J., Miliauskiene J., Novicˇkovas A., Duchovskis P. (2019). Response of Mustard Microgreens to Different Wavelengths and Durations of UV-A LEDs. Frontier in Plant Science. Volumen (10):1153.

Di Gioia, F., Renna, M., Santamaria, P. (2017). Sprouts, Microgreens and "Baby Leaf" Vegetables. Minimally Processed Refrigerated Fruits and Vegetables. 403–432.

Di Gioia, F., Santamaria, P. (2015). The nutritional properties of microgreens. In book: Microgreens: Novel, fresh and functional food to explore all the value of biodiversity. 41-50.

Kopsell D. A., Sams C. E., Casey T. (2014). Barickman Sprouting Broccoli Accumulate Higher Concentrations of Nutritionally Important Metabolites under Narrow-band Light-emitting Diode Lighting. Journal of the American Society for Horticultural Science. Volumen (139): 469–477.

Lobiuc A., Vasilache V., Pintilie O., Stoleru T., Burducea M., Oroian M., Zamfirache M.M. (2017). Blue and Red LED Illmination Improves Growth and Bioactive Compounds Contents in Acyanic and Cyanic Ocimum basilicum L. Microgreens. Molecules. Volumen (22): 2111.

Nam T.G., Nam T., Lim Y. J., Lim J., Hyun Eom S. (2018). Flavonoid accumulation in common buckwheat (*Fagopyrum esculentum*) sprout tissues in response to light. Horticulture, environment and biotechnology. Volumen (59): 19-27.

Niroula A., Khatri S., Timilsina R., Khadka D., Khadka A., Ojha, P. (2019). Profile of chlorophylls and carotenoids of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) microgreens. Journal of Food Science and Technology. Volumen (56): 2758– 2763.

Ouzounis T., Rosenqvist E., Ottosen C.-O. (2015). Spectral Effects of Artificial Light on Plant Physiology and Secondary Metabolism: A Review. Department of Food Science. Volumen 50: 1128-1135.

Olle M., Viršilė A. (2013). The effects of light-emitting diode lighting on greenhouse plant growth and quality. Agricultural and Food Science. Volumen (22): 223–234.

Rajan P., Lada R.R., MacDonald M.T. (2019). Advancement in Indoor Vertical Farming for Microgreen Production. American Journal of Plant Sciences. Volumen (10): 1397 – 1408.

SAS/STAT (2010). SAS Institute, Cary, NC, USA.

Toscano S., Cavallaro V., Ferrante A., Romano D., Patané C. (2021). Effects of Different Light Spectra onFinal Biomass Production and Nutritional Quality of Two Microgreens. Plants. Volumen (10): 1584.

Vastakaite V., Virsile A., Brazaityte A., Samuoliene G., Jankauskiene J., Novickovas A., Duchovskis P. (2017). Pulsed Light-Emitting Diodes for a Higher Phytochemical Level in Microgreens. Journal of Agricultural Food Chemistry. Volumen 65(31): 6529–6534.

Zhang X., Bian Z., Yuan X., Chen X., Lu C. (2020). A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. Trends in Food Science & Technology. Volumen (99): 203-216.

Influence of light on mineral composition, chlorophyll and carotenoid content in buckwheat microgreens

Abstract

In recent years, there is a growing interest in consuming microgreens. Microgreens are extremely rich in minerals, chlorophyll and vitamins. Production method significantly affects their nutritional composition. The type of light is considered a very important environmental factor influencing the photochemical processes in plants, which affect the composition of minerals, chlorophyll and carotenoids. The aim of the study was to determine the mineral composition and content of chlorophyll and carotenoids in young shoots of buckwheat grown under FLUO and LED lighting. Young shoots grown under FLUO lighting had a higher content of P, K, Mg and chlorophyll *b*, while young shoots grown under LED lighting had a higher content of Fe and chlorophyll *a*.

Key words: buckwheat, microgreens, light, minerals, plant pigments

IZVORNI ZNANSTVENI RAD

Utjecaj pH vrijednosti vodenih otopina kiselina na svojstva sjemena i klijanaca bijele djeteline kultivara 'Apolo'

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Sažetak
Istraživanje utjecaja vodene otopine sumporne i klorovodične kiseline različitih pH vrijednosti provedeno je u laboratorijskim uvjetima sa sjemenom bijele djeteline (kultivar 'Apolo'). Naklijavanje sjemena provedeno je metodom vlažnog filter papira. Dodavanjem klorovodične odnosno sumporne kiseline, pripremljene su vodene otopine pH vrijednosti 4, 5, 6, 7. Ispitivana su sljedeća svojstva: energija klijanja i klijavost, te svojstva klijanaca (dužina korijena, stabljike i ukupna dužina). Vrijednosti pH nisu utjecale na svojstva sjemena. Međutim, utvrđen je značajan utjecaj pH vrijednosti ($p = 0,05$; $p = 0,01$) vodene otopine kiselina na svojstva klijanaca. Najveće vrijednosti kod svih ispitivanih svojstava klijanaca utvrđene su pri pH 6.

Ključne riječi: bijela djetelina, sjeme, klijanci, pH

Uvod

Bijela djetelina (*Trifolium repens* L.) višegodišnja je krmna leguminoza. Najbolje uspjeva u subtropskom i tropskom području, na glinovitim i ilovastim tlima (Smith i Valenzuela, 2002.). Sije se u smjesama za ispašu domaćih životinja, uglavnom sa višegodišnjim ljuljem (Black i sur., 2006.), kako bi se osigurala visokokvalitetna stočna hrana, uz istodobno poboljšanje plodnosti tla fiksacijom dušika simbiotskim bakterijama (Elgersma i sur., 2010.). Iako bijela djetelina može uspjevati u velikom rasponu pH vrijednosti (Chu i sur., 2022.), na vrlo kiselim tlima dolazi do smanjenja simbiotske fiksacije dušika (Brauer i sur., 2002.), kao i rasta djeteline (Shah i sur., 2021.). Stoga je prilikom sjetve bijele djeteline važno poznavati svojstva klijavosti sjemena pri različitim agroekološkim uvjetima. Metoda ispitivanja standardne klijavosti sjemena (ISTA) odvija se u idealnim uvjetima, pa se visoki rezultati klijavosti mogu očekivati samo pri optimalnim uvjetima u polju (Siddique i Wright, 2004.). Test standardne klijavosti često nadilazi vrijednosti poljskog nicanja (Hamman i sur., 2002.), osobito pri niskim pH vrijednostima tla. Rezultati svojstava sjemena i klijanaca bijele djeteline uzgojene u vanjskom kontroliranom pokusu u polistirenskim kontejnerima, bili su u skladu sa rezultatima dobivenim pri uzgoju u laboratorijskim uvjetima (Bukvić i sur., 2008.[a]). Također, Kendall i sur. (1994.) prilikom istraživanja utjecaja različite temperature, kiselosti tla i dostupnosti vode kod različitih leguminoza, utvrdili su povezanost laboratorijskih i poljskih mjerenja klijavosti. Istraživanja su pokazala kako primjena sumporne ili klorovodične kiseline može imati pozitivan utjecaj na klijavost sjemena. Fallah i sur. (2014.) utvrdili su pozitivan utjecaj sumporne kiseline na klijavost sjemena te duljinu korijena i stabljike. Durrant i sur. (1992.) utvrdili su poboljšanu klijavost sjemena nakon tretmana klorovodičnom kiselinom.
Cilj istraživanja bio je utvrditi utjecaj vodene otopine sumporne i klorovodične kiseline različitih pH vrijednosti na svojstva sjemena i klijanaca bijele djeteline.

Materijal i metode

Istraživanje je provedeno u kontroliranim laboratorijskim uvjetima sa sjemenom bijele djeteline, kultivar 'Apolo'. Istraživan je utjecaj vodenih otopina sumporne i klorovodične kiseline podešenih na pH vrijednosti 4, 5, 6, 7. Pomoću pH metra (Mettler Toledo, USA) utvrđena je pH vrijednost vodovodne vode, a pH vrijednosti otopina podešavale su se dodavanjem klorovodične odnosno sumporne kiseline na sve četiri razine pH. Sjeme bijele djeteline naklijavano je na navlaženom filter papiru (50 ml vodene otopine). Za svaku pH razinu i vodenu otopinu obje kiseline zasijano je 100 sjemenki u četiri ponavljanja. Rolani filter papir sa sjemenom, stavljen je u prozirne pvc vrećice, a potom u klima komoru tijekom 10 dana pri 20 °C. Test klijavosti sjemena bijele djeteline proveden je sukladno pravilima ISTA (2003.). Određena su svojstva sjemena: energija klijanja i klijavost, te svojstva klijanaca: dužina korijena, dužina stabljike i ukupna dužina klijanaca. Pomoću programa SAS Software-a 9.4 provedena ja analiza varijance (ANOVA), a značajnosti istraživanih svojstava testirane su LSD testom.

Rezultati i rasprava

Vodene otopine kiselina, kao i pH vrijednosti nisu utjecale na energiju klijanja i klijavost sjemena bijele djeteline (Tablica 1). Prosječna vrijednost energije klijanja u prosjeku za sve pH vrijednosti i obje kiseline bila je 81 %, dok je prosječna klijavost iznosila 82 %. Dobiveni rezultati u skladu su sa deklariranim vrijednostima klijavosti bijele djeteline, kao i istraživanju (Bukvić i sur., 2008[b].) o utjecaju pH vrijednosti i temperature na klijavost tri kultivara bijele djeteline.

Chu i sur. (2022.) ispitujući utjecaj pH vrijednosti, također nisu utvrdili značajan utjecaj pH vrijednosti na klijavost sjemena bijele djeteline. Hampton i sur. (1987.) pri optimalnoj temperaturi uzgoja ovisno o kultivaru, utvrdili su klijavost bijele djeteline od 82 do 92 %. Klijavost sorte 'Apolo' u našem istraživanju kretala se od 80 do 85 % ovisno o tretmanu pH vrijednosti i kiseline.

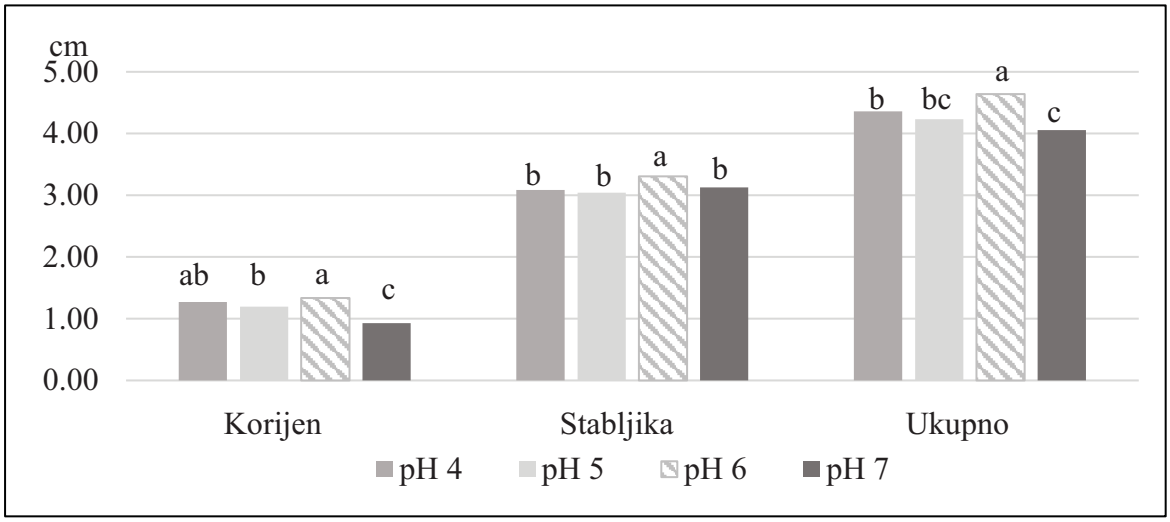
Tablica 1. Utjecaj pH vrijednosti i kiselina na energiju klijanja i klijavost bijele djeteline

Energija klijanja %					
Kiselina	pH vodene otopine				Prosjek
	4	5	6	7	
HCl	82	84	82	81	82
H_2SO_4	84	83	78	80	81
Prosjek	83	83	80	80	81
Klijavost %					
Kiselina	pH vodene otopine				Prosjek
	4	5	6	7	
HCl	81	80	85	80	81
H_2SO_4	82	85	81	81	82
Prosjek	81	82	83	80	82

Statističkom obradom podataka, dobiven je značajan utjecaj ($p = 0,05$; $p = 0,01$) pH vrijednosti vodene otopine, te interakcija pH vrijednosti i kiseline na morfološka svojstva klijanaca.

Dužina korijena klijanaca značajno je ovisila o pH vrijednosti vodene otopine ($p = 0,01$) (Tablica 2), a kretala se u zavisnosti od pH vrijednosti vodene otopine od 0,9 do 1,33 cm

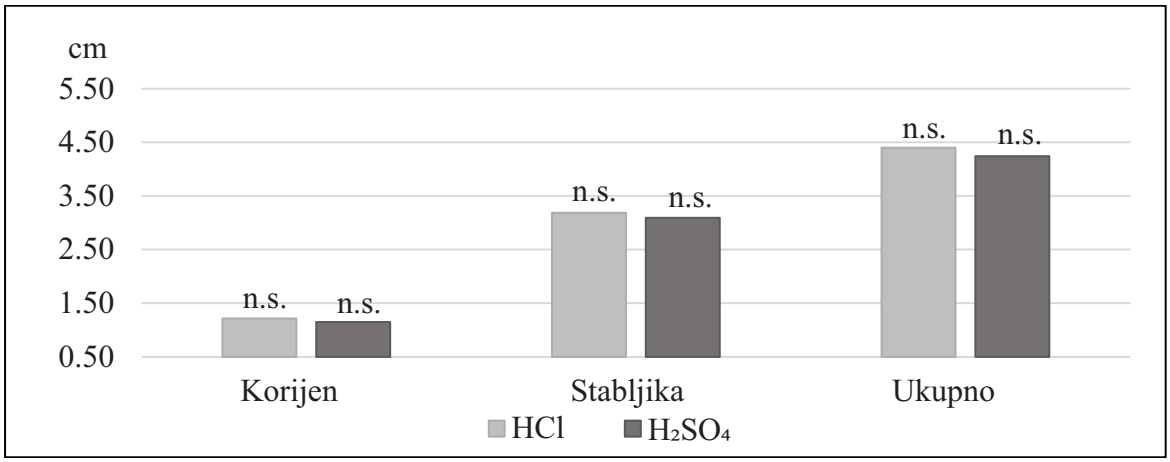
(Grafikon 1). Najveću dužinu korijena imali su klijanci naklijavani pri pH 6, a najkraću pri pH 7.
U prosjeku za ispitivane pH vrijednosti dužina stabljika klijanaca kretala se od 3,04 do 3,30 cm. Vodene otopine različite pH vrijednosti imale su statistički značajan utjecaj (p = 0,01) na dužinu stabljike klijanaca (Grafikon 1). Značajno veću dužinu stabljike imali su klijanci uzgojeni u vodenoj otopini pH 6, a najmanju pri pH 5 (Grafikon 1). Vrijednosti dužine stabljike u skladu su s istraživanjem Grljušić i sur. (2007.). Navedeni autori istraživali su utjecaj pH vrijednosti vodene otopine na tri kultivara soje, te utvrdili najveću dužinu korijena i stabljike pri pH 6.



* vrijednosti označene različitim slovom statistički su značajno različite (p < 0,01)

Grafikon 1. Utjecaj pH vrijednosti vodene otopine na dužinu korijena, stabljike i ukupnu dužinu klijanaca bijele djeteline

Ukupna dužina klijanaca bila je pod značajnim utjecajem pH vrijednosti otopina, a najveće dužine dobivene su pri pH 6 (4,64 cm) i statistički su se razlikovale od ostalih promatranih pH vrijednosti (Grafikon 1).



* n.s. – nema statistički značajne razlike (p < 0,01)

Grafikon 2. Utjecaj vrste vodene otopine kiseline na dužinu korijena, stabljike i ukupnu dužinu klijanaca bijele djeteline

Vodene otopine korištenih kiselina nisu imale značajan utjecaj na svojstva sjemena i klijanaca (Grafikon 2). Neznatno veće morfološke vrijednosti imali su klijanci uzgojeni u vodenoj otopini klorovodične kiseline, dok je neznatno veća prosječna klijavost utvrđena pri vodenim otopinama sumporne kiseline.

Tablica 2. Prikaz LSD vrijednosti promatranih pH reakcija, kiselina i njihove interakcije za ispitivana morfološka svojstva klijanaca

p	pH	Kiselina	pH x Kiselina
		Korijen	
0,05	0,099	n.s.	0,137
0,01	0,134	n.s.	0,186
		Stabljika	
0,05	0,147	n.s.	0,202
0,01	0,198	n.s.	0,274
		Ukupno	
0,05	0,188	n.s.	0,236
0,01	0,254	n.s.	0,319

* n.s. – nema statistički značajne razlike

Zaključak

Ispitivana svojstva sjemena nisu se značajno mijenjala ovisno o pH vrijednosti i kiselini vodene otopine. Svojstva klijanaca bijele djeteline značajno su ovisila o pH vrijednosti, te interakciji pH vrijednosti i vodene otopine kiselina. Utvrđen je utjecaj pH vrijednosti i vodene otopine kiselina na dužinu korijena, stabljike i ukupnu dužinu klijanaca. Klijanci bijele djeteline imali su najdulji korijen, stabljiku, a samim tim i ukupnu dužinu pri vodenoj otopini pH 6. Najmanje vrijednosti dužine korijena i ukupne dužine klijanaca izmjerene su kod pH 7, a dužine stabljike kod pH 5.

Dobiveni rezultati istraživanja mogli bi poslužiti kao preliminarna procjena pogodne reakcije tla za uzgoj bijele djeteline kultivara 'Apolo'.

Literatura

Black, A. D., Moot, D. J., Lucas, R. J. (2006). Development and growth characteristics of Caucasian and white clover seedlings, compared with perennial ryegrass. Grass and Forage Science. 61(4): 442-453.

Brauer, D., Ritchey, D., Belesky, D. (2002). Effects of lime and calcium on root development and nodulation of clovers. Crop science, 42(5): 1640-1646.

Bukvić, G., Grljušić, S., Karalić, K., Nikolić, S., Bukvić, A. (2008[a]). Produkcija nadzemne biomase kultivara bijele djeteline na različitim tlima i supstratu. Poljoprivreda. 14(1): 15-20.

Bukvić, G., Ravlić, M., Grljušić, S., Rozman, V., Popović, B., Tkalec, M. (2008[b]). Utjecaj temperature i pH vrijednosti na klijavost sjemena i dužinu klijanaca bijele djeteline. Sjemenarstvo. 25(3-4): 179-192.

Chu, L., Gao, Y., Chen, L., McCullough, P. E., Jespersen, D., Sapkota, S., Yu, J. (2022). Impact of Environmental Factors on Seed Germination and Seedling Emergence of White Clover (*Trifolium repens* L.). Agronomy. 12(1): 190.

Durrant, M. J., Mash, S. J., Payne, P. A. (1992). The use of hydrochloric acid to improve the germination of sugar-beet seed. Plant growth regulation, 11(4): 363-369.

Elgersma, A., Nassiri, M., Schlepers, H. (1998). Competition in perennial ryegrass-white clover mixtures under cutting. 1. Dry-matter yield, species composition and

nitrogen fixation. Grass Forage Science, 53: 353-366.
Fallah, I. A., Salehi, S. A., Shahdadneghad, M. (2014). Effect of H2SO4 on seed germination and viability of *Canna Indica* L. ornamental plant. International Journal of Advanced Biological and Biomedical Research. 2(1): 223-229.
Grljušić, S., Bukvić, G., Vratarić, M., Antunović, M., Sudarić, A., Prepelec, I. (2007). Utjecaj pH vodene otopine na klijavost sjemena soje. Poljoprivreda, 13(2): 5-9.
Hamman, B., Egli, D. B., Koning, G. (2002). Seed vigor, soil borne pathogens, preemergent growth, and soybean seedling emergence. Crop Science, 42(2): 451-457.
Hampton, J. G., Charlton, J. F. L., Bell, D. D., Scott, D. J. (1987). Temperature effects on the germination of herbage legumes in New Zealand. In Proceedings of the New Zealand Grassland Association, 48: 177-183.
ISTA (2003). Handbook on seedling evaluation, 3rd edition.
Kendall, W. A., Shaffer, J. A., Hill Jr, R. R. (1994). Effect of temperature and water variables on the juvenile growth of lucerne and red clover. Grass and Forage Science. 49(3): 264-269.
SAS 9.4; SAS Institute Inc., Cary, North Carolina, USA.
Shah, A. S., Wakelin, S. A., Moot, D. J., Blond, C., Laugraud, A., Ridgway, H. J. (2021). *Trifolium repens* and *T. subterraneum* modify their nodule microbiome in response to soil pH. Journal of Applied Microbiology. 131(4): 1858-1869.
Siddique, A. B., Wright, D. (2004). Effects of date of sowing on seed yield, seed germination and vigour of peas and flax. Seed Science and Technology. 32(2): 455-472.
Smith, J., Valenzuela, H. (2002). White Clover (Cover Crops). Raspoloživo: https://scholarspace.manoa.hawaii.edu/bitstream/10125/12750/SA-CC-7.pdf

Influence of aqueous acid solutions pH on the properties of seeds and seedlings of white clover cultivar 'Apolo'

Abstract
The influence of aqueous solution of sulfuric and hydrochloric acid of different pH values was carried out in laboratory conditions with white clover seeds (cultivar 'Apolo'). Seed germination was performed using the method of wet filter paper. By adding hydrochloric or sulfuric acid, aqueous solutions were adjusted to the desired pH of 4, 5, 6, 7. The following properties were investigated: germination energy and germination, and seedling properties (root length, stem length and total length). pH values did not affect seed properties. However, a significant effect of pH value ($p = 0.05$; $p = 0.01$) of aqueous acid solution was recorded for all seedlings traits. The highest values for all tested seedling traits were determined at pH 6.

Key words: white clover, seed, seedlings, pH

ORIGINAL SCIENTIFIC PAPER

Does the water content affect the performance index in the sunflower grain filling stage?

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Abstract
The study aimed to determine the justification for irrigation in the grain filling stage and determine the difference in sunflower hybrids' sensitivity to the lack of water. Hybrids 7, 11 and 12 were singled out as being more sensitive to drought conditions than other hybrids according to the maximum quantum yield of photosystem II (TR_0/ABS) and hybrids 7 and 11 were considered sensitive to drought conditions according to PI_{ABS}. As water regimes proved to be significant sources of variation, irrigating sunflower plants in the grain filling stage is considered justified. Also, the significant differences between tested genotypes justify using chlorophyll *a* fluorescence parameters to select breeding material that could supplement the conventional process of testing sunflower genotypes during breeding in dry conditions.

Key words: *Helianthus annuus*, chlorophyll *a* fluorescence, drought, hybrid

Introduction

As one of the most valuable sources of edible oils, sunflower is a very important oilseed for human consumption. According to FAOSTAT data (2021), in 2020, was produced 50.229,567 tons of sunflowers in the world. Given the ongoing situation with very variable weather conditions, breeders are faced with problems that arise during the sunflower vegetation. Recently, the production has been affected by frequent changes in temperature with unevenly distributed precipitation, which leaves plants, in key developmental stages, without sufficient water for development. This causes changes in plants observed at the biochemical, biological and morphogenetic levels leading to alterations in crop development, growth and productivity (Agüera and de la Haba, 2021). Therefore, to increase the productivity of this important oilseed crop, various abiotic factors that could affect the plant should be considered carefully. Plant growth in field environments faces several dares, including drought, waterlogging, heat, cold and salinity (Ashraf et al., 2018). Drought is one of the leading limiting abiotic factors as it dominantly limits agricultural productivity (Bhardwaj et al., 2014). Drought occurs when a plant's water needs are not fully met. This phenomenon happens when the amount of water transported exceeds the amount taken by the roots, caused by insufficient rainfall, water retention by soil particles or low groundwater levels (Fernández et al., 2011). Lack of water leads to stomata closing, which reduces CO_2 inflows and photosynthetic activity (Saharan and Nehra, 2011).

Considering that a lack of precipitation most often characterises the cultivation in Slavonia, the study was conducted to investigate the impact of two water regimes on sunflower hybrids in the grain filling stage. The aim was to determine the justification for irrigating plants in the grain filling stage and prove the sensitivity of sunflower hybrids to the lack of water.

Material and methods

The experiment was conducted in vegetation pots on 13 sunflower hybrids that differed in agronomic characteristics. The 12-litre pots contained two plants per pot in three replications per treatment for each hybrid. The experiment included two treatments with different water regimes. The control treatment represented 80% of the field water capacity (FWC), while the second treatment represented mild drought with 60% FWC. FWC was determined using the modified gravimetric method daily by weighing pots (Schinner et al. 1993). During the experiment, reference pots were used to consider the increasing plant biomass during plant growth. According to the analysis (Ayers and Westcot, 1994), the used water was safe for use, and it was pumped from a well (37 m deep), after which water was stored in a tank to reach ambient temperature before watering.

Chlorophyll *a* fluorescence data, measured by a plant efficiency analyser (Handy PEA, Hansatech, UK) needed for calculating the maximum quantum yield of photosystem II (TR_0/ABS) and photosynthetic performance index (PI_{ABS}) according to Strasser et al. (2004) were measured in the morning hours on the three youngest leaves per hybrid and treatment (n = 3) in the R6 stage (Schneiter and Miller, 1981).

Analyses of variance for TR_0/ABS and PI_{ABS} were done with a significance level of $p < 0.05$. The differences in values between genotypes and treatment were tested with the LSD test at $p < 0.05$.

Results and discussion

For both TR_0/ABS and PI_{ABS}, ANOVA showed a significant difference between hybrids and treatments, but their interaction was not a significant source of variation (Table 1).

Table 1. ANOVA for sunflower hybrids, treatments and their interactions for a maximum quantum yield of photosystem II (TR_0/ABS) and performance index (PI_{ABS}).

Source of variability	Degree of freedom	Mean square		F value	
		TR_0/ABS	PI_{ABS}	TR_0/ABS	PI_{ABS}
Hybrid (H)	12	0.0007	13.92	2.70 *	5.25 *
Treatment (T)	1	0.0034	35.59	13.50 *	13.42 *
H x T	12	0.0004	4.24	1.50 ns	1.60 ns

* significant at $p < 0.05$, ns - not significant

According to numerous studies, TR_0/ABS and PI_{ABS} are considered the most sensitive to environmental factors among the chlorophyll *a* fluorescence parameters. The optimal range of maximum quantum yield for most plants was determined to be 0.80-0.83 (Pfündel, 1988), with the limit value of 0.75 (Bolhar-Nordenkampf et al., 1989), below which the plant mechanism changes and the functionality of photosystem II being disturbed. TR_0/ABS values in the control treatment ranged between 0.814 (hybrid 7) and 0.844 (hybrid 1) (Figure 1). In the drought treatment, the values were slightly higher and ranged between 0.827 (hybrid 9) and 0.850 (hybrid 8). The presented results show that drought did not affect the function of the photosynthetic apparatus in the sunflower grain filling stage, although TR_0/ABS had increased in most hybrids in the drought conditions. Nevertheless, a significant difference was found only for 7, 11 and 12.

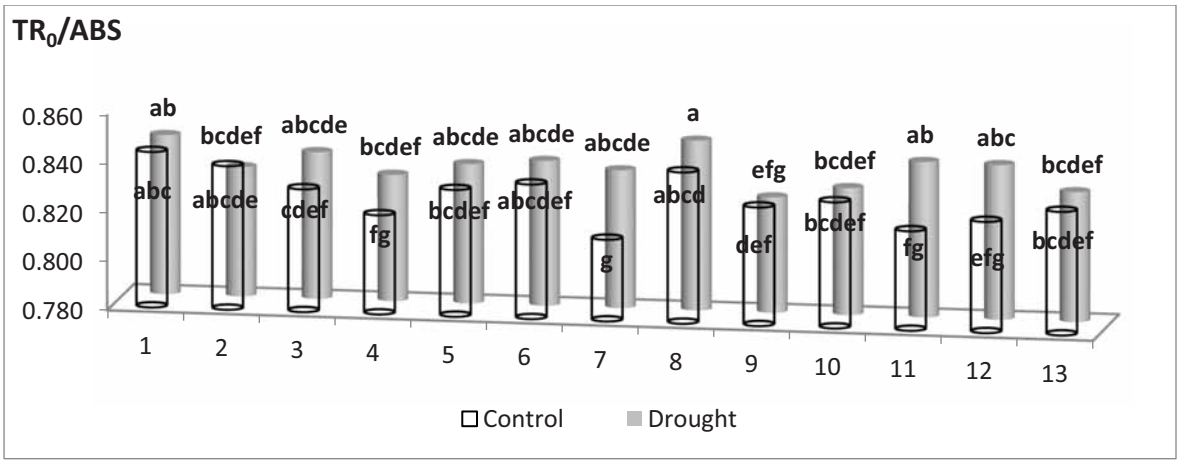

Figure 1. The maximum quantum yield of photosystem II (TR_0/ABS) in leaves of 13 sunflower genotypes in two treatments. Mean values marked with the same letter did not differ statistically at the $p < 0.05$, representing differences in interaction genotype and treatment.

PI_{ABS} is considered a parameter that provides information on the vitality of the tested plants (Kalaji et al., 2016). Its values in the control treatment ranged between 3.823 (hybrid 7) and 7.759 (hybrid 8) (Figure 2). The same drought reaction of the hybrids as with TR_0/ABS was repeated, i.e. there was an increase in almost all PI_{ABS} values in drought. The minimum PI_{ABS} in drought was determined in hybrid 13 (5.631) and the maximum value in hybrid 11 (8.078). A significant difference between treatments was found only for hybrids 7 and 11.

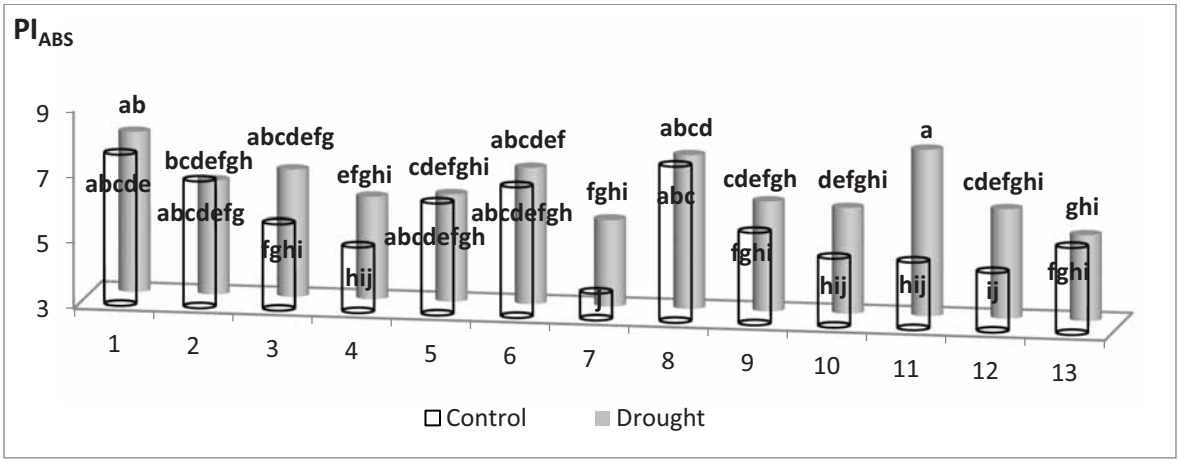

Figure 2. Performance index (PI_{ABS}) in leaves of 13 sunflower genotypes in two treatments. Mean values marked with the same letter did not differ statistically at the $p < 0.05$, representing differences in interaction genotype and treatment.

In previously published papers of this same study, lower values of TR_0/ABS and PI_{ABS} were found in most hybrids in the drought treatment occurring in the initial stages of growth and development of sunflower plants, i.e. the stage of development of six pairs of leaves (V6) (Markulj Kulundžić et al., 2016a) and butonisation (R2) (Markulj et al., 2014). In flowering (R3/R4), TR_0/ABS and PI_{ABS} increased as a result of drought conditions in most hybrids (Markulj Kulundžić et al., 2016b), the same as in this study in the grain filling stage. Different direction of parameter change depending on the developmental stage indicates that the photosynthetic process in the leaves of 13 sunflower hybrids depends on the timing of the drought, which is contrary to the results of Viljevac Vuletić et al. (2019), who confirmed the same direction of the change in wheat flag leaves during three developmental stages.

Combined results of previously mentioned published papers and the results of this study, it can be noticed that TR_0/ABS was similar in all stages of development in contrast to PI_{ABS}, for which the values increased as growth and development progressed from maximal 4.76 determined in V6 (Markulj Kulundžić et al., 2016a) to maximal 8.078 in R6. Increasing PI_{ABS} values with plant senescence were previously determined by Vuletić Vuletić et al. (2019) in wheat.

Conclusions

This study proved the significant influence of hybrids and treatments as sources of variation for tested chlorophyll *a* fluorescence parameters. According to both TR_0/ABS and PI_{ABS}, hybrids 7 and 11 were singled out to be more sensitive to drought conditions than other hybrids. In addition, to the two mentioned hybrids, hybrid 12 was considered sensitive to drought conditions according to TR_0/ABS. As water regimes proved to be significant sources of variation, irrigating sunflower plants in the grain filling stage is considered justified. At the same time, the significant differences between tested genotypes justify the use of tested chlorophyll *a* fluorescence parameters to select breeding material that could shorten the conventional process of testing sunflower genotypes during breeding in dry conditions.

Acknowledgement

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References

Agüera E., de la Haba P. (2021). Climate Change Impacts on Sunflower (*Helianthus annuus* L.) Plants. Plants. 10: 2646.

Ashraf M. A., Akbar A., Askari S. H., Iqbal M., Rasheed R., Hussain, I. (2018). Recent Advances in Abiotic Stress Tolerance of Plants Through Chemical Priming: An Overview. In Advances in Seed Priming, Rakshit, A., Singh, H. (eds), 51–59 Springer, Singapore.

Ayers R. S., Westcot D. W. (1994). Water quality for agriculture. Food and agricultural organisation of united nations, FAO. Rome, Italy.

Bhardwaj D., Ansari M. W., Sahoo R. K., Tuteja N. (2014). Biofertilizers Function as Key Player in Sustainable Agriculture by Improving Soil Fertility, Plant Tolerance and Crop Productivity. Microbial Cell Factories. 13: 66.

Bolhar-Nordenkampf H. R., Long S. P., Baker N. R., Öquist G., Schreiber U., Lechner E.G. (1989). Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: A review of current Instrumentation. Functional Ecology. 3: 497–514.

FAOSTAT data (2021). Food and Agriculture Organization of the United Nations.

Fernández M. C., Gutiérrez B., Flavio H., Rubio G. (2011). Effect of Indigenous Mycorrhizal Colonization on Phosphorus-Acquisition Efficiency in Soybean and Sunflower. Journal of Plant Nutrition and Soil Science. 174: 673–677.

Kalaji H. M., Jajoo A., Oukarroum A., Brestic M., Zivcak M., Samborska I. A., Cetner M. D., Łukasik I., Goltsev V., Ladle R. J. (2016). Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiologiae Plantarum. 38: 102.

Markulj A., Viljevac Vuletić M., Kovačević J., Josipović A., Liović I., Mijić A., Sudarić A., Matoša Kočar M. (2014). Water deficiency effects on photosynthetic performance in leaves of sunflower plants at developmental stage of butonisation. 7th international scientific/professional conference "Agriculture in nature and environment protection", Baban, M., Đurđević, B. (ed.), 191–195. Osijek: Glas Slavonije d.d., Osijek, Hrvatska.

Markulj Kulundžić A., Kovačević J., Viljevac Vuletić M., Josipović A., Liović I., Mijić A., Lepeduš H., Matoša Kočar M. (2016). Impact of abiotic stress on photosynthetic efficiency and leaf temperature in sunflower. Poljoprivreda. 22 (2): 17–22

Markulj Kulundžić A., Viljevac Vuletić M., Jocić S., Cvejić S., Matoša Kočar M., Mijić A., Liović I., Sudarić A., Lepeduš H., Kovačević J., Josipović A. (2016). Effect of different soil water content effect on genotype expession in photosynthetic efficiency and leaf temperature in sunflower. Genetika-Belgrade. 48 (3): 971–982.

Pfündel E. (1998). Estimating the contribution of photosystem I to total leaf chlorophyll fluorescence. Photosynthesis Research. 56: 185–195.

Sahara, B. S., Nehra V. (2011). Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sciences and Medicine Research. 21: 30.

Schinner F., Öhlinger R., Kandeler E., Margesin R. (1993). Bodenbiologische Arbeitsmethoden, 2nd ed.; Springer: Berlin/Heidelberg, Germany.

Schneiter A. A., Miller J. F. (1981). Description of sunflower growth stages. Crop Science. 21: 901–903.

Strasser R. J., Tsimilli-Michael M., Srivastava A. (2004). Analysis of the fluorescence transient. In *Chlorophyll Fluorescence: A Signature of Photosynthesis*, George C., Papageorgiou C., Govindjee (eds). Advances in Photosynthesis and Respiration Series. Springer, Dordrecht, 321–362.

Viljevac Vuletić M., Marček T., Španić V. (2019). Photosynthetic and antioxidative strategies of flag leaf maturation and its impact to grain yield of two field-grown wheat varieties. Theoretical and Experimental Plant Physiology. 31: 387–399.

ORIGINAL SCIENTIFIC PAPER

Trends in breeding soybean for abiotic stress at the Agricultural Institute Osijek

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Abstract
As water shortage can significantly limit soybean production in Europe, breeding drought-tolerant cultivars while reducing the input burden of additional selection criteria is important, especially in the conventional, GMO-free breeding programmes. This study aimed to compare the photosynthetic efficiency of four genotypes in sufficient soil water supply (T1) and drought (T2) conditions, determining genotypes potentially more tolerant to drought occurring during seed filling. The results indicated that the maximum quantum yield of PSII photochemistry (TR_0/ABS) remained at optimal levels, irrelevant of soil water status. The photosynthetic performance index on absorption basis (PI_{ABS}) and the performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{TOTAL}) significantly changed only in genotype G3. Based on T2 PI_{ABS} and T2 PI_{TOTAL}, as well as stress tolerance indices (STI) for both parameters, genotype G3 was determined to have the least efficient photosynthetic apparatus in drought, while genotype G4 was determined to have the most efficient. Although PI_{ABS} and PI_{TOTAL} could be useful in eliminating genotypes with the most unfavourable trait values, especially in large scale genotype screenings for drought stress tolerance in early generations, stress tolerance of genotypes with superior photosynthetic performance should be confirmed in multi-environment yield trials.

Key words: soybean, photosynthesis, drought, performance index

Introduction

Adverse weather events are becoming more frequent, imposing stress on plants, limiting crop production and causing instabilities on the food and feed markets. In Europe, soybean is mainly grown in rainfed areas with little or no possibility for irrigation (FAO, 2021), so drought can have significant negative economic effects. The effects of adverse weather need to be mitigated not only with appropriate agro-technical measures but also with stable and adaptable cultivars tolerant to abiotic stress. At the conventional, GMO-free soybean breeding programme of the Agricultural Institute Osijek (AIO), genotype adaptability is commonly evaluated via yield data collected from input-heavy multi-environment trials. For early-generation screening of abiotic stress tolerance more cost-effective methods are needed. One such method is Chlorophyll *a* (Chl *a*) fluorescence measuring. It is commonly used for predicting, monitoring and evaluating abiotic stress effects in plants and determining adaptability (Strasser et al., 2004; Kalaji et al., 2016). This easy to use, relatively fast and reliable method facilitates the decision-making process and increases breeding efficiency without increasing the input burden of additional selection criteria. Among numerous Chl *a* fluorescence parameters, the maximum quantum yield of PSII photochemistry (TR_0/ABS, Strasser et al., 2004) is one of the most frequently used for determining the effects of abiotic stress in plants (Kalaji et al., 2016). The photosynthetic

performance index on absorption basis (PI_{ABS}) provides information on the photosynthetic apparatus functioning, the electron transport chain, the efficiency of the PSII (Strasser et al., 2004), and it can be used in the breeding of stress-tolerant genotypes (Kalaji et al., 2016; Kovačeviće et al., 2017; Bano et al., 2020; Killi et al., 2020; Markulj Kulundžić et al., 2021). The performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{TOTAL}) is known to have a higher sensitivity to the unfavourable environmental changes compared to other fluorescence parameters, and it is closely related to plant's vitality (Yusuf et a., 2010; Pavlović et al., 2019; Mihaljević et al., 2021). All of the above justifies the use of chosen Chl *a* parameters in the selection of drought-tolerant genotypes. This study aimed to compare the photosynthetic efficiency of four genotypes in different soil water contents and to determine genotypes potentially more tolerant to drought occurring during seed filling.

Material and methods

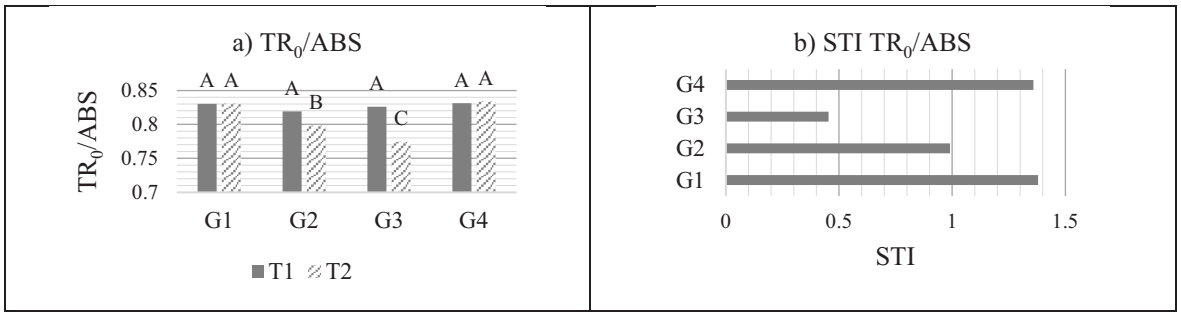
The research with two treatments (T1, T2) and three repetitions per treatment and genotype was conducted in plant pots with four 0–I maturity group soybean genotypes (G1 – G4), created at and in the property of the Agricultural Institute Osijek (AIO, Osijek, Croatia). The first treatment had sufficient water supply (T1, 80% available water holding capacity, AWC), while the second was drought-stressed (T2, 50% AWC). Drought was initiated in the beginning seed (R5) stage of development (Fehr and Caviness, 1977). The soil for the experiment, taken from the arable soil layer, was classified as anthropogenic eutric cambisol (FAO, 2015), silty clay loamy texture with 36.6% AWC and 23.7% permanent wilting point (PWP). Each pot was filled with sieved, air-dried soil, and the amount of water needed to be compensated by watering was determined by weighing the pots every day. Six seeds per plot were sown and 500 cm^3 of sand was added on top. Plants were thinned to theree per pot in the second-node (V2) stage and kept inside the greenhouse until beginning bloom (R1) stage (Fehr and Caviness, 1977) after which they were taken outside and placed under a polyethene foil roof. The plants were irrigated when soil water content (SWC) reached 29.3% (management allowable depletion, MAD) in T1 and 23.7% (PWP) in T2. The Chl *a* fluorescence was determined according to the saturation pulse method (Kalaji et al., 2014), measured with the Handy Plant Efficiency Analyzer (PEA, Hansatech Instruments, King's Lynn, Norfolk, UK) on three plants per repetition when SWC reached PWP in T2. The measurements were taken on a cloudless day between 7:00 and 9:00 AM. Data recorded by measuring Chl a fluorescence, expressed in relative units, were used for calculating TR_0/ABS, PI_{ABS}, PI_{TOTAL} according to Strasser et al. (2004) and Yusuf et al. (2010) in Microsoft Excel. The differences between individual values were determined with the analysis of variance (ANOVA), followed by Fisher's least significant difference test (LSD test, $P < 0.05$) preformed in Statistica 12.0 software (StatSoft Inc., 2013). Stress tolerance index (STI) was calculated according to Fernandez (1992) for all three tested parameters by the following formula:

$$STI = \frac{Y_s \; x \; Y_p}{\bar{Y}_p}$$

where Ys and Yp are values of the investigated parameters for each genotype in conditions with sufficient water supply (50% FWC) and drought conditions (80% FWC) respectivelly, and $\bar{Y}p$ is the mean parameter value of all genotypes evaluated in nonstress conditions. Genotypes with higher STI are considered more stress tolerant.

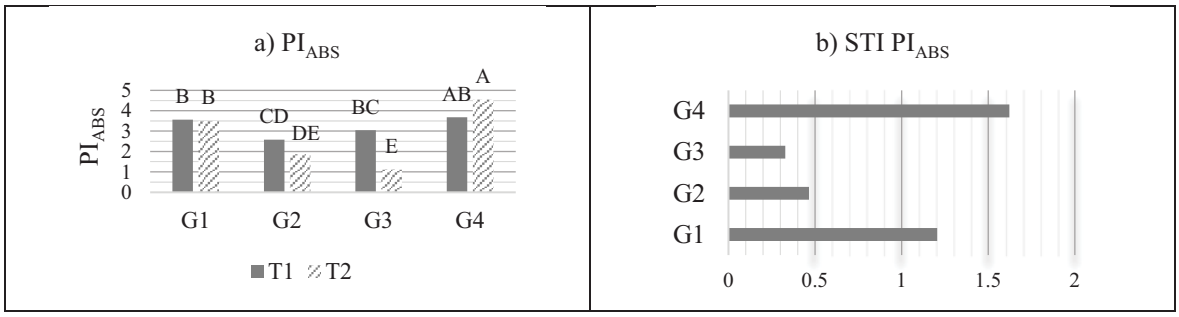
Results and discussion

The differences between individual values were noted in all three parameters used for evaluating the photosynthetic efficiency in different soil water contents (Graph 1-3). A significant TR_0/ABS decrease in drought was determined only in genotypes G2 and G3 (Graph 1a), indicating a change in the maximal efficiency of the excitation energy capture by active PSII RCs occurred (Strasser et al., 2004). In plants susceptible to abiotic stress, TR_0/ABS usually decreases (Bano et al., 2020; Markulj Kulundžić et al., 2021), which leads to a conclusion that photosynthetic apparatuses of genotypes G2 and G3 are less tolerant to drought compared to those of genotypes G1 and G4. Their T2 values being significantly lower than genotype G1's and G4's (Graph 1a), and lower STIs (Graph 1b), considered a good indicator of abiotic stress tolerance in different plants (Khokhar et al., 2012; Abdi et al., 2013,) lead to the same conclusion. However, all genotypes had the same level of maximum quantum yield of PSII photochemistry in drought conditions (T2, Graph 1a). Furthermore, all TR_0/ABS values (Graph 1a) ranged between 0.75 and 0.85 relative units, which according to Bolhar-Nordenkampf et al. (1989), indicates a well functional photosynthetic apparatus. It could be explained by the fact that TR_0/ABS is reportedly not appropriate for determining the early drought-stress symptoms in plants (Bukhov and Carpentier, 2004; Ohashi et al., 2006), indicating that stress in this research did not last long enough to cause impairment of the excitation energy capture in PSII.



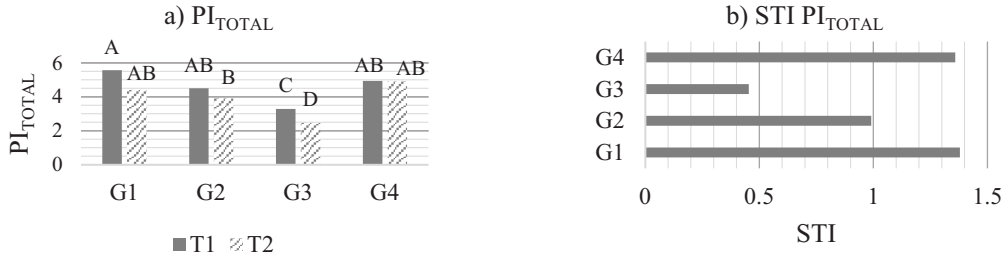
Graph 1. a) The maximum quantum yield of PSII photochemistry (TR_0/ABS) and b) stress tolerance index (STI) for four soybean genotypes (G1 - G4) tested in sufficient water supply (T1) and drought (T2) conditions.

PI_{ABS} can be used for evaluating the total photosynthetic efficiency in variable environmental conditions (Strasser et al., 2004), it quantifies general plant condition and vitality and is thought to be able to detect changes in plants as a result of stress very early (Kalaji et al., 2016). Drought caused a significant PI_{ABS} change only in genotype G3 (Graph 2a). PI_{ABS} decrease caused by abiotic stress is a sign of susceptibility (Bano et al., 2020; Killi et al., 2020), indicating genotypes G1, G4, and even G2 are more drought-tolerant than G3. Furthermore, significant differences between genotypes in their T2 PI_{ABS} and differences in STI values indicate there is room for the selection of genotypes with superior photosynthetic efficiency in drought stress. The positive correlation between the PI_{ABS} and seed yield determined previously (Franić et al., 2015; Kovačević et al., 2017) makes this parameter even more important for early generation screening. Based on parameter values and STI, genotype G4 could be considered the most tolerant in drought conditions, while genotype G3 is the least tolerant.



Graph 2. a) The photosynthetic performance index on absorption basis (PI_{ABS}) and b) stress tolerance index (STI) for four soybean genotypes (G1 - G4) tested in sufficient water supply (T1) and drought (T2) conditions.

PI_{TOTAL} detects the combined changes at each fluorescence transient functional step (Strasser et al., 2004; Yusuf et al., 2010) and has higher sensitivity to abiotic stress compared to other fluorescence parameters (Yusuf et a., 2010; Pavlović et al., 2019; Mihaljević et al., 2021). Although drought-induced changes of PI_{TOTAL}, the difference was significant only in genotype G3 (Graph 3a). In susceptible plants, abiotic stress usually causes a decrease in PI_{TOTAL}, indicating inhibition of the PSII activity and structural and/or functional damage of the PSI, e.i. decreased ability for energy conservation (Yusuf et al., 2010; Pavlović et al., 2019; Mihaljević et al., 2021). As genotype G3 was the only genotype with the impaired ability for energy conservation in drought, in addition to having the lowest T2 PI_{TOTAL} and STI (Graph 3), it can be considered as having the least efficient photosynthetic apparatus in stress. On the other hand, genotypes G1 and G4 have the most stable ability for energy conservation (Graph 3).



Graph 3. a) The performance index for energy conservation from exciton to the reduction of PSI end acceptors (PI_{TOTAL}) and b) stress tolerance index (STI) for four soybean genotypes (G1 - 4) tested in sufficient water supply (T1) and drought (T2) conditions.

Conclusions

Among the investigated parameters, PI_{ABS} and PI_{TOTAL} proved useful for detecting variability in photosynthetic efficiency. According to both, genotype G4 had the most efficient photosynthetic apparatus, while genotype G3 had the least efficient. The use of mentioned Chl a fluorescence parameters could be beneficial for eliminating genotypes with the most unfavourable trait values, especially in large scale genotype screenings for drought stress tolerance in early generations. However, stress tolerance of genotypes with superior photosynthetic performance should be confirmed in multi-environment yield trials.

References

Abdi N., Darvishzadeh R., Hatami Maleki H. (2013). Effective selection criteria for screening drought tolerant recombinant inbred lines of sunflower. Genetika. 45(1), 153-166.

Bano H., Athar H., Zafar Z.U., Kalaji H.M., Ashraf M. (2020). Linking changes in chlorophyll a fluorescence with drought stress susceptibility in mung bean [*Vigna radiata* (L.) Wilczek]. Physiologia Plantarum. 172, 1244-1254.

Bolhar-Nordenkampf H.R., Long S.P., Baker N.R., Öquist G., Schreiber U., Lechner E.G. (1989). Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: A review of current instrumentation. Functional Ecology, 3, 497-514.

Bukhov N. G., Carpentier R. (2004). Effects of water stress on the photosynthetic efficiency of plants. In Chlorophyll fluorescence: A signature of photosynthesis. Advances in photosynthesis and respiration series, Papageorgiou G.C., Govindjee G. (eds). Dordrecht, Netherlands: Springer, pp. 321-362.

FAO. (2015). World reference base for soil resources 2014. World Soil Resources Report 106 (Update 2015). Food and Agriculture Organization of the United Nations: Rome, Italy. [Online] Available at: https://www.fao.org/3/i3794en/I3794en.pdf

FAO. (2021). AQUASTAT Core Database. Food and Agriculture Organization of the United Nations. [Online] Available at: https://www.fao.org/aquastat/statistics/query/index.html

Fehr W.R., Caviness C.E. (1977). Stages of soybean development. Special report No. 87.

Fernandez G.C.J. (1992). Effective selection criteria for assessing stress tolerance. Proceedings of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress. Tainan, Taiwan, pp. 257-270.

Franić M., Mazur M., Volenik M., Brkić J., Brkić A., Šimić D. (2015). Effect of plant density on agronomic traits and photosynthetic performance in the maize IBM population. Poljoprivreda. 21 (2), 36-40.

Kalaji H.M., Schansker G., Ladle R.J., Goltsev V., Bosa K., Allakhverdiev S.I., Brestic M., Bussotti F., Calatayud A., Dabrowski P., Elsheery N.I., Ferroni L., Guidi L., Hogewoning S.W., Jajoo A., Misra A.N., Nebauer S.G., Pancaldi S., Penella C., Poli D.B., Pollastrini M., Romanowska-Duda Z.B., Rutkowska B., Serodio J., Suresh K., Szulc W., Tambussi E., Yanniccari M., Zivcak M. (2014). Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. Photosynthesis Research. 122, 121-158.

Kalaji H.M., Jajoo A., Oukarroum A., Brestic M., Zivcak M., Samborska I. A., Cetner M.D., Łukasik I., Goltsev V., Ladle R. J. (2016). Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiologiae Plantarum. 38, 1-11.

Khokhar M.I., Teixeira da Silva J.A. (2012). Evaluation of drought tolerance and yield capacity of barley (*Hordeum vulgare*) genotypes under irrigated and water-stressed conditions. Pakistan Journal of Agricultural Science. 49 (3), 307-313.

Killi D., Raschi A., Bussotti F. (2020). Lipid peroxidation and chlorophyll fluorescence of photosystem II performance during drought and heat stress is associated with the antioxidant capacities of C3 sunflower and C4 maize varieties. International Journal of Molecular Sciences. 21, 4846.

Kovačević J., Mazur M., Drezner G., Lalić A., Sudarić A., Dvojković K., Viljevac Vuletić M., Josipović M., Josipović A., Markulj-Kulundžić A., Lepeduš H. (2017). Photosynthetic efficiency parameters as indicators of agronomic traits of winter wheat cultivars in different soil water conditions. Genetika. 49 (3), 891-910.

Markulj Kulundžić A., Viljevac Vuletić M., Matoša Kočar M., Mijić A., Varga I., Sudarić A., Cesar V., Lepeduš H. (2021). The Combination of increased temperatures and high irradiation causes changes in photosynthetic efficiency. Plants. 10 (10), 2076.

Mihaljević I., Viljevac Vuletić M., Šimić D., Tomaš V., Horvat D., Josipović M., Zdunić Z., Dugalić K., Vuković D. (2021). Comparative study of drought stress effects on traditional and modern apple cultivars. Plants. 10 (3), 561.

Ohashi Y., Nakayama N., Saneoka H., Fujita K. (2006). Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. Biologia Plantarum. 50, 138-141.

Pavlović I., Mlinarić S., Tarkowska D, Oklestkova J., Novak O., Lepeduš H., Vujčić Bok V., Radić Brkanac S., Strnad M., Salopek-Sondi B. (2019). Early Brassica crops responses to salinity stress: A comparative analysis between Chinese cabbage, white cabagge, and kale. Frontiers in Plant Science. 10, 450.

StatSoft Inc. (2013): STATISTICA Data analysis software system (Release 12) [Software]. Tulsa, OK: StatSoft Inc.

Strasser, R.J., Srivastava, A., Tsimilli-Michael, M. (2004). Analysis of chlorophill a fluorescence transient. In Chlorophyll fluorescence: A signature of photosynthesis. Advances in photosynthesis and respiration series, Papageorgiou, G.C., Govindjee, G., (eds.), Dordrecht, Netherlands: Springer, pp. 321-362.

Yusuf M.A., Kumar D., Rajwanshi R., Strasser R. J., Tsimilli-Michael M., Govindjee S., Sarin N. B. (2010). Overexpression of g-tocopherol methyl transferase gene in transgenic Brassica juncea plants alleviates abiotic stress: Physiological and chlorophyll a fluorescence measurements. Biochimica et Biophysica Acta. 1797, 1428–1438.

ORIGINAL SCIENTIFIC PAPER

Effect of seed priming on germination of common bean (*Phaseolus vulgaris* L.) under drought stress

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Abstract
The aim of this study was to determine the effect of hydropriming (dH_2O; PT1) and osmopriming (-0.8 MPa PEG; PT2) on germination of two common bean cultivars ('Biser' and 'Trešnjevac') under induced drought stress. The germination capacity was tested on the germination paper soaked with dH_2O (T1) and -0.6 MPa PEG (T2) for nine days. The results showed that there was no significant difference between two common bean cultivars in terms of germination success, but there were significant differences between pretreatments (PT1 and PT2) and treatments (T1 and T2) in terms of germination success for these cultivars. Seeds of both cultivars germinate significantly faster on dH_2O (T1) when pretreatment is PEG -0.8.MPa (PT2), but dH_2O (PT1) is a better seed priming technique to use under induced drought stress (-0.6 MPa PEG; T2).

Key words: drought tolerance, hydropriming, PEG, water stress

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption worldwide and has an important role in human nutrition (Murube et al., 2021). Drought stress is one of the abiotic stressors caused by climate change that limits the germination, growth and productivity of crops worldwide (Nadeem et al., 2019) and has a significant impact on food security, especially in developing countries (Hussaini et al., 2021). Drought affects various aspects of legume growth and development, and it is well known that rapid germination and establishment of seedlings provides competitive advantages over weeds and improves yields (Nadeem et al., 2019; Lei et al., 2021). Therefore, researches are focused on improving drought tolerance and production of common bean through selection for various physiological and genetic traits (Hussaini et al., 2021; Lei et al., 2021). Priming is one of the pre-sowing treatments that results in a physiological state that enables the seed to germinate more efficiently (Dhal et al., 2019). Polyethylene glycol (PEG) is a popular osmopriming agent that can mitigate the negative effects of abiotic stress and hydropriming is a simple and economical technique useful in areas with adverse environmental conditions including heat and drought stress (Waqas et al., 2019).

The aim of this study was to determine the effect of hydropriming and osmopriming on germination of two common bean cultivars ('Biser' and 'Trešnjevac') under induced drought stress.

Material and methods

This research was conducted at the University of Zagreb Faculty of Agriculture, Department of Seed Science and Technology, in 2021. Seeds of two common bean cultivars 'Biser' and

'Trešnjevac', bought on the market, were used. One hundred seeds of each cultivar were soaked in distilled water (dH_2O; PT1) and 100 seeds in -0.8 MPa polyetilene glycol (PEG 8000; PT2) for eight hours. Then the seeds were taken out, washed under running water, then under distilled water and dried between paper towels. One hundred seeds without seed priming treatment represented the control. The laboratory test for germination of common bean seeds was conducted as per the ISTA (1993) Rules. The seeds were germinated on germination paper (Munktell 21/N, 580x580mm, 80g/qm) in 10-cm-diameter Petri dishes (Steriplan®, DURAN®, DWK Life Sciences GmbH, Germany). Fifty seeds from each pretreatment [dH_2O (PT1), -0.8 MPa PEG (PT2)] and control were placed on filter paper soaked with dH_2O (T1) and -0.6 MPa PEG (T2) (Table 1) in five replicates (five Petri dishes) with 10 seeds each. Petri dishes were placed in a germination chamber at a constant temperature (22 °C ± 1 °C) with a photoperiod of 16 h of light and 8 h of darkness. The number of germinated seeds (seeds with root size ≥ 2 mm) was determined every 24 h for nine days.

Table 1. Seed priming treatments (pretreatments) and treatments used in reserch

Pretreatment		Treatment	
PT1	100 seeds dH_2O	T1	50 seeds on dH_2O
		T2	50 seeds on PEG -0.6 MPa
PT2	100 seeds PEG -0.8 MPa	T1	50 seeds on dH_2O
		T2	50 seeds on PEG -0.6 MPa

PT1 – hydropriming (dH_2O); PT2 – osmopriming (-0.8 MPa PEG); T1 –germination – (dH_2O); T2 – drought stress (-0.6 MPa PEG);

Statistical analysis

The differences among cultivars, seed priming treatments (pretreatments) and treatments in terms of seed germination were tested by survival analysis using Kaplan-Meier method with log-rank test (Kaplan and Meier, 1958) as implemented in R package ′survival′ (Therneau and Grambsch, 2000; Therneau, 2015). Mean germination time was estimated as restricted mean survival time with upper limit equals 9. The impact of cultivars, seed priming treatments (pretreatments) and treatments on germination success was tested by Cox proportional-hazards model (Cox, 1972) using ′coxph′ function from R package ′survival′ (Therneau and Grambsch, 2000; Therneau, 2015).

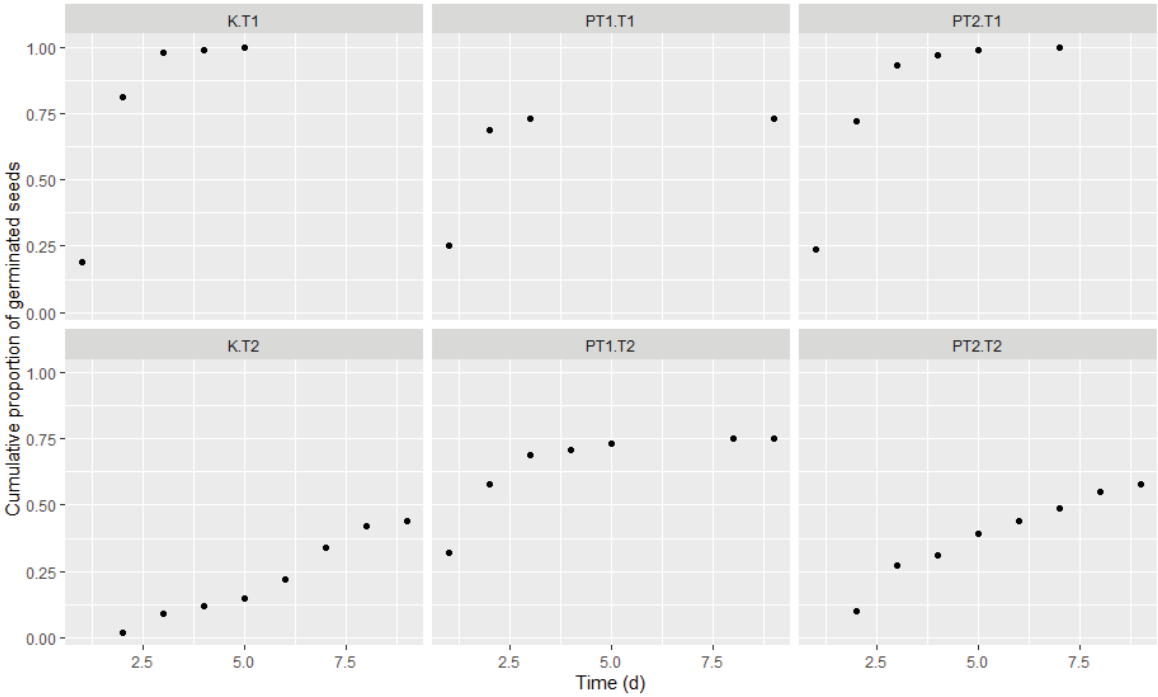
Results and discussion

The results of Kaplan-Meier analysis with log-rank test showed that there was no significant difference between two common bean cultivars ('Biser' and 'Trešnjevac') in germination success, but there were significant differences ($P < 0.05$) between seed priming treatments in germination success for these cultivars.

Seed from control treatment (K; without seed priming treatment) had the longest mean germination time (4.83 days), while seed in PT2 (-0.8 MPa PEG) had a mean germination time of 4.30 days, whereas seed in PT1 (dH_2O) had the shortest mean germination time (3.72 days). According to the Cox regression model for germination success, there were significant differences ($P < 0.01$) between seed priming treatment PT1 (dH_2O), which resulted in a relatively higher germination success of 40% compared to the control (K), and there were no significant differences between PT2 (-0.8 MPa PEG) and the control (K). In the T2 (-0.6 MPa PEG) treatment, germination time was 71% significantly ($P < 0.001$) longer than in T1 (dH_2O), unless the seed was previously primed with dH_2O (PT1 + T2). Germination is

generally slower with T1 (dH_2O) compared to T2 (-0.6 MPa PEG), except in combination with PT1 (dH_2O) when there is almost no difference between the treatments (Graph 1).

This study provides useful information on seed germination of common bean cultivars ('Biser' and 'Trešnjevac') under hydropriming (dH_2O) and osmopriming (-0.8 MPa PEG). Although osmopriming has more advantages than hydropriming, especially better response to stress (Marthandan et al., 2020), hydropriming improved germination of common bean cultivars under drought stress in our study. In agreement with studies on other crop species, seed hydropriming improved germination parameters of chickpea (Kuar et al., 2002) and Malaysian Indica rice (Kalhori et al., 2018) under drought stress and of sunflower under salt and drought stress conditions (Moghanibashi et al., 2012). Hydropriming is a simple, cheap and environmentally friendly technique and therefore can be used to improve seed performance of common beans under different conditions and in field trials.



Graph 1. Cumulative proportion of germinated seeds by category (pretreatment-treatment combinations) and days (over two cultivars).
K – control; PT1 – hydropriming (dH_2O); PT2 – osmopriming (-0.8 MPa PEG); T1 – treatment 1 – (dH_2O); T2 – treatment 2 (-0.6 MPa PEG).

Conclusion

It can be concluded that there was no significant difference between two common bean cultivars ('Biser' and 'Trešnjevac') in terms of germination success and that seeds of both cultivars germinate significantly faster on dH_2O (T1) when seed priming treatment is PEG -0.8.MPa (PT2), but dH_2O (PT1) is a better seed priming treatment under induced drought stress (T2; -0.6 MPa PEG).

Acknowledgement

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Literature

Cox D.R. (1972). Regression Models and Life-Tables. Journal of the Royal Statistical Society. Series B (Methodological). 34 (2): 187-220.

Dhal P., Sahu G.S., Mohanty S., Dhal A. (2019). Effect of priming on seed characters, disease incidence & yield in french bean (*Phaseolus vulgaris* L.). Journal of Pharmacognosy and Phytochemistry. 9 (1): 1028-1032.

Hussaini S.M.B., Sidle R.C., Kazimi Z., Khan A.A., Rezaei A.Q., Ghulami Z., Buda T., Rastagar R., Fatimi A.A., Muhmmadi Z. (2021). Drought Tolerant Varieties of Common Beans (*Phaseolus vulgaris*) in Central Afghanistan. Agronomy. 11 (11): 2181.

ISTA (1993.). International Rules for Seed Testing. International Seed Testing Association, Zürich, Switzerland.

Kaplan E.L., Meier P. (1958). Nonparametric Estimation from Incomplete Observations. Journal of the American Statistical Association. 53 (282): 457-481.

Kalhori N., Nulit R., Azizi P., Abiri R., Atabki N. (2018). Hydro priming stimulates seedling growth and establishment of Malaysian Indica rice (MR219) under drought stress. Acta Scientific Agriculture. 2 (11): 9-16.

Kaur S., Gupta A.K., Kaur, N. (2002). Effect of osmo- and hydropriming of chickpea seeds on seedling growth and carbohydrate metabolism under water deficit stress. Plant Growth Regulation. 37: 17-22.

Lei C., Bagavathiannan M., Wang H., Sharpe S.M., Meng W., Yu J. (2021). Osmopriming with Polyethylene Glycol (PEG) for Abiotic Stress Tolerance in Germinating Crop Seeds: A Review. Agronomy. 11 (11): 2194.

Marthandan V., Geetha R., Kumutha K., Renganathan V.G., Karthikeyan A., Ramalingam, J. (2020). Seed Priming: A Feasible Strategy to Enhance Drought Tolerance in Crop Plants. International Journal of Molecular Sciences. 21 (21): 8258.

Moghanibashi M., Karimmojeni H., Nikneshan P., Behrozi D. (2012). Effect of hydropriming on seed germination indices of sunflower (*Helianthus annuus* L.) under salt and drought conditions. Plant Knowledge Journal. 1 (1): 10-15.

Murube E., Beleggia R., Pacetti D., Nartea A., Frascarelli G., Lanzavecchia G., Bellucci E., Nanni L., Gioia T., Marciello U., Esposito S., Foresi G., Logozzo G., Frega G.N., Bitocchi E., Papa R. (2021). Characterization of Nutritional Quality Traits of a Common Bean Germplasm Collection. Foods. 10 (7):1572.

Nadeem M., Li J., Yahya M., Sher A., Ma C., Wang X., Qui L. (2019). Research Progress and Perspective on Drought Stress in Legumes: A Review. International Journal of Molecular Sciences. 20: 2541.

Therneau. T.M. (2015). Mixed Effects Cox Models. BMC Genetics. 6: S127.

Therneau T.M., Grambsch P.M. (2000). Modeling Survival Data: Extending the Cox Model. Springer, Berlin.

Waqas M., Korres N.E., Khan M.D., Nizami A.-S., Deeba F., Ali I., Hussain H. (2019). Advances in the Concept and Methods of Seed Priming. In: Priming and Pretreatment of Seeds and Seedlings. Implication in Plant Stress Tolerance and Enhancing Productivity in Crop Plants. Hasanuzzaman M., Fotopoulos V. (eds.), 11-41. Singapore, Singapore: Springer Nature Singapore Pte Ltd.

Croatian 57 International Symposium on Agriculture 2022

Povrćarstvo, ukrasno, aromatično i ljekovito bilje

04

Vegetable Growing, Ornamental, Aromatic and Medicinal Plants

PROFESSIONAL PAPER

Studies on the behaviour of *Begonia semperflorens* in different culture systems, in the conditions of North East of Romania

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Abstract
Begonia semperflorens is an annual flowering species frequently used to decorate parks and urban gardens due to its constant decorative appearance throughout the growing season and its resistance to outdoor conditions. This paper aims to study the behaviour, adaptability and decorative qualities of *Begonia semperflorens* in vertical green façades systems in the climatic conditions of the NE area of Europe. The control variant consisted of plants planted in field. The vertical experiment was carried out on high levels, applied on façades facing the four cardinal points. The study showed that *B. semperflorens* grown in vertical systems grows harmoniously regardless of the orientation of the wall on which it is planted. Also, in vertical systems, there is a higher degree of coverage and a higher number of flowers than the control variant throughout the monitoring period.

Key words: *Begonia semperflorens*, urban design, green façades, annual decoration

Introduction

The genus *Begonia* is distinguished by its decorative elements either by leaves or flowers, or both in equal measure. The fleshy leaves are positioned asymmetrically on the stem whose shape and colour varies among species of the genus *Begonia* (Chelariu, 2015). *Begonia semperflorens* needs a lot of heat to reach its full potential. It grows and blooms beautifully in sunny or partially shady, fertile soils with constant humidity (Toma, 2009). It survives even in areas with considerable shade, but with less flowers. With thick, waxy leaves that help the plant minimize water loss in hot weather, it can tolerate periods of drought. On the other hand, prolonged high humidity can lead to plant loss. It also has a compact appearance, abundant flowering throughout the growing season (from May to the first frost). It is well known that most urban areas have a shortage of green areas and the lack of free construction land is a major impediment to solving this problem. That is why covering the façades and roofs of buildings with plant species is a good solution both aesthetically (Dascălu and Cojocariu, 2016) and ecologically (Francis and Lorimer, 2011). Plants also act as a sunscreen that has the effect of reducing the heat outside the building, thus preventing the absorption of thermal radiation by building materials (Pérez et al., 2011). In this context, the study of the behaviour of various ornamental species, perennials and annuals, planted vertically in green façades systems becomes a topical idea that can be used (Cojocariu et al., 2020). *B. semperflorens* is a flowering species often used in the decoration of parks and gardens in Romania due to its decorative potential and its resistance to environmental conditions. These qualities suggest it's appropriate species for the study of its behaviour in vertical systems for green façades.

Material and methods

The study material used for the experiment is the species *B.semperflorens*. The plant has erect, slightly branched, succulent stems with red leaves and flowers. Experiment consisted

of purchased uniform seedlings from local flower producers. The average seedlings dimensions at the establishment were: diameter = 12.9 cm; height = 11.5 cm; number of inflorescences 1.2; number of leaves = 26.8. *Begonia* specimens were planted on an experimental structure built specifically for study to monitor the behaviour of several vertically planted flower species. The façades of the experimental structure, oriented towards the four cardinal points, are made up of four overlapping equal levels. A small intermediate space was left between the bearings to facilitate watering (Cojocariu et al., 2022). *Begonia* specimens were arranged one below the other, grouped on the same column. A ground control variant has also been set up. The experiment was carried out from the end of May to the end of September 2021 in the didactic field of the Faculty of Horticulture within ULS Iași. The levels of the experimental scheme were watered monthly, each level benefiting from 25 litres of water. The control variant did not benefit from additional water supply. No fertilizers were used. Three sets of data were collected at intervals of one month, monitoring, in addition to the percentage of attachment, the following characteristics: plant diameter, plant height, number of leaves and number of inflorescences. At the end of the monitoring, a plant survival rate was also calculated. Anova Single-Factor was used for statistical interpretation of the data.

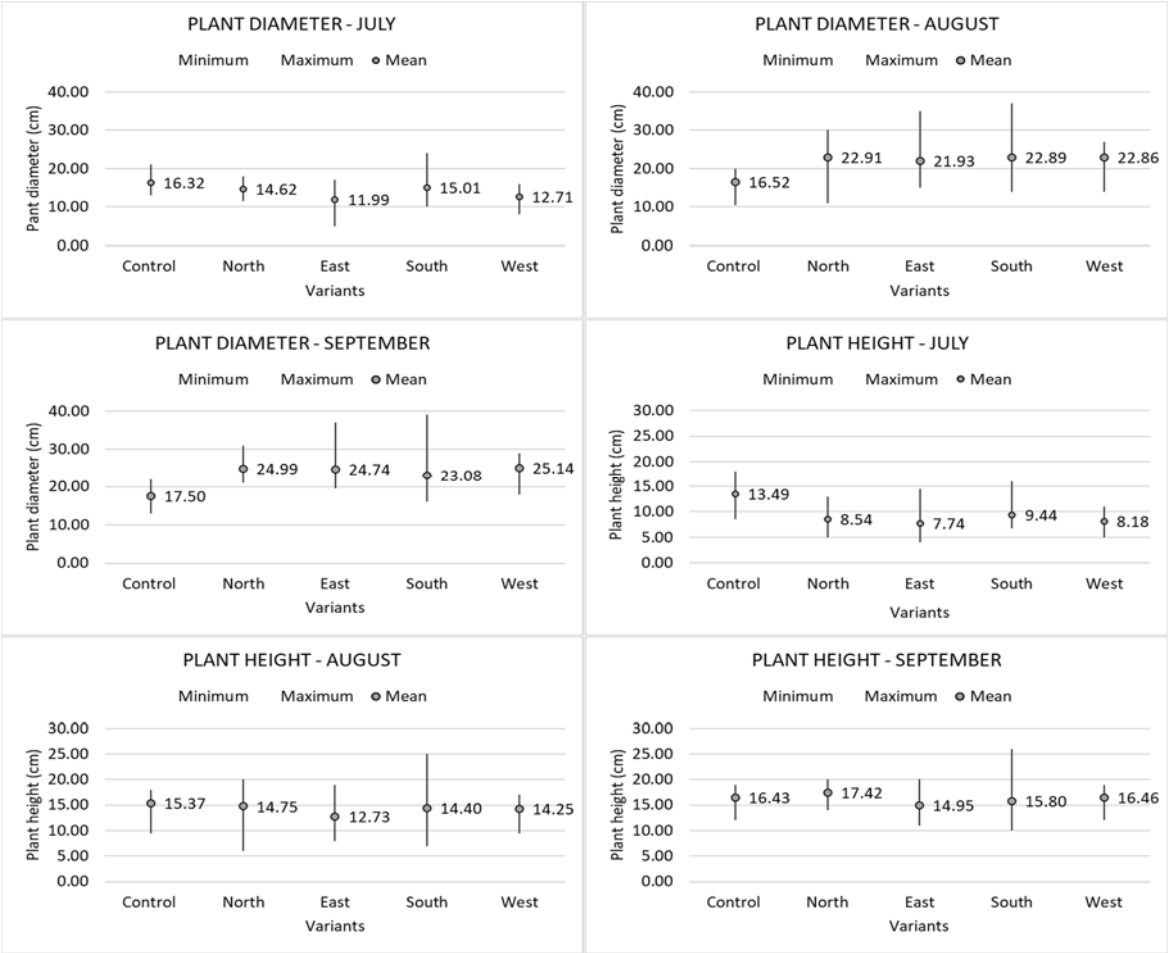
Results and discussion

The façades of the experimental scheme were oriented towards the four cardinal points, so that they benefit from different light intensity depending on the month and the cardinal point. Behaviour of the *B. semperflorens* both in vertical systems and in the in the field (control variant) were followed. Previous research conducted under similar conditions on the 'Big' hybrid of *Begonia semperflorens* pointed out that certain biometric features were higher on the north façade, where the light intensity is lower. However, the 'Big' hybrid has maintained its aesthetic value on all four façades (Cojocariu et al., 2020). At the end of July survival rate of *Begonia* plants in field (control) was 100 % compared to 77.17%, the total percentage on the entire vertical system. Divided by each cardinal orientation, the percentage of attachment recorded the following values: 78.26% on the North and East façades, 77.27% on the South and 75 % on the West façade. At the end of the monitoring period, at the end of September, a survival rate of 50% compared to 73.23% calculated on the entire vertical system was recorded in the control version. On the façades of the experimental scheme, the highest survival rate was noted on the South façade of 88.23%, and the lowest on the East façade of only 55.55 %. The survival rate was reported as a percentage of the values obtained in July (Table 1).

Table 1. The survival rate reported as a percentage of the values obtained in July

	North	East	South	West	Control
July	78.26%	78.26%	77.27%	75.00%	100.00%
August	83.33%	61.11%	94.12%	83.33%	64.29%
September	72.22%	55.56%	88.24%	77.78%	50.00%

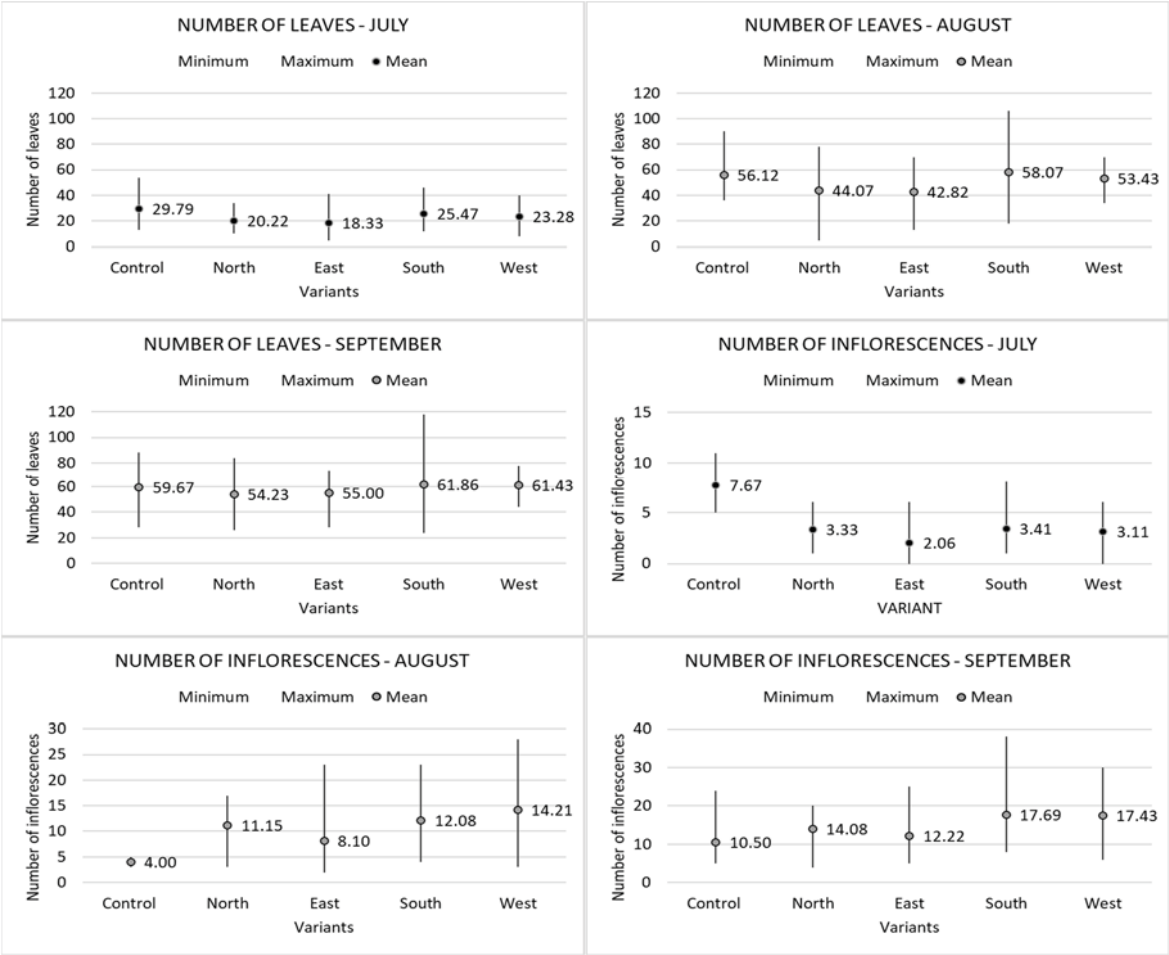
The percentage of attachment represents what percentage of the total number of planted specimens resisted transplantation. It was calculated at the first monitoring after planting (July). The survival rate is percentage of the total number of specimens that survived transplantation survived to the end of monitoring. The highest losses were recorded, on the experimental module, in the interval from planting to the first monitoring (5 plants on the North, East and South façades and 6 on the West).



Graph 1. Monthly evolution of plant diameter and plant height in respone to growing system and cardinal point

This indicates that *Begonia* plants need more attention after transplanting. Otherwise, except for the East façade where the survival rate was the lowest (8 plants withered between the first and the last monitoring) on the other façades the survival rate was over 70% (2 plants withered on the South and 5 on the North). At control variant, percentage of attachment was 100 % (in July - at the first monitoring), but at the end of the study period, the survival rate was 50 % (half of the planted *Begonia* plants withered). One month after the establishment of the experiment, highest average value of plant diameter was recorded in the control treatment, 16.32 cm. On the façades of the experimental scheme recorded values of this parameter were between 11.99 cm (value obtained on the Eastern orientation) and 15.01 cm (value obtained on the Southern orientation). In August and September, the average values of plant diameter on the vertical system were higher on all four façades orientations compared to the control variant in the field. A larger data dispersion was found on the Eastern and Southern façades which indicates that the plants did not develop uniformly (Graph 1). In terms of plant height, on the control variant the highest average values in all three months of experiment were recorded. The exception is the variant on the North façade, in September, where the average height of *B. semperflorens* plants was 17.42 cm compared to 16.43 cm obtained in the control variant (Graph 1). The average number of leaves per plant in the control variant was 29.79 in July and reaching a 59.67 in September. The values obtained on the façades of the experimental scheme for this parameter were usually below the values in the control variant except for the Southern façade where, in August and September, the calculated averages were slightly higher. In August and September, the highest dispersal

interval of the obtained values is registered on the Southern façade, which shows that the number of leaves per plant was not uniform (Graph 2).



Graph 2. Monthly evolution of number of leaves and inflorescences in respone to growing system and cardinal point

The average number of inflorescences, for all façades of the experimental scheme, in all three months of monitoring, registers increasing values. Thus, in September, most inflorescences were recorded on the Southern and Western façades, where the average values were 17.69 and 17.43, respectively, also recording the largest increases in the number of inflorescences per plant compared to July (14.28 and 14.32 respectively). The control variant, after a good start in July when the highest number of inflorescences compared to façades was recorded, had a considerable decline of 47.84 % in August. In September, number of inflorescences increased significantly, approaching the average of the Eastern façade of the experimental scheme (Graph 2). Data collected for plant diameter and number of inflorescences were statistically processed using the Anova Single Factor test for all those 4 façades and control. Thus, for the plant diameter p *value* < 0.05 in all three months of monitoring which indicates that the averages obtained by each variant (North, East, South, West and control) were significantly different. For the number of inflorescences p *value* < 0.05 for July and August and p *value* > 0.05 for September which shows that in the first two months of monitoring the averages obtained for this parameter are significantly different and for the last month the averages are equal (North, East, South, West and control) (Table 2).

Table 2. *p-value* obtained from the single factor ANOVA test

Variable	July	August	September
Plant diameter	0.000311	0.007643	0.020319
Number of inflorescences	3.75E-06	0.027929	0.089626

Conclusions

Cultivated vertically, in green façades systems, *B. semperflorens* is distinguished by a good degree of coverage and by an abundant and long-lasting flowering. It also has a compact appearance, a thick and healthy foliage and good resistance to environmental conditions. Although the plants did not have a uniform development, especially on the Southern façade of the experimental scheme, overall the decorative qualities were properly expressed. The visual evaluation of the ornamental characters did not reveal major differences between the façades of the experimental scheme. After the plants in the vertical system adapted to it, the values obtained at two of the monitored parameters (plant diameter and number of inflorescences) equalled and even exceeded the values obtained at the same parameters in the control variant, in the field. These results recommend *B. semperflorens* for use not only in the classic floor system or in planters but also vertically, in various green façades systems. They can be planted in groups of colour spots, as a single species or in combination with other annual or perennial flowering species ensuring an attractive long-lasting annual decoration.

References

Chelariu E.L. (2015). Floricultură – plante de apartament. Ed. “Ion Ionescu de la Brad” Iași, Romania, ISBN 978-973-147-177-8, 270

Cojocariu M., Chelariu E.L., Chiruţă C., Amişculesei P., Bulgariu E. (2020). The behaviour of *Begonia semperflorens* in vertical systems used for green façades, in climatic conditions specific to the North-Eastern part of Romania. Horticulture Series, 63(2).

Cojocariu M., Chelariu E.L., Chiruţă C. (2022). Study on behavior of some perennial flowering species used in vertical systems for green façades in Eastern European climate. Applied Sciences, 12(1): 474. https://doi.org/10.3390/app12010474

Dascălu D.M., Cojocariu M. (2016). Design peisagistic. Ed. “Ion Ionescu de la Brad” Iaşi, Romania, ISBN 978-973-147-223, 367

Draghia L., Chelariu E.L. (2011). Floricultură. Ed. “Ion Ionescu de la Brad” Iaşi, ISBN 978-973-147-074-0

Francis A.R., Lorimer J. (2011). Urban reconciliation ecology: The potential of living roofs andwalls, Journal of Environmental Management 92(6): 1429-1437.

Pérez G., Rincón Lidia, Vila Anna, González M.J., Cabeza Luisa F. (2011). Green vertical systems for buildings as passive systems for energy savings. Applied Energy, 88(12): 4854–4859.

Toma F. (2009). Floricultură și artă florală. Specii utilizate pentru decorul parcurilor și grădinilor, vol. 4, Otopeni, Romania, Ed. Invel Multimedia, 377.

IZVORNI ZNANSTVENI RAD

Utjecaj parametara sušenja na fizikalna svojstva ploda muškatne tikve (*Cucurbita moschata*)

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Sažetak
Cilj ovog rada bio je utvrditi utjecaj parametara sušenja (temperatura, vrijeme sušenja i tlak) na fizikalna svojstva ploda muškatne tikve, boju i vlažnost, odnosno gubitak mase mesa (mezokarp) ploda tijekom sušenja u kondukcijskoj vakuum sušari. Smanjenje tlaka u sustavu i povećanje temperature značajno utječu na brzinu sušenja i konačnu vlažnost uzoraka, pri čemu je postignuta razlika u smanjenju vlažnosti uzoraka od 6,9 puta. Porast temperature ima nepoželjne uočljive, a smanjenje tlaka slabo uočljive do uočljive promjene u boji (ΔE^*) mesa ploda muškatne tikve. Povećanjem temperature s 40 na 70 °C uz smanjenje tlaka od atmosferskog na 50 mbar pri vremenu sušenja od 300 minuta dolazi do značajne razlike u vrijednosti ΔE^*, odnosno vrlo uočljive razlike u boji sa 6,60 na 13,43 ($d_{\Delta E^*}$=6,83). Temperatura sušenja od 70 ºC pri 1 bar uz vrijeme sušenja od 120 min pokazala se optimalnom za postizanje vlažnosti od 16,40 % uz promjenu boje ΔE^* od 4,03.

Ključne riječi: boja i vlažnost ploda, vakuum sušenje, temperatura, tlak i vrijeme sušenja

Uvod

Sušenje je jedan od postupaka konzerviranja koji se provodi od davnina (sušenje na suncu) s ciljem očuvanje hrane, a temelji se na djelomičnom uklanjanju sadržaja vode. Uslijed uklanjanja vode iz namirnice dolazi do smanjenja aktiviteta vode, te se sprječava rast mikroorganizama. Konvencionalne metode sušenja mogu dovesti do nepoželjnih pojava poput promjene fizikalnih karakteristika (boje i teksture) i djelomičnog gubitka nutritivnih sastojaka, arome, gubitka aminokiselina i degradacije proteina, tj. kemijske reakcije enzimskog i neenzimskog posmeđivanja, karamelizacije te oksidacije lipida (Lovrić, 2000). Sve veći zahtjevi potrošača za kvalitetnijom hranom u pogledu nutritivnih i fizikalnih karakteristika i potrebe za povećanom konkurentnosti na tržištu dovodi do unaprjeđenja konvencionalnih postupaka obrade sirovina kombiniranjem inovativnih netoplinskih tehnologija i primjenom hibridnog postupka sušenja. Ultrazvučna predobrada i predobrada visokim tlakovima mogu dovesti do pozitivnih fizikalno-kemijskih promjena, kao i poboljšanog prijenosa mase (Fuente-Blanco i sur., 2006). Hibridno sušenje predstavlja kombinirani postupak sušenja koji koristi više tehnika sušenja, a može djelovati sinergijski te rezultirati kraćim vremenom sušenja i manjom potrošnjom energije uz zadržavanje kvalitativnih svojstava sušenog materijala. Osim hibridnog sušenja vakuum sušenje povrća ili sušenje pod sniženim tlakom je jedan od potencijalno iskoristivih postupaka kojim se može unaprijediti konvencionalni proces sušenja u prehrambenoj industriji. Vakuum postupak sušenja omogućava primjenu nižih temperatura čime se smanjuje utjecaj temperature na termolabilne nutritivne sastojke i fizikalne karakteristike namirnice, prvenstveno boje (Marelja i sur., 2020.), a uslijed smanjenja volumne koncentracije kisika osigurava se bolja oksidacijska stabilnost sušenog materijala (Fan i sur., 2012.). Muškatna tikva (*Cucurbita moschata* Duchesne) pripada porodici tikvenjača (Cucurbitaceae) koja obuhvaća važne vrste povrća kao što su krastavac, dinja, bundeva, tikvica/buča i lubenica

(Habtemariam, 2019.). Boja kore muškatne tikve varira od tamno zelene do svijetlo žute te može biti glatka ili naborana, a meso je jarko žute ili narančaste boje (Jacobo-Valenzuela i sur., 2011.).

Materijal i metode

Svježi plodovi muškatne tikve (*Cucurbita moschata* Duchesne) ručno su oguljeni, uklonjene su sjemenke sa sjemeništem, a mezokarp je narezan na ploške debljine 3 - 4 mm koje su korištene za pripremu 72 uzoraka od 360 g potrebnih za 24 varijante sušenja (2 temperature × 3 razine tlaka × 4 duljine sušenja) u 3 ponavljanja. Uzorci mesa ploda muškatne tikve sušeni su u kondukcijskoj vakuum sušari VO200 PM200 (Memmert GmbH + Co. KG, Njemačka) pri različitim uvjetima sušenja: temperatura 40 i 70 ºC, tlak 50, 250 i 1000 mbar) i vrijeme 120, 180, 240 i 300 min. Za određivanje parametara boje mesa ploda muškatne tikve primijenjena je objektivna CIE LAB metoda koja je ujedno najbliža vizualnoj percepciji. Premda takva metoda ne daje u potpunosti točnu definiciju boje, vrlo je učinkovita u određivanju razlika boja i praćenju promjene boje tijekom sušenja i drugih postupaka obrade. CIE LAB prostor boja prikazuje shematski prostorni dijagram poznat kao L*a*b* koordinatni sustav, odnosno kvantitativni odnos boja na tri osi koji se temelji na Heringtovoj teoriji suprotnih parova boja. Radi prihvaćanja od strane potrošača i praćenja kvalitete gotovog proizvoda, nužna je ujednačenost boje proizvoda, stoga je vrlo važno određivanje parametra ΔE* koji označava odstupanje od referentne boje svježeg uzorka (Setchell, 2017.). Za određivanje boje korišten je kolorimetar PCE-CSM 4 (PCE Instruments UK Ltd., Ujedinjeno Kraljevstvo). Tablica 1. prikazuje izračunate vrijednosti odstupanja u boji u odnosu na referentni uzorak (ΔE*) s obzirom na sposobnost uočavanja razlika između boja ljudskim okom. Statistička obrada podataka provedena je u programu GraphPad Prism 9.3.1. Korištena je multi i jednofaktorijalna ANOVA metoda, uz Tukey post-hoc test kako bi se utvrdio utjecaj parametara sušenja (tlak, vrijeme i temperatura) na boju i vlažnost mesa ploda muškatne tikve.

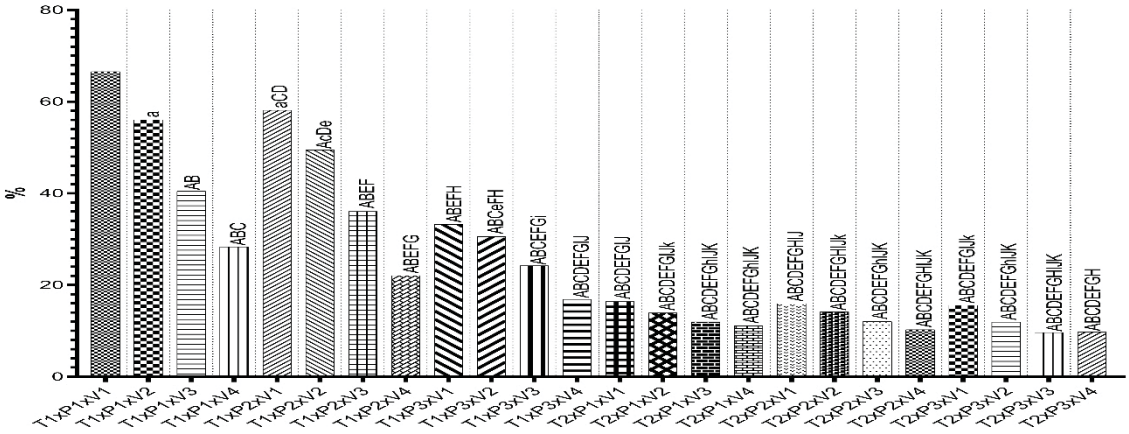
Tablica 1. Prikaz odnosa vrijednosti ΔE* i sposobnosti uočavanja razlike boja ljudskim okom (Candan i sur., 2021.)

ΔE*	Razlika između boja
0-0,2	Nema vidljive razlike
0,2-2,0	Vrlo slabo uočljiva
2,0-3,0	Primjetna (slabo uočljiva)
3,0-6,0	Uočljiva (značajna)
6,0-12,0	Vrlo uočljiva (velika)
>12	Vrlo velika

Rezultati i rasprava

Rezultati ovisnosti vlažnosti (%) mesa ploda muškatne tikve o uvjetima sušenja prikazani su u Grafikonu 1. Vidljivo je da se primjenom nižeg tlaka i više temperature postiže statistički značajno bolji učinak sušenja pri svim promatranim vremenima sušenja. Uz iste vrijednosti tlaka i vremena sušenja, usporedbom ispitivanih temperatura sušenja potvrđena je očekivana manja vlažnost mesa ploda muškatne tikve kod uzoraka sušenim višom temperaturom. Razlika iznosi 42 % kod kombinacije s najnižim tlakom i najdužim vremenom sušenja, odnosno 75 % kod kombinacije s najvišim tlakom i najkraćim vremenom sušenja. Najveću vlažnost imali su uzorci sušeni pri nižoj temperaturi, atmosferskom tlaku i najkraćem vremenu sušenja (kombinacija 40 °C × 1000 mbar × 120 min), a najmanju uzorci sušeni pri višoj temperaturi, najnižem testiranom tlaku i dužim vremenima sušenja (kombinacije 70 °C × 50 mbar × 240 i 300 min), pri čemu je utvrđena razlika u vlažnosti od 6,9 puta. Znatno

veći gubitak mase, odnosno manja vlažnost kod uzoraka sušenih pri sniženom tlaku u odnosu na sušenje pri atmosferskom tlaku, u skladu je s istraživanjima autora Arévalo-Pinedo i Murr (2006.). Učinak sušenja se pri nižem tlaku znatno povećava budući da molekule vode lakše difundiraju prema površini i u konačnici brže isparavaju (Sokač i sur., 2022.).

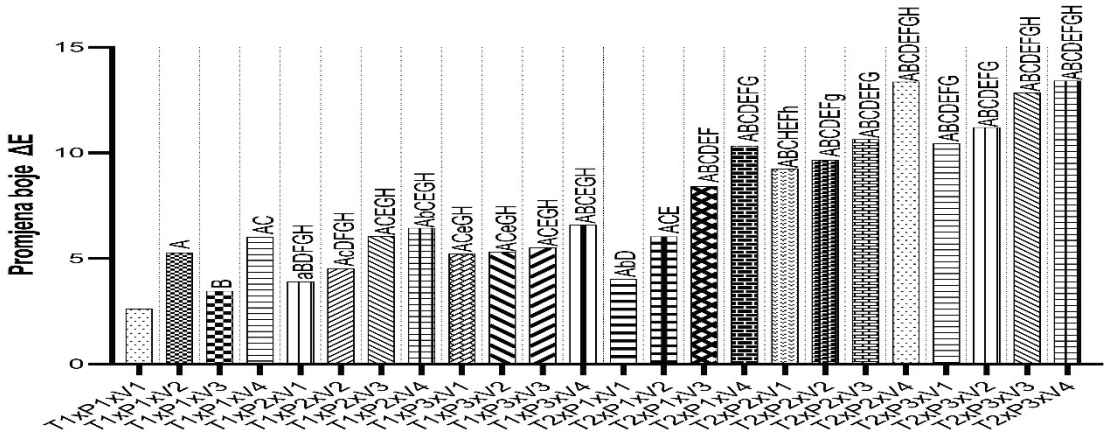


Grafikon 1. Učinak temperature (°C), tlaka (mbar) i vremena sušenja na vlažnost (%) mesa ploda muškatne tikve.

Statistička usporedba s uzrokom T1xP1xV1 - oznaka A; T1xP1xV2 – B; T1xP1xV3 -C; T1xP1xV4 -D; T1xP2xV1 – E; T1xP2xV2 – F; T1xP2xV3 – G; T1xP2xV4 – H; T1xP3xV – I; T1xP3xV2- -J; T1xP3xV3 – K, statistička značajnost usporedbe p≤0,001 označena je velikom slovom , dok utvrđena značajnost od p≤0,01 označena je malim slovom.

Legenda: T1–40 °C, T2–70 °C ; P1–1000 mbar, P2–250 mbar, P3–50 mbar; V1–120 min, V2–180 min, V3–240 min, V4–300 min.

Analizom varijance utvrđeno je da postoji statistički značajna razlika u svim parametrima boje između svježih i osušenih uzoraka, što je u skladu s rezultatima ukupne promjene boje osušenih uzoraka mesa ploda muškatne tikve prikazanim u grafikonu 2. Iz grafikona 2 je vidljivo da ukupna razlika u boji između kontrolnog svježeg uzorka i uzoraka sušenih pri 40 °C varira od primjetne do uočljive (ΔE* 2,0 - 6,0) te uzoraka sušenih pri 70 °C od uočljive do vrlo velike (ΔE* 4,0 - 15,0). Također, uočeno je da produljenje vremena sušenja rezultira nepoželjnom promjenom boje, pri čemu najveći utjecaj ima komponenta boje L*.



Grafikon 2. Učinak temperature (°C), tlaka (mbar) i vremena sušenja na promjenu boje (%) mesa ploda muškatne tikve

Statistička usporedba s uzrokom T1xP1xV1 - oznaka A; T1xP1xV2 – B; T1xP1xV3 -C; T1xP1xV4 -D; T2xP1xV1 – E; T2xP1xV2 – F; T2xP1xV3 – G; T2xP1xV4 – H. statistička značajnost usporedbe p≤0,001 označena je velikom slovom , dok utvrđena značajnost od p≤0,01 označena je malim slovom.

Promjena boje do koje je došlo uslijed povećanja temperature u skladu je s rezultatima istraživanja Uribe i sur. (2018.) koji navode da povećanje temperature nepovoljno djeluje na promjenu ΔE vrijednosti kod vakuum sušenja algi *Pyropia orbicularis,* a isto su utvrdili i autori Cox i sur., (2012) u postupku sušenja Irskih smeđih algi. Također, Swasdisevi i sur. (2007.) temeljem istraživanja utjecaja različitih vrijednosti podtlaka, vremena sušenja i temperature na svojstva kriški banane, navode da povećanje temperature negativno utječe na promjenu boje. Rezultati provedenog istraživanja s muškatnom tikvom u skladu su s navodima Salehi i sur. (2017.) vezanim za istraživanje kinetike vakuum sušenja narezanih gljiva, s obzirom na utvrđen izražen nepoželjan utjecaj temperature na promjenu ΔE vrijednosti kao i neznatan utjecaj promjene tlaka, tj. smanjenje vakuuma.

Zaključak

Za vođenje procesa vakuum sušenja neophodno je poznavanje fizikalnih svojstava materijala koji se suši te njegovog ponašanja pri različitim uvjetima sušenja. Smanjenjem tlaka u sustavu i povećanjem temperature dolazi do značajnog utjecaja na smanjenje vlažnosti mesa ploda muškatne tikve. Najveća razlika u vlažnosti od 6,9 puta postignuta je između uzorka sušenog pri kombinaciji 40 °C×1000 mbar×120 min i uzoraka sušenih pri kombinaciji 70 °C×50 mbar×240 i 300 min. Porastom temperature s 40 na 70 °C uz tlak od 50 mbar i vrijeme sušenja od 300 minuta dolazi do značajne razlike u vrijednosti ΔE* od 6,83, odnosno vrlo uočljive razlike u boji s 6,60 na 13,43. Kod uzoraka koji su sušeni pri temperaturi od 40 °C, neovisno o vremenu sušenja i tlaku, uočene su slabo uočljive do uočljive nepoželjne promjene u boji $d_{(\Delta E^*)}$ od 0,5 do 4 između uzoraka. Kod uzoraka koji su sušeni pri temperaturi od 70 °C, neovisno o primijenjenom tlaku, uočena je tendencija blago nepovoljnog utjecaja vremena sušenja. Pri tlaku od 50 mbar i temperaturi od 70 °C, produljenjem vremena sušenja dolazi do jedva primjetne promjene u boji ΔE* s 10,45 (120 min) na 13,43 (300 min). Na temelju dobivenih rezultata optimalna temperatura sušenja bila je 70 ºC pri atmosferskom tlaku, pri čemu je vrijeme sušenja iznosilo 120 min. Navedeni parametri pokazali su se optimalnim pri čemu je postignuta minimalna vlažnost u iznosu od 16,40 %, uz promjenu boje ΔE od 4,03.

Napomena

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Literatura

Arévalo-Pinedo A., Murr F. E. X. (2006). Kinetics of vacuum drying of pumpkin (Cucurbita maxima): Modeling with shrinkage. Journal of Food Engineering. 76 (4), 562-567.

Candan, Z., Gonultas, O., Gorgun, H.V. i Unsal, O. (2021). Examining Parameters of Surface Quality Performance of Paulownia Wood Materials Modified by Thermal Compression Technique. Drvna industrija, 72 (3), 231-236.

Delazio A., Israr A., Klatzky R. L. (2017). Cross-modal correspondence between vibrations and colors. World Haptics Conference (WHC), 219-224.

Fan L., Li J., Deng K., Ai L. (2012). Effects of drying methods on the antioxidant activities of polysaccharides extracted from Ganodermalucidum. Carbohydrate Polymers 87, 1849–1854.

Fuente-Blanco, S., Sarabia, E. R., Acosta-Aparicio, V. M., Blanco-Blanco, A., & Gallego-Juarez, J. A. (2006). Food drying process by power ultrasound. Ultrasonics Sonochemistry, 44, 523-527.

Habtemariam S. (2019). The chemical and pharmacological basis of pumpkins (Cucurbita species) as potential therapy for type-2 diabetes, Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases, Academic Press, str. 473-502.

Jacobo-Valenzuela N., Maróstica-Junior M.R., Zazueta-Morales J., Gallegos- Infante J.A. (2011). Physicochemical, technological properties, and health-benefits of Cucurbita moschata. Food Research International 44, 2587-2593.

Lovrić, T. (2000). Procesi u prehrambenoj industriji s osnovama prehrambenog inženjerstva. Zagreb: Hinus, 179-226.

Marelja M., Dujmić F., Ježek D., Škegro M., Bosiljkov T., Karlović S., Lasić M., Brnčić M. (2020). Vakuum sušenje u prehrambenoj industriji. Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam. 15 (3-4), 94 – 101.

Salehi F., Kashaninejad M., Jafarianlari A. (2017). Drying kinetics and characteristics of combined infrared-vacuum drying of button mushroom slices. Heat and Mass Transfer, 53(5), 1751-1759.

Setchell J.S. (2017). 4 - Colour description and communication. U: Colour Design (Second Edition), (Best J., ured.), Woodhead Publishing, United Kingdom, str. 99-129

Sokač T., Gunjević V., Pušek A., Tušek A.J., Dujmić F., Brnčić M., Ganić K.K., Jakovljević T., Uher D., Mitrić G., Redovniković I.R. (2022). Comparison of Drying Methods and Their Effect on the Stability of Grasevina Grape Pomace Biologically Active Compounds. Foods, 11, 112.

Swasdisevi T., Devahastin S., Ngamchum R., Soponronnarit S. (2007). Optimization of a drying process using infrared-vacuum drying of Cavendish banana slices. Optimization, 29(3), 810.

Uribe E., Vega-Gálvez A., Heredia V., Pastén A., Di Scala K. (2018). An edible red seaweed (Pyropia orbicularis): Influence of vacuum drying on physicochemical composition, bioactive compounds, antioxidant capacity, and pigments. Journal of Applied Phycology, 30(1), 673-683.

Influence of hybrid drying on physical properties of „*Cucurbita moschata*“squash

Abstract

This study aimed to determine the influence of drying parameters (temperature, drying time, and pressure) on the physical properties of squash such as color, and weight loss of pumpkin samples during drying. Decreasing the pressure and increasing the temperature of the dryer significantly affected the drying rate and moisture content of pumpkin samples, with a significant difference in the moisture content with a decrease of 6.9 times. Increasing the temperature resulted in an undesirable noticeable change in color and decreasing the pressure resulted in a noticeable ΔE* color change. An increase in temperature from 40 to 70 °C with a decrease in pressure from atmospheric to 50 mbar for a drying time of 300 minutes has a negative correlation with ΔE*, resulting in a significant increase in ΔE* value from 6.60 to 13.43 ($d_{\Delta E^*}$=6,83). A drying temperature of 70 °C at 1 bar with a drying time of 120 min proved to be optimal to achieve a moisture content of 16.40% with a color change ΔE* of 4.03.

Key words: colour and humidity, vacuum drying, temperature, pressure, drying time

IZVORNI ZNANSTVENI RAD

Morfološka svojstva koprive (*Urtica dioica* L.) u plutajućem hidroponu

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Sažetak

Hidroponski uzgoj koprive u zaštićenom prostoru omogućuje dostupnost biljnog materijala kroz duži period godine uz veće prinose te bolju kvalitetu herbe u odnosu na uzgoj na otvorenom. Cilj istraživanja bio je odrediti komponente prinosa te prinos svježe herbe koprive uzgajane tehnikom plutajući hidropon u dvije hranive otopine različitog sastava kroz dva roka košnje. Bolji utjecaj na promatrana morfološka svojstva imala je otopina s manjom količinom hraniva (Jensen, EC = 1,7 mS cm^{-1}) u odnosu na otopinu s većom količinom hraniva (Cooper, EC = 3,0 mS cm^{-1}). Kombinacija pojedine otopine i 2. roka košnje ostvarila je značajno veći prinos koprive u odnosu na 1. rok košnje (2,3 kg m^{-2} i 1,8 kg m^{-2}).

Ključne riječi: hidroponski uzgoj, hraniva otopina, prinos, morfologija

Uvod

Kopriva (*Urtica dioica* L.) je višegodišnja samonikla biljna vrsta koja uz farmakološku upotrebu postaje sve popularnija među potrošačima kao prehrambena namirnica. Najčešće se sakuplja iz prirode, no takav biljni materijal je neujednačene kvalitete zbog čega se preporučuje njena kultivacija. Sve je više istraživanja vezanih za introdukciju koprive u poljoprivrednu proizvodnju i tehnologiju uzgoja. Osim uzgoja koprive na tlu, veliku pozornost stječu i hidroponski sustavi uzgoja bilja bez tla, od kojih se plutajući hidropon ističe kao tehnika koja osigurava bolje uvjete za rast i razvoj koprive (Opačić i sur., 2022.). U odnosu na uzgoj na otvorenom, hidroponski uzgoj omogućuje kontrolu abiotskih čimbenika zaštićenog prostora i hranive otopine uz racionalno korištenje vode i hraniva (D'Anna i sur., 2003.; Nicola i sur., 2006.; Toth i sur., 2012.). S obzirom da je kopriva višegodišnja vrsta koja ima sposobnost retrovegetacije, primjenom ove tehnike uzgoja moguće ju je uzgajati tokom cijele godine što rezultira većim brojem košnji i prinosom. Proizvođači koji posjeduju zaštićene prostore opremljene za hidroponski uzgoj lisnatog povrća lako mogu prilagoditi pogon uzgoju koprive te time ostvariti dodatni prihod i veću konkurentnost na tržištu (Radman i sur., 2014.).

Cilj ovog istraživanja bio je odrediti morfološke komponente (visina biljaka, broj listova, indeks lisne površine i prinos) hidroponski uzgojene koprive u dvije otopine različitog sastava hraniva prema Jensen i Cooper recepturi (Lorenz i Maynard, 1988.) u 1. i 2. roku košnje.

Materijal i metode

Istraživanje je postavljeno na pokušalištu Agronomskog fakulteta Sveučilišta u Zagrebu u negrijanom zaštićenom prostoru u proljeće 2021. godine. Pokus je postavljen prema slučajnom bloknom rasporedu u tri ponavljanja i uključivao je uzgoj koprive tehnikom plutajući hidropon u bazenima s 2 različite hranive otopine kroz dva roka košnje.

Sjetva sjemena koprive (B&T World Seeds, Francuska) u količini od 50 sjemenki/prorezu (1,2 g m^{-2}) provedena je 11. ožujka u polistirenske ploče (0,96 m × 0,6 m) sa 102 proreza ispunjene inertnim supstratom, perlitom. Ploče su nakon naklijavanja u periodu od 7 dana nakon sjetve postavljene u bazene (3 m × 1,2 m) ispunjene hranivim otopinama prema

Rad je izvod iz diplomskog rada Lucijana Sikirića, mag. ing. agr. naslova 'Bioaktivni spojevi i morfološka svojstva hidroponski uzgojene koprive pod utjecajem otopina različitog sastava'.

recepturama Jensen i Cooper, koje su se međusobno razlikovale u količini dodanih soli (Tablica 1). Svaki bazen bio je zastupljen sa po 6 ploča pri čemu su jednu repeticiju predstavljale dvije ploče.

Tablica 1. Količine hraniva za pripremu otopina

	B1	B2
Otopina	**Jensen**	**Cooper**
Soli	mg L^{-1}	
KNO_3	203,4	583,9
KH_2PO_4	272,1	261,6
$Ca(NO_3)_2 \times 4H_2O$	548,7	1093,0
$MgSO_4 \times 7H_2O$	494,1	512,6
H_3PO_4 (75 %)	25,4	79,3
FeEDTA (13%)	2,64	1,59
H_3BO_3	1,32	0,26
$CuSO_4 \times 5H_2O$	0,13	0
$CuCl_2 \times 2H_2O$	2,38	6,08
$MnSO_4 \times 4H_2O$	0,40	0,45
$ZnSO_4 \times 7H_2O$	0,05	0
$Na_2MoO_4 \times 2\ H_2O$	0	0,37
EC (mS cm^{-1})	1,7	3,0

B1 – bazen 1; B2 – bazen 2; EC - elektroprovodljivost

Tijekom uzgoja redovito su praćeni abiotski čimbenici zaštićenog prostora i hranive otopine. U cilju određivanja prinosa i morfoloških karakteristika koprive uzgojene u plutajućem hidroponu pod utjecajem otopina različitog sastava, provedene su dvije košnje. Kopriva se kosila prije cvatnje (u prehrambenu svrhu), a biljke su rezane iznad prva dva nodija, kako bi se omogućila retrovegetacija. Prva košnja obavljena je 20. svibnja 2021. (70 dana nakon sjetve) dok je druga košnja bila 8. lipnja 2021. Prilikom svake berbe utvrđen je ukupan prinos po bazenu (kg m^{-2}) te je sa svake uzgojne ploče odabrano 5 reprezentativnih biljaka na kojima su izvršena sljedeća mjerenja: visina biljke (cm), broj listova, širina i duljina lista (cm). Iz podataka za širinu i duljinu lista izražen je indeks lisne površine (ILP) izračunat pomoću formule: dužina × širina lista (cm^2).
Statistička obrada podataka provedena je u programu SAS Software v. 14.3 (2017.), prema proceduri PROC GLM (opći linearni model). Rezultati su podvrgnuti dvofaktorijalnoj analizi varijance (ANOVA), a utvrđene razlike između srednjih vrijednosti uspoređene su t-testom (LSD) na razini značajnosti $p \leq 0,05$.

Rezultati i rasprava

Prosječna minimalna temperatura zraka u zaštićenom prostoru bila je u rasponu od 8,3 °C (1. dekada travnja) do 18,1 °C (1. dekada lipnja). Prosječna maksimalna temperatura varirala je između 29,0 °C (2. dekada travnja) i 40,6 °C (1. dekada lipnja). Prosječna vrijednost srednje temperature zraka bile je od 19,6 °C (2. dekada travnja) do 24,9 °C (3. dekada svibnja), a prosječna vrijednost relativne vlage zraka varirala je od 39 do 51 %. Optimalne temperature za rast koprive su 20 do 25 °C (Radman, 2015.) te su visoke vrijednosti maksimalnih temperatura vjerojatno izazvale temperaturni stres što se očitovalo klorotičnim promjenama i smanjenim rastom pojedinih biljaka što je vjerojatno rezultat smanjenog usvajanja hraniva uslijed visoke temperature. pH i EC vrijednosti obje hranive otopine bile su u rangu preporučenih vrijednosti (tablica 1) te su se po potrebi korigirale. U bazenu 1 (B1) prosječna EC vrijednost iznosila je 1,5 mS cm^{-1} dok je pH varirao od 6,6 do 7,2. U bazenu 2 (B2) prosječna EC vrijednost iznosila je 3,0 mS cm^{-1}, a pH vrijednost otopine

kretala se od 6,3 do 7,1. Prema Toth i sur. (2012.) preporučeni pH otopine za uzgoj lisnatog povrća iznosi 5,8 do 6,2, što su nešto niže vrijednost od onih ostvarenih tijekom ovog istraživanja. Tijekom uzgoja također su mjerene temperatura i količina otopljenog kisika u otopinama. Temperature otopina varirale su od 23,2 do 28,0 °C (B1), odnosno, 23,3 do 29,0 °C (B2) i rasle su s toplijim danima. Količina otopljenog kisika također se mijenjala (2,0 do 3,5 mg L^{-1} u B1; 2,7 do 3,5 mg L^{-1} u B2) te je u nekim slučajevima viša temperatura otopine utjecala na smanjenje količine otopljenog kisika što je u skladu s Toth i sur. (2012.).
U tablici 2 prikazan je utjecaj otopina, košnji te njihovih interakcija na prinos i morfološka svojstva koprive uzgojene u plutajućem hidroponu. Vidljiv je opravdan utjecaj glavnog faktora, otopine, na ILP dok nije utvrđen statistički opravdan utjecaj na visinu biljaka, broj listova i prinos. Podfaktor košnja statistički je značajno utjecao na visinu biljaka, broj listova i prinos, no nije utvrđen opravdan utjecaj ovog faktora na ILP. Interakcija različitih otopina i rokova košnje imala je značajan utjecaj na sva promatrana svojstva.

Tablica 2. Utjecaj otopina, košnji i njihove interakcije na prinos i morfološka svojstva hidroponski uzgojene koprive

Faktori	Tretmani	Visina biljaka (cm)	Broj listova	ILP (cm^2)	Prinos (kg/m^2)
Otopina	Jensen (J)	$30,2^{ns}$	$12,3^{ns}$	$48,6^{A}$	$1,5^{ns}$
	Cooper (C)	$26,9^{ns}$	$10,5^{ns}$	$38,6^{B}$	$1,5^{ns}$
Košnja	1. košnja (1)	$20,0^{B}$	$13,0^{A}$	$40,1^{ns}$	$0,9^{B}$
	2. košnja (2)	$37,0^{A}$	$9,8^{B}$	$47,1^{ns}$	$2,1^{A}$
Interakcija	J×1	$17,6^{C}$	$13,3^{A}$	$37,33^{B}$	$0,7^{B}$
	J×2	$42,8^{A}$	$11,33^{BA}$	$59,90^{A}$	$2,3^{A}$
	C×1	$22,5^{C}$	$12,67^{BA}$	$42,93^{B}$	$1,2^{B}$
	C×2	$31,3^{B}$	$8,33^{B}$	$34,20^{B}$	$1,8^{A}$

Različita slova pridodana vrijednostima unutar stupca predstavljaju značajno različite vrijednosti prema LSD testu, $p \leq 0,05$; ns – nije signifikantno.

Premda razlika nije bila statistički opravdana, biljke uzgajane u B1 (30,2 cm) bile su više u odnosu na biljke iz B2 (26,9 cm). Podfaktor košnja pozitivno je utjecala na visinu biljaka te su više biljke izmjerene nakon 2. košnje (37,0 cm), dok je prosječna visina biljaka u 1. košnji bila 17 cm manja (20,0 cm). Interakcija J×2 rezultirala je statistički najvišim biljkama (42,8 cm), dok su kombinacije obje otopine i 1. roka košnje rezultirale najnižim biljkama (J×1 = 17,6 cm; C×1 = 22,5 cm). Prema Javornik (2016.), za razliku od rezultata ovog istraživanja, hraniva otopina je imala statistički značajan utjecaj na visinu biljaka koprive. Biljke uzgajane u otopini slabijeg sastava (EC = 2,3 mS cm^{-1}) ostvarile su veću visinu u 2. roku košnje u odnosu na 1. rok što je u skladu s ovim istraživanjem. Isti autor navodi kako su biljke uzgajane u otopini jačeg sastava (EC = 2,5 mS cm^{-1}) bile podjednake visine u oba roka košnje. Prema Radman i sur. (2014.) visina koprive uzgajane u plutajućem hidroponu ovisi o gustoći sjetve i vrsti supstrata, a u prosjeku više biljke izmjerene su nakon 1. košnje što je suprotno rezultatima ovog istraživanja.
Broj listova bio je u prosjeku nešto viši kod biljaka uzgajanih u B1 (12,3) u usporedbi s biljkama iz B2 (10,5), no razlika nije bila statistički značajna. Statistički opravdana razlika uočena je kod faktora košnja pri čemu su biljke nakon 1. košnje imale više listova (13,0) u odnosu na 2. košnju (9,8). Pri interakciji promatranih faktora, statistički najviše listova zabilježeno je kod biljaka u kombinaciji J×1 (13,3) dok su najmanje listova imale biljke u kombinaciji C×2 (8,3). Interakcije J×2 i C×1 bile su statistički istog ranga. Javornik (2016.) navodi kako otopina nije imala statistički opravdan utjecaj na broj listova koprive što je sukladno ovom istraživanju. Biljke uzgajane u otopinama različitog sastava hraniva ostvarile

su veći broj listova u 2. roku košnje u odnosu na 1. rok. U istraživanju Radman i sur. (2014.) također su više listova imale biljke nakon 2. košnje, što je u skladu s ovim istraživanjem.
ILP znatno utječe na intenzitet fotosinteze i brzinu transpiracije, a time i na ukupni prinos koprive te je važno tijekom rasta i razvoja biljaka pratiti ovo svojstvo (Sabouri, 2015.). Biljke uzgajane u B1 (48,6 cm^2) imale su statistički opravdano veći ILP u odnosu na biljke iz B2 (38,6 cm^2) što nije bilo očekivano s obzirom da prema Radman (2015.) veće količine dušika rezultiraju značajno višim vrijednostima većine promatranih morfoloških svojstava koprive. Košnja nije statistički značajno utjecala na ILP, ali su biljke u 2. roku košnje imale veći prosječni ILP u odnosu na biljke iz 1. roka košnje. Interakcija J×2 rezultirala je opravdano najvećim ILP (59,9 cm^2), dok je statistički najmanji ILP zabilježen kod kombinacije C×2 (34,2 cm^2) u čijem su rangu, premda nešto veće, bile i vrijednosti iz kombinacija J×1 i C×1 (37,3 cm^2 i 42,9 cm^2). U istraživanju Rutto i sur. (2012.) kopriva posađena u lončiće tretirana je različitim količinama dušika po posudi te pri tome nije uočen statistički značajan utjecaj gnojidbe na ILP. Očekivano, viši ILP zabilježen je kod biljaka tretiranim većom količinom N po posudi, dok je najmanji ILP izmjeren u biljaka kojima nije dodan N. Navedeno je suprotno ovom istraživanju u kojem je otopina, koja sadrži manju količinu N, imala je statistički značajan pozitivan učinak na ILP. Taylor (2009.) navodi kako je za povećanje ILP potrebna veća količina dušika u hranivoj otopini ili tlu, što ovo istraživanje nije pokazalo s obzirom da su bolji rezultati ostvareni u otopini s manjom količinom dušika. Navedeno je možda rezultat slabijeg usvajanja N u otopini više EC vrijednosti koja je mogla negativno utjecati na rast i razvoj korijenskog sustava, a time i na usvajanje dušika.
Otopina nije imala opravdan utjecaj na prinos (kg m^{-2}) koji je u oba bazena iznosio 1,5 kg m^{-2}. Faktor košnja značajno je utjecala na prinos te je dvostruko veći prinos ostvaren pri 2. roku košnje (2,1 kg m^{-2}) u odnosu na 1. rok košnje (0,9 kg m^{-2}). Kombinacija otopine Jensen i 2. roka košnje rezultirala je najvećim prinosom (2,3 kg m^{-2}), no nije bilo statističke razlike u odnosu na kombinaciju istog roka košnje i otopine Cooper (1,8 kg m^{-2}). Kombinacije pojedine otopine i 1. roka košnje ostvareni su statistički najniži prinosi (J×1 = 0,7 kg m^{-2} i C×1 = 1,2 kg m^{-2}). Sukladno ovom istraživanju, rezultati istraživanja Javornik (2016.) ukazuju da hraniva otopina nije imala statistički opravdan utjecaj na prinos koprive uzgojene u plutajućem hidroponu. Kopriva uzgojena u otopini slabijeg sastava (EC = 2,3 mS cm^{-1}) ostvarila je veći prinos u 2. roku košnje, što je jednako trendu u ovom pokusu. Uzgoj koprive u otopini jačeg sastava (EC = 2,5 ms cm^{-1}) rezultirao je statističkim jednakim prinosom u oba roka košnje, što nije u skladu s provedenim istraživanjem. Suprotno navedenim istraživanjima, Radman i sur. (2014.) navode veći prinos koprive uzgajane u otopini Tesi (EC = 2,3 mS cm^{-1}) nakon 1. roka košnje u odnosu na 2. rok košnje.

Zaključak

Otopina Jensen, koja je u sastavu imala manju količinu hraniva (posebice N, K i Ca), imala je bolji utjecaj na visinu biljaka, broj listova i indeks lisne površine, dok je otopina Cooper imala izraženiji utjecaj na prinos. Nakon 1. košnje biljke su imale više listova, ali je u 2. roku košnje ostvaren veći prinos, biljke su bile više i s većim indeksom lisne površine. Statistički veći utjecaj na većinu morfoloških svojstava imao je podfaktor košnja u odnosu na otopine različitog sastava hraniva. Interakcija J×2 rezultirala je višim vrijednostima većine promatranih svojstava u odnosu na ostale tri kombinacije. Uz morfološka svojstva, vrlo je važna i nutritivna kvaliteta biljnog materijala te bi u budućim istraživanjima trebalo ispitati utjecaj otopina različitog sastava hraniva i rokova košnje na sadržaj specijaliziranih metabolita, minerala te akumuliranih nitrata u svježem listu koprive.

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Literatura

D'Anna F., Miceli A., Vetrano F. (2003). First results of floating system cultiovation of *Eruca sativa* L. Acta Horticulturae. 609: 361-364.

Javornik M. (2016). Utjecaj sastava hranjive otopine i gustoće sjetve na komponente prinosa hidroponski uzgojene koprive. Agronomski fakultet u Zagrebu. Doktorski rad.

Lorenz O. A., Maynard D. N. (1988). Knotts Handbook for Vegetable Growers. John Wiley Sons, New York.

Nicola S., Hoeberechts J., Fontana E. (2006). Ebb-and-flow and floating systems to grow leafy vegetables: a review for rocket, corn salad, garden cress and purslane. In VIII International Symposium on Protected Cultivation in Mild Winter Climates: Advances in Soil and Soilless Cultivation. 747: 585-593.

Opačić N., Radman S., Fabek Uher S., Benko B., Voća S., Žlabur J. Š. (2022). Nettle Cultivation Practices—From Open Field to Modern Hydroponics: A Case Study of Specialized Metabolites. Plants. 11 (4): 483.

Radman S. (2015). Utjecaj gnojidbe dušikom i načina uzgoja na kemijska svojstva dvodomne koprive (*Urtica dioica* L.). Agronomski fakultet u Zagrebu. Doktorski rad.

Radman S., Fabek S., Žutić I., Benko B., Toth N. (2014). Stinging netlle cultiovation in floating hydropon. Conteporary Agriculture: The Serbian Journal of Agricultural Sciences. 63 (3): 215-223.

Rutto L. K., Ansari M. S., Brandt M. (2012). Biomass yield and dry matter partitioning in greenhouse-grown stinging nettle under different fertilization regimes. HortTecnology. 22 (6): 751-756.

Sabouri A., Hassanpour Y. (2015). Predication of leaf area, fresh ad dry weight in stinging nettle (*Urtica dioica*) by linear regression models. Medicinal and aromatic plants. 4 (2):1-6.

SAS®/STAT 14.3. (2017). SAS Institute Inc., Cary, NC, USA

Taylor K. (2009). Biological flora od British Isles: *Urtica dioica* L. Journal of Ecology. 97 (256): 1436-1458.

Toth N., Fabek S., Benko B., Žutić I., Stubljar S., Zeher S. (2012). Učinak abiotskih čimbenika, gutoće sjetve i višekratne berbe na prinos rige u plutajućem hidroponu. Glasnik zaštite bilja. 35 (5): 24-34.

Morphological characteristics of stinging nettle (*Urtica dioica* L.) in floating hydropon

Abstract
Hydroponic cultivation of nettle in greenhouses allows the availability of plant material for a longer period of the year with higher yields and better quality of the herb compared to outdoor cultivation. The aim of this study was to determine the yield components and yield of fresh nettle herb grown in floating hydroponics in two nutrient solutions with different compositions during two harvests. A solution with a lower amount of nutrients (Jensen, EC = 1.7 ms cm^{-1}) had a better effect on the observed morphological characteristics than a solution with a higher amount of nutrients (Cooper, EC = 3.0 mS cm^{-1}). The combination of each solution and the 2nd harvest produced a significantly higher nettle yield compared to the 1st harvest (2.3 kg m^{-2} and 1.8 kg m^{-2}, respectively).

Key words: hydroponic cultivation, nutrient solution, yield, morphology

PRETHODNO PRIOPĆENJE

Sadržaj vitamina C u različitim vrstama krstašica (Brassicaceae) tijekom skladištenja

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Sažetak

Povrtne vrste iz porodice krstašica značajno su zastupljene u svakodnevnoj prehrani, a prije svega bogat su izvor nutrijenata važnih za ljudsko zdravlje, poput vitamina C. Cilj ovog istraživanja bio je utvrditi sadržaj vitamina C kao i utjecaj skladišenja na sadržaj istog u različitim vrstama iz porodice Brassicaceae. Prema rezultatima, najviši sadržaj vitamina C utvrđen je za kelj (60,83 mg 100 g^{-1} sv.t.), dok najniži u kupusu (18,49 mg 100 g^{-1} sv.t.). Skladištenje pri 8 °C u trajanju od 7 dana utjecalo je na smanjenje sadržaja vitamina C u svim istraživanim vrstama uz izuzetak kupusa kod kojeg nije utvrđen trend smanjenja istog. Analizirane vrste krstašica bogat su izvor vitamina C te pokazuju dobar skladišni kapacitet.

Ključne riječi: kineski kupus, brokula, cvjetača, kelj, hlađeni prostor

Uvod

Varijacija u sadržaju vitamina C među vrstama iz porodice Brassicaceae pripisuje se njihovoj karakterističnoj genetskoj pozadini. Naime, porodica Brassicaceae obuhvaća više od 300 rodova, a od kojih je najvažniji rod *Brassica*, s oko 40 vrsta, među koje ubrajamo i različite lisnate i korjenaste povrtne vrste značajno zastupljene u svakodnevnoj prehrani (Lešić i sur., 2004.). Ovisno o preferencijama potrošača za konzumacijom ovih vrsta, povrtne vrste roda *Brassica* mogu osigurati čak 50 % dnevnog preporučenog unosa vitamina C (Domínguez-Perles i sur., 2014.; Sanlier i Guler Saban, 2018.). S obzirom kako su vrste ove porodice najčešće integrirane u navikama tržišta i prehrani ljudi, nameće se potreba istraživanja i uzgoja sortimenata najboljih varijacija koji povezuju genetski faktor odgovoran za sintezu i akumulaciju pojednih fitokemikalija poput vitamina C. Tako je omogućen uzgoj sortimenata visoke nutritivne vrijednosti, visokog sadržaja biaoktivnih spojeva, ali i značajne održivosti (Domínguez-Perles i sur., 2014.). S obzirom na specifičnosti vitamina C kao nutrijenta, odnosno njegovoj topljivosti u vodi, nestabilnosti u prirodnoj formi i iznimnoj osjetljivosti na okolišne uvjete poput povišene temperature, utjecaja svjetlosti, enzima, teških metala i alkalnog medija, očuvanje ovog vitamina u svježoj namirnici predstavlja izazov i iziskuje optimizaciju ključnih čimbenika, od temperature skladištenja, vlage zraka, izloženosti utjecaju kisika, svjetlosti i drugom (Šic Žlabur i sur., 2016). Također, i brojna istraživanja dokazuju kako krstašice namijene za konzumaciju u svježem stanju sadrže značajno više vitamina C od procesiranih ili uskladištenih namirnica (Raiola i sur., 2017). Gubitak vitamina C kod krstašica započinje već u početnoj fazi pripreme za tržište, a što prije svega uključuje podrezivanje i rezanje biljnih organa. Intenzivno smanjenje vitamina C koje nastaje kao posljedica tih procesa nažalost doprinosi slabijoj nutritivnoj i zdravstvenoj vrijednosti sirovine namijenjene za tržište (Domínguez-Perles i sur., 2014).

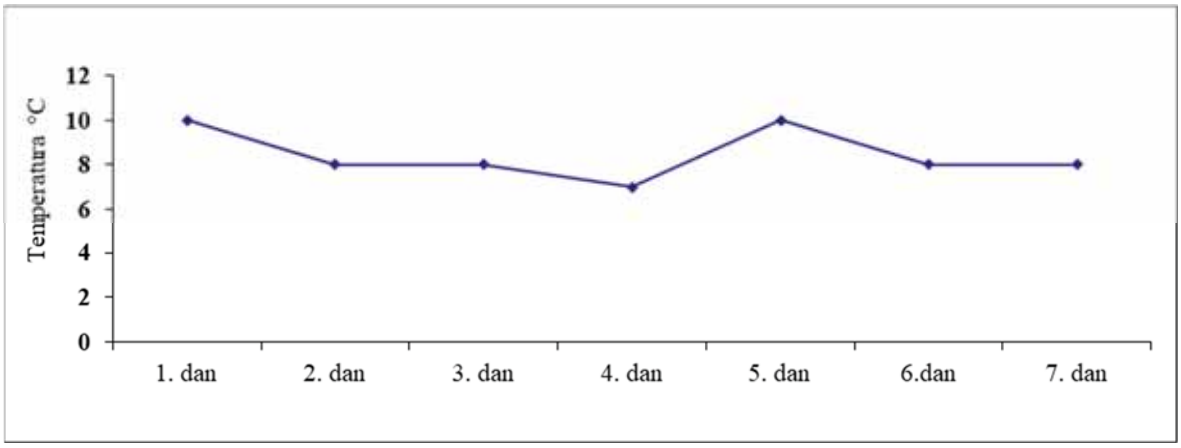
Razlike između kratkog i dugog vremenskog razdoblja kao i temperatura skladištenja ključni su za sadržaj vitamina C. Među krstašicama, ovisno o dijelu biljke, kratko vrijeme skladištenja pri temperaturama ispod 8 °C ne utječe značajnije na gubitak vitamina C, dok dugotrajnija skladištenja značajno smanjuju sadržaj istog (Domínguez-Perles i sur., 2014), a

što dokazuje kako osim temperature i vrijeme čuvanja predstavlja značajan čimbenik u održavanju kvalitete gotovog proizvoda.
Temeljem navedenog, cilj ovog istraživanja bio je utvrditi razlike u sadržaju vitamina C između različitih vrsta iz porodice Brassicaceae kao i utvrditi sadržaj vitamina C u istim vrstama nakon perioda skladištenja.

Materijal i metode

U pokusu su analizirane sljedeće vrste roda *Brassica*: kupus (*Brassica oleracea* var. *capitata*), kineski kupus (*Brassica rapa* var. *pekinensis*), brokula (*Brassica oleracea* var. *italica*), cvjetača (*Brassica oleracea* var. *botrytis*) i kelj (*Brassica oleraceae* var. *sabauda*). Sve navedene kulture nabavljene su istog dana u trgovačkom centru te odmah dopremljeni do laboratorija za analizu. S obzirom kako su uzorci prikupljeni s tržišta, dodatni podaci o vremenu berbe, agrotehničkim mjerama i ostali nisu dostupni. Za svaku kulturu s deklaracije proizvoda očitani su podaci o sorti i klasi. Zeleni kupus sorte 'Agressor' klasificiran je u II. klasi, kineski kupus sorte 'Enduro F1' klasificiran je u II. klasi, brokula sorte 'Naxos' klasificirana je u I. klasi, cvjetača sorte 'Meridot' klasificirana je u II. klasi, dok kelj sorte 'Famos' klasificiran je u II. klasi.

Svježi uzorci kupusa, kineskog kupusa, brokule, cvjetače i kelja odmah po kupovini dostavljeni su u laboratorij Zavoda za poljoprivrednu tehnologiju, skladištenje i transport, Sveučilišta u Zagrebu Agronomskog fakulteta gdje su odmah sortirani te odvojeni oni s eventulanim mehaničkim oštećenjima ili znakovima kvarenja. Samo zdrava sirovina u optimalnoj fazi zrelosti korištena je u daljenjem dijelu pokusa. Od svake vrste nabavljeno je ukupno po pet glavica kupusa, kineskog kupusa i kelja, odnosno kod brokule i cvjetače po pet cvata, a od kojih je za potrebe analize vitamina C u vježem stanju napravljen uniforman uzorak od ukupno tri glavice po svakoj sorti. Nakon analize svježih uzoraka krstašica, provedeno je skladištenje ostalih uzoraka (po dvije glavice kupusa, kineskog kupusa i kelja i dva cvata brokule i cvjetače) u hladnjaku tijekom 7 dana. Svakodnevno je u hladnjaku očitavana temperatura (Grafikon 1), prema čemu je prosječna temperatura skladištenja uzoraka u periodu od 7 dana iznosila 8 °C (prosječna vlažnost zraka hlađenog prostora iznosila je 74 %).



Grafikon 1. Temperatura (°C) u hlađenom prostoru tijekom 7 dana čuvanja uzoraka krstašica

Sadržaj vitamina C određen je titracijski s 2,6- diklorindolfenolom prema standardnoj laboratorijskoj metodi (AOAC, 2002.). Odvagano je 10±0,01 g svježeg uzorka i homogenizirano sa 100 mL oksalne kiseline (2% v/v) u odmjernoj tikvici od 100 mL. Takav uzorak ostavljen je stajati oko jedan sat nakon čega je preko Whatmanovog filter papira

sadržaj profiltiran. Filtrat u ukupnom volumenu od 10 mL korišten je za titraciju s otopinom 2,6- diklorindolfenola do pojave ružičastog obojenja. Iz volumena 2,6-diklorindolfenola utrošenog za titraciju, prema formuli 1 izračunat je sadržaj L-askorbinske kiseline (vitamina C) izražen u mg 100 g^{-1} svježe tvari.

$$vitamin\ C = \frac{V \times F}{D} \times 100 \quad (1)$$

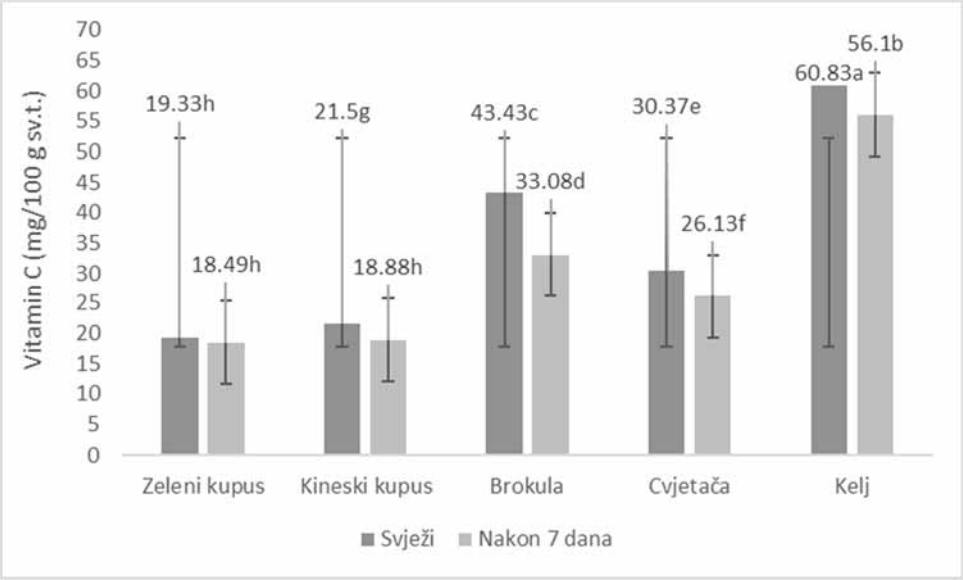
Gdje je: V - mL utrošenog 2,6-diklorindolfenola pri titraciji; F - faktor otopine 2,6-diklorindofenola; D - masa uzorka u filtratu u gramima.

Za statističku analizu rezultata korišten je generalizirani linearni model, s uključenim ponavljanjem, vrstom i skladištenjem kao kategorijskim prediktorima. Za analizu je korištena procedura PROC GLM iz SAS programskog paketa, verzija 9.4. (2010). Dobiveni podaci obrađeni su analizom varijance, dok su razlike između srednjih vrijednosti testirane LSD testom, pri čemu je P= 1% smatrana statističkim pragom značajnosti.

Rezultati i rasprava

Rezultati sadržaja vitamina C odabranih vrsta krstašica (kupus, kineski kupus, brokula, cvjetača i kelj) u svježem stanju te nakon čuvanja u periodu od 7 dana prikazani su u Grafikonu 2. Dobiveni rezultati ukazuju na signifikantnu razliku sadržaja vitamina C unutar istraživanih vrsta u svježem stanju i tijekom skladištenja. Od istraživanih vrsta u svježem stanju, najviši sadržaj vitamina C (60,83 mg/100 g sv.t.) utvrđen je za kelj, zatim redom slijede brokula (43,43 mg 100 g^{-1} sv.t.), cvjetača (26,13 mg/100 g sv.t.), kineski kupus (21,50 mg 100 g^{-1} sv.t.) i kupus (18,49 mg 100 g^{-1} sv.t.). Zabilježen trend sadržaja vitamina C u svježem uzorcima istraživanih vrsta krstašica u sklopu ovog istraživanja poklapa se i s ostalim literaturnim navodima, a prema kojima je također u kelju zabilježen najviši sadržaj vitamina C (prosječne vrijednosti oko 120 mg 100 g^{-1} sv.t.), zatim nešto niži u brokuli (prosječne vrijednosti oko 93 mg 100 g^{-1} sv.t.), cvjetači (prosječne vrijednosti oko 48 mg 100 g^{-1} sv.t.), te zelenom i kineskom kupusu najniže vrijednosti i to prosječno redom oko 31 i 27 mg 100 g^{-1} sv.t (Domínguez-Perles i sur., 2014.; Raiola i sur., 2017.; Sanlier i Guler Saban, 2018.). U ovom istraživanju su općenito zabilježene nešto niže vrijednosti vitamina C analiziranih svježih uzoraka u odnosu na druga istraživanja, a što se može objasniti nizom čimbenika koji mogu značajno utjecati na devijaciju sadržaja vitamina C, od genetskih karakteristika (velik broj varijeteta, podvrsta i sorti), fizioloških čimbenika (dospjelost, dio biljke za konzumaciju, vrijeme ubiranja) te ekoloških (klimatske prilike) i agrotehničkih (mjere tijekom uzgoja) čimbenika (Domínguez-Perles i sur., 2014). Također, osim spomenutih uvjeta prije berbe, na sadržaj vitamina C značajan utjecaj imaju i uvjeti nakon berbe i to prije svega skladištenje (utjecaj temperature i perioda čuvanja), zatim upotreba različitih fizikalnih predtretmana prije skladištenja, čuvanje pri niskim temperaturama odnosno smrzavanje kao i metode pakiranja u modificiranoj amtosferi. Adekvatna primjena svih spomenutih ključnih čimbenika u očuvanju kvalitete proizvoda nakon berbe, omogućit će očuvanje nutritivnih svojstava proizvoda namijenjenog za konzumaciju, a time posljedično utjecati na smanjenu degradaciju vrijednih nutrijenata poput vitamina C. U tom smislu, vitamin C smatra se i jednim od bioindikatora adekvatnih postupaka rukovanja i obrade sirovine upravo zbog svoje osjetljivosti na razgradnju (lako oksidira enzimskim i neenzimskim putevima). Temperatura i vrijeme tijekom perioda čuvanja svježih sirovina ključni su faktori za održavanje razine sadržaja vitamina C, a prilikom čega temperaturni režimi hlađenja, od 4 do 8 °C, osiguravaju smanjenu degradaciju, dok više temperature značajno utječu na smanjenje sadržaja. Temeljem dobivenih rezultata sadržaja vitamina C u

analiziranim krstašicama tijekom skladištenja (Grafikon 1), evidetno je kako je čuvanje plodova u periodu od 7 dana utjecalo na smanjenje sadržaja vitamina C, uz izuzetak kupusa kod kojeg nije utvrđena značajna statistička razlika u sadržaju vitamina C između sirovine u svježem stanju i nakon čuvanja u hlađenom prostoru. Najveće smanjenje sadržaja vitamina C tijekom perioda čuvanja zabilježeno je za brokulu i to čak 32 % niži sadržaj vitamina C nakon 7 dana čuvanja, dok najmanje smanjenje vitamina C u kelju, samo 8 % niži sadržaj nakon perioda čuvanja.



Grafikon 2. Sadržaj vitamina C (mg 100 g^{-1} sv.t.) u svježim i skladištenim uzorcima analiziranih vrsta krstašica

Prema rezultatima signifikatnosti utjecaja pojedinačnih faktora (vrste i skladištenja) te njihovoj interakciji (V × S) (Tablica 1) može se zaključiti kako svaki od istraživanih faktora značajno utječe ($p \leq 0{,}0001$) na sadržaj vitamina C, odnosno da sadržaj vitamina C u istraživanim krstašicama značajno ovisi i o genetskim karakteristikama, kao i o periodu čuvanja u hlađenom prostoru.

Tablica 1. Signifikantnost utjecaja pojedinačnih faktora i njihova interakcija na sadržaj vitamina C u različitim krstašicama

Utjecaj faktora	Vitamin C
Vrsta (V)	0,0001
Skladištenje (S)	0,0001
V × S	0,0001

V × S – interakcija faktora vrsta (V) i skladištene (S)

Zaključak

Istraživanjem je utvrđeno kako kelj i brokula imaju viši sadržaj vitamina C u usporedbi sa cvjetačom, kineskim kupusom i kupusom. Skladištenje pri prosječnoj temperaturi od 8 °C u periodu od 7 dana značajno je utjecalo na sadržaj vitamina C. Kod svih analiziranih vrsta utvrđeno je smanjanje sadržaja, uz izuzetak kupusa kod kojeg se sadržaj vitamina C nije

mijenjao tijekom čuvanja, a temeljem čega se može istaknuti kako u tom smislu kupus pokazuje najbolju održivost. Skladišni kapacitet svih istraživanih kultura relativno je visok s obzirom kako degradacija vitamina C nije bila tako izražena, uz izuzetak brokule kod koje je uočen najveći pad sadržaja tijekom čuvanja. Također, valja naglasiti kako su rezultati ovog istraživanja dokazali kako su sve istraživane kulture iz porodice krstašica bogat izvor vitamina C te kako ih se može smatrati vrijednim izvorom ovog nutrijenta.

Literatura

AOAC (2002). Official Methods of Analysis 2002-02. Association of Official Analytical Chemist, Washington DC, USA.

Domínguez-Perles R., Mena P., García-Viguera C., Moreno D.A.. (2014). Brassica Foods as a Dietary Source of Vitamin C: A Review. Critical Reviews in Food Science and Nutrition. 54(8): 1076-1091.

Lešić R., Borošić J., Butorac I., Herak-Ćustić M., Poljak M., Romić D. (2004). Povrćarstvo, 2. izdanje, ZRINSKI, 169 – 228.

Sanlier N, Guler Saban, M. (2018). The Benefits of Brassica Vegetables on Human Health. Journal of Human Health Research 1(1): 1-13.

SAS Institute. SAS®/STAT 9.4; SAS Institute Inc: Cary, NC, SAD, 2011.

Šic Žlabur J., Voća S., Dobričević N. (2016). Kvaliteta voća, povrća i prerađevina - priručnik za vježbe. Sveučilište u Zagrebu Agronomski fakultet, Zagreb. pp: 78-80.

Raiola A., Errico A., Petruk G., Monti D.M., Barone A., Rigano M.M. (2017). Bioactive Compounds in Brassicaceae Vegetables with a Role in the Prevention of Chronic Diseases. Molecules. 23(1): 1-10.

Vitamin C content in different species of the Brassicaceae family during storage

Abstract

Vegetable species belonging to the Brassicaceae family are an important part of the daily diet and, above all, a rich source of nutrients important for human health, such as vitamin C. The aim of this study was to determine the vitamin C content and the effect of storage on the content in different Brassicaceae species. According to the results, the highest vitamin C content was determined in kale (60.83 mg 100 g^{-1} fw), while the lowest content in cabbage (18.49 mg 100 g^{-1} fw). Storage at 8 °C for 7 days had an effect on the reduction of vitamin C content in all species studied, except cabbage, which showed no decreasing trend. The studied varieties are a rich source of vitamin C and have a satisfactory shelf life.

Key words: Chinese cabbage, broccoli, cauliflower, kale, cooled space

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Ratarstvo

05

Field Crop Production

ORIGINAL SCIENTIFIC PAPER

Stability valuation of some antigraminaceous and combined herbicides for grain yields of durum wheat (*Triticum durum* Desf.)

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Abstract

The research was conducted during 2018 - 2020 on pellic vertisol soil type with Bulgarian durum wheat (cultivar Predel). Factor A included the years of investigation and factor B included 18 variants: hand weeded control, 4 antigraminaceous herbicides (Imaspro 7.5 EB, Sword 240 EC, Traxos 50 EC, Axial 050 EC) and 13 combined herbicides (Axial one, Zerrate, Palace 75 WG, Corello duo 85 WG, Hussar max OD, Pacifica expert, Atlantis flex 20.25 WG, Tolurex 500 SC, Constell, Battle delta, Eagle 75 WG, Prol aqua and Krum). All of antigraminaceous herbicides and foliar-applied combined herbicides were treated during tillering stage of the durum wheat. Soil-applied combined herbicides were treated during preemergence period of the durum wheat. The herbicides Tolurex and Constell have phytotoxicity on durum wheat by soil treatment during preemergence period. All foliar-applied antigraminaceous herbicides (Imaspro, Sword, Traxos, Axial), foliar-applied combined herbicides (Axial one, Zerrate, Palace, Corello duo, Hussar max, Pacifica expert, Atlantis flex) and some soil-applied combined herbicides (Battle delta, Eagle, Prol aqua and Krum) have high selectivity on durum wheat. The herbicides Tolurex and Constell are the most unstable for grain yield, followed by herbicides Sword, Traxos and Zerrate. The herbicides Eagle, Atlantis flex, Axial, Palace, Hussar max, Axial one, Corello duo, Pacifica expert and Prol aqua are technological the most valuable. They combine high grain yield with high stability with relation to different years.

Key words: durum wheat, antigraminaceous and combined herbicides, grain yield, selectivity, stability

Introduction

The herbicides have specific effect on the physiological and biochemical characteristics on the plants (Liu et al, 1994). They affected on the growth and the development to the weeds from the different biological groups, but also on the cultural plants (Orr, 1996; Delchev, 2018, 2018a, 2021). The weed associations are not inactive; they are changed by influence the climatic conditions, used agrotechnical management and crop rotations (Hartmann et al., 2000; Labrada, 2000; Mennan and Zandstra, 2005, Delchev, 2020). Weeds have acquired mechanisms to adapt to adverse conditions in its evolutionary. Therefore they are particularly harmful in drought due to their high ecological plasticity and adaptability compared to cultivated plants, and this makes them more competitive. Based on the above, it is necessary to analyze new herbicides constantly in laboratory and open field conditions.

The specific sensitivity of the durum wheat (*Triticum durum* Desf.) to some of the herbicides used at the common wheat (*Triticum aestivum* L.) necessitates studies on the efficacy and selectivity of new chemicals to control weeds in durum wheat crops.

The purpose of the investigation was to establish the selectivity and stability of some antigraminaceous and combined herbicides on the durum wheat under different meteorological conditions.

Material and methods

The research was conducted during 2018 - 2020 on pellic vertisol soil type with Bulgarian durum wheat cultivar Predel (*Triticum durum* Desf.). A field experiment was conducted under the block method in 4 repetitions. Basic size of the crop plot was 15 m^2. Factor A included the years of investigation and factor B included 18 variants: hand weeded control, 4 antigraminaceous herbicides – Imaspro 7.5 EB (1 l ha^{-1}), Sword 240 EC (250 ml ha^{-1}), Traxos 50 EC (1.20 l ha^{-1}), Axial 050 EC (900 ml ha^{-1}) and 13 combined herbicides - Axial one (1 l ha^{-1}), Zerrate (250 g ha^{-1}), Palace 75 WG (250 g ha^{-1}), Corello duo 85 WG (250 g ha^{-1}), Hussar max OD (1 l ha^{-1}), Pacifica expert (500 g ha^{-1}), Atlantis flex 20.25 WG (330 g ha^{-1}), Tolurex 500 SC (4 l ha^{-1}), Constell (4.5 l ha^{-1}), Battle delta (600 ml ha^{-1}), Eagle 75 WG (20 g ha^{-1}), Prol aqua (l ha^{-1}), Krum (5 l ha^{-1}). Herbicide Zerrate was used in addition with adjuvant Adigor (1.5 l ha^{-1}), herbicides Palace 75 WG and Corello duo 85 WG – with adjuvant Dasoil 26-2N (500 ml ha^{-1}), and herbicides Pacifica expert and Atlantis flex 20.25 WG – with adjuvant Biopower (1 l ha^{-1}). To study the effects of herbicides only and to eliminate the negative effects of weeds, they were removed by manual weeding throughout the growing season. The selectivity of herbicides has been established through their influence on grain yield. The math processing of the data was done according to the method of analyses of variance (Shanin 1977; Barov, 1982; Lidanski 1988). The stability of herbicides and herbicide combinations for grain yield with relation to years was estimated using the stability variances σ_i^2 and S_i^2 of Shukla (1972), the ecovalence W_i of Wricke (1962) and the stability criterion YS_i of Kang (1993).

Results and discussion

The results for the obtained grain yields (Table 1) show that the greatest reduction in grain yield relative to the hand weeded control was registered by use of herbicide Tolurex on soil application during preemergence period - with 280 kg ha^{-1} or 5.3 % average for the period. Soil application of the herbicide Constell during the preemergence period also has a negative effect on grain yield. The yield reduction was 252 kg ha^{-1} or 4.8 % on average over the period.

Reducing the percentage of active substance chlorotoluron in 1 kg of product and adding to it the active substance diflufenican in herbicide Constell increases the selectivity of the herbicide to grain yield compared in herbicide Tolurex containing chlorotoluron only.

High selectivity to durum wheat of the other herbicides included in the investigation was found: the antigraminaceous herbicides (Imaspro, Sword, Traxos and Axial), the combined herbicides Axial one, Zerrate, Palace, Corello duo, Hussar max, Pacifica expert, Atlantis flex), treated during tillering stage of the durum wheat and Battle delta, Eagle, Prol aqua, Krum, applied during preemergence period. With these herbicides grain yields do not prove differ from those in hand weeded control. Practically equal to the yields of the hand weeded control are the yields obtained by soil application of herbicide Eagle.

A two-factor analysis of variance for grain yield (Table 2) shows that the investigated antigraminaceous and combined herbicides have a proven influence on this index (96.2 %). The years also have high influence on grain yield (92.0 %) on the variants, while the strength of influence of herbicides is lower (only 2.6 %). The influence of variants as a whole and of the meteorological conditions of years was proven at $p \leq 0.1\%$, and that of herbicides was proven at $p \leq 1\%$. Also there is an interaction between years x herbicides (A x B), proven at $p \leq 5$ %. This means that the meteorological conditions during the vegetation period of the three years of the experiment influenced on the degree of herbicides selectivity in regard to the investigated durum wheat cultivar.

Table 1. Influence of some antigraminaceous and combined herbicides on grain yield of durum wheat (mean 2018 - 2020)

Herbicides	2018		2019		2020		Mean (Factor B)	
	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%
Control – hand weeded	5354	100	5286	100	5100	100	5247	100
Imaspro	5240	97.9	5036	95.3	5067	99.3	5114	97.4
Sword	5138	96.0	5161	97.6	5067	99.3	5122	97.6
Traxos	5240	97.9	5127	97.0	5049	99.0	5139	97.9
Axial	5331	99.6	5061	95.7	5033	98.5	5142	98.0
Axial one	5300	99.0	5022	95.0	4998	98.0	5107	97.3
Zerrate	5290	98.8	5075	96.0	4988	97.8	5118	97.5
Palace	5311	99.2	5085	96.2	5033	98.5	5143	98.0
Corello duo	5300	99.0	5085	96.2	4998	98.0	5128	97.7
Hussar max	5190	97.0	5161	97.6	5117	100.4	5156	98.2
Pacifica expert	5183	96.8	5127	97.0	5090	99.8	5133	97.8
Atlantis flex	5252	98.1	5170	97.8	5100	100	5174	98.6
Tolurex	5129	95.8	4937	93.4	4835	94.8	4967	94.7
Constell	5140	96.0	4995	94.5	4850	95.1	4995	95.2
Battle delta	5161	96.4	5127	97.0	4942	96.9	5077	96.8
Eagle	5194	97.1	5311	100.5	5133	100.7	5213	99.3
Prol aqua	5194	97.1	5261	99.5	4967	97.0	5141	97.9
Krum	5183	96.8	5211	98.6	4867	94.8	5087	97.0
Mean (Factor A)	**5229**	**-**	**5125**	**-**	**5013**	**-**	**-**	

LSD:

A	p≤5%=61	p≤1%=81	p≤0.1%=104
B	p≤5%=180	p≤1%=238	p≤0.1%=307
A x B	p≤5%=211	p≤1%=311	p≤0.1%=430

Table 2. Analysis of variance for durum wheat grain yield

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean squares	Fisher's criterion	Level of significance
Total	161	2592494	100	-	-	-
Tract of soil	2	24549	1.7	19381.3	7.3	**
Variants	53	2496734	96.2	45219.5	23.0	***
Factor A – Years	2	2434466	92.0	107239.3	26.0	***
Factor B – Herbicides	17	32385	2.6	1616.8	0.5	**
A x B	34	14897	1.6	256.3	0.3	*
Pooled error	106	47222	2.1	282.4	-	-

*p≤5% **p≤1% ***p≤0.1%

This is observed in regard to herbicides containing chlorotoluron (Tolurex and Constell), as well as in regard to herbicides containing clodinafop-propargyl (Sword, Traxos and Zerrate). Their selectivity varies with the meteorological conditions over the years. The highest negative effect on yield had herbicide Tolurex. In herbicide Constell the phytotoxicity are poorly expressed, resulting in a smaller decrease in grain yield.

Based on year x herbicide interaction, it was evaluated stability parameters for each antigraminaceous and combined herbicide for grain yield of durum wheat with relation to years (Table 3).

Stability variances (σ_i^2 and S_i^2) of Shukla, which recorded respectively linear and nonlinear interactions, unidirectional evaluate the stability of the variants. These variants which showed lower values are considered to be more stable because they interact less with the environmental conditions. Negative values of the indicators σ_i^2 and S_i^2 are considered 0.

Table 3. Stability parameters of some antigraminaceous and combined herbicides for grain yield with relation to years

Herbicides	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Control – hand weeded	5247	223.9	25.7	422.4	23+
Imaspro	5114	141.6	222.0	270.1	12+
Sword	5122	333.9*	263.8	441.1	13+
Traxos	5139	329.1*	244.7	358.3	14+
Axial	5142	320.4	239.0	231.0	18+
Axial one	5107	333.3	254.1	345.6	17+
Zerrate	5118	318.2*	245.6	349.8	14+
Palace	5143	265.4	102.5	654.3	18+
Corello duo	5128	288.6	111.1	687.2	17+
Hussar max	5156	271.3	104.6	666.6	18+
Pacifica expert	5133	300.63	108.7	678.9	17+
Atlantis flex	5174	215.1	100.1	555.5	19+
Tolurex	4967	1497.5***	607.0**	3537.3	-7
Constell	4995	545.9*	405.2*	1009.1	5
Battle delta	5077	123.4	78.9	345.6	8
Eagle	5213	27.9	-28.4	59.6	21+
Prol aqua	5141	44.7	-26.0	90.5	17+
Krum	5087	119.6	66.6	229.4	9
Mean	**5123**				**14.1**
LSD (p=0.05)	211				

Using the first three parameters of stability (σ_i^2, S_i^2 and W_i), it is found that the most unstable are herbicide Tolurex, followed by herbicide Constell. In these variants values of stability variance σ_i^2 and S_i^2 of Shukla and ecovalence W_i of Wricke are the highest and mathematically proven. At these two herbicides there is except instability from linear type - proven values of σ_i^2, there is also instability from nonlinear type - proven values of S_i^2. The reason for this high instability is greater variation in grain yields during years of experience as weather conditions affect those most. While the decrease in grain yield by Constell has not been proven during some years, the decrease in grain yield by Tolurex has always been proven. At herbicides Sword, Traxos and Zerrate there is instability from linear type - proven values σ_i^2. The values of S_i^2 are not proven. Foliar-applied antigraminaceous herbicides Imaspro and Axial, foliar-applied combined herbicides Axial one, Palace, Corello duo, Hussar max, Pacifica expert, Atlantis flex as well as soil-applied combined herbicides Battle delta, Eagle, Prol aqua and Krum exhibit high stability for durum wheat grain yield because they interact poorly with the conditions of years.

To evaluate the complete efficacy of each antigraminaceous and combined herbicide should be considered as its effect on grain yield of durum wheat and its stability - the reaction of wheat to this variant during the years. Valuable information about the value of technologic value of the variant give the stability criterion YS_i of Kang for simultaneous assessment of yield and stability, based on the reliability of the differences in yield and variance of interaction with the environment. The value of this criterion is experienced that using nonparametric methods and warranted statistical differences we get a summary assessment aligning variants in descending order according to their economic value.

Generalized stability criterion YS_i of Kang, taking into accounts both the stability and value of yields gives a negative assessment of herbicide Tolurex, characterizing it as the most unstable and low yields. It combines lower levels of grain yield and higher instability of this index during some years. According to this criterion, technologically the most valuable are herbicides Eagle, Atlantis flex, Axial, Hussar max, Axial one, Corello duo, Pacifica expert and Prol aqua. These herbicides combine high levels of grain yield - equal or close to hand weeded control - and high stability of this index during the years. Herbicides Constell, Battle

delta and Krum get lower ratings. Constell combines low grain yield and high instability of this index during some years. Battle delta and Krum combine low grain yield and high stability of this index during the years.

Conclusions

The herbicides Tolurex and Constell have phytotoxicity on durum wheat by soil treatment during preemergence period. All foliar-applied antigraminaceous herbicides (Imaspro, Sword, Traxos, Axial), foliar-applied combined herbicides (Axial one, Zerrate, Palace, Corello duo, Hussar max, Pacifica expert, Atlantis flex) and some soil-applied combined herbicides (Battle delta, Eagle, Prol aqua and Krum) have high selectivity on durum wheat. The herbicides Tolurex and Constell are the most unstable for grain yield, followed by herbicides Sword, Traxos and Zerrate. The herbicides Eagle, Atlantis flex, Axial, Palace, Hussar max, Axial one, Corello duo, Pacifica expert and Prol aqua are technological the most valuable. They combine high grain yield with high stability with relation to different years.

References

Barov V. (1982). Analysis and schemes of the field experience. NAPO, Sofia, pp. 668.

Delchev G. (2018). Late use of herbicides in durum wheat crop (*Triticum durum* Desf.). Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 141.

Delchev G. (2018a). Mixability of herbicides with growth regulators and foliar fertilizers. Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 329.

Delchev G. (2020). Winter resistance of oilseed canola and reseeding with spring crops. Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 129.

Delchev G. (2021). Efficacy and selectivity of some herbicides in five field crops. Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp 225.

Hartmann F., Pal B., Dellei A., Toth A. (2000). Atrazine resistant biotype of *Senecio vulgaris* in Hungary. Novenyvedelem, 36 (10): 529-532.

Kang M. (1993). Simultaneous selection for yield and stability: Consequences for growers. Agronomy Journal, 85: 754-757.

Labrada R. (2000). Development of resistance to herbicides in various countries. Informatore Fitopatologie, 50 (7/8): 35-38.

Lidanski T. (1988). Statistical methods in biology and agriculture, Sofia, pp. 376.

Liu S., Hsiao A., Quick W. (1994). Interaction between imazamethabenz and feroxapropethyl in wild oat control and crop tolerance. Crop Protection, 13 (7): 525-530.

Mennan H., Zandstra B. (2005). Influence of wheat seedling rate and cultivars on competitive ability of *Bifora radians*. Weed Technology, 19 (1): 128-136.

Orr P. (1996). Post emergence herbicides and application time affect wheat wield. California Agriculture, 50 (4): 32-36.

Shanin Yo. (1977). Methodology of the field experience. BAS, pp. 384

Shukla G. (1972). Some statistical aspects of partitioning genotype - environmental components of variability. Heredity, 29: 237-245.

Wricke G. (1962). Über eine Methode zur Erfassung der ökologischen Streikbreiten Feldersuchen. Pflanzen zu Recht, 47: 92-96.

ORIGINAL SCIENTIFIC PAPER

Productivity of lentil (*Lens culinaris* Medik.) influenced by some herbicides, herbicide combinations and herbicide tank mixtures

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Abstract

The research was conducted during 2019 – 2021 in order to investigate changes in the seed yield and structural elements of yield of lentil (*Lens culinaris* Medik.) influenced by some herbicides, herbicide combinations and herbicide tank mixtures. Research included 23 variants: untreated control, 8 herbicides – Pendistar 40 SC, Dual gold 960 EC, Lentagran VP, Challenge 600 SC, Zencor 600 SC, Wish top, Zetrola and Passat 40, as well as combinations and tank mixtures between them. Soil-applied herbicides were applied during the period after sowing before emergence. Foliar-applied herbicides were applied during 2-3, 4-5 or 6-7 real leaf stage of the lentil. The highest yields of lentil seeds were obtained using herbicide combinations Pendistar 5 l ha^{-1} + Lentagran 500 + 500 ml ha^{-1} and Pendistar 5 l ha^{-1} + Lentagran 1 l ha^{-1}. High yields were also obtained when combining Challenge 3 + 1 l ha^{-1} with Wish top 1.25 l ha^{-1}, as well as with the herbicide combination Challenge 4 l ha^{-1} + Wish top 1.25 l ha^{-1}. Increase in seed yield was due to the greatest degree of increase in indexes beans number per plant, seeds number per bean and 1000 seeds weight. First beans height was the highest with use of combinations of soil-applied herbicide Pendistar with foliar-applied herbicide Lentagran in doses of 500 + 500 ml ha^{-1} and 1 l ha^{-1}, followed by combinations of herbicide Challenge in doses of 3 + 1 l ha^{-1} and 4 l ha^{-1} with the herbicide Wish top.

Key words: lentil, herbicides, herbicide combinations, seed yield, structural elements

Introduction

Weed control in lentil is important because the crop is poor competitor to weeds due to its slow initial development and limited vegetative growth (Sultan and Nasir, 2007; Lhungdim et al., 2013). Yield reductions can reach 80 %. The low competitiveness of lentil increases at low temperatures and insufficient moisture during the growing season (Tepe et al., 2004; Sharara et al., 2011). Practices that reduce weed infestation in lentil crops include tillage, cultivar selection, proper sowing, proper fertilization and irrigation (Elkoca et al., 2004; Mojeni et al., 2005; Chandrakar, 2011). Increasing the sowing density of lentil leads to reduced weed infestation and increased seed yield (Baird et al., 2009; Manjunath et al., 2010). However, these practices are not sufficient to efficiently control weeds in lentil fields (Malik et al., 2001; Chandrakar et al., 2016).

Crop rotation is one of the most powerful pest management tools. It is very important to know the history of crop rotation before growing lentil, as this crop is very sensitive to the many herbicides used in its predecessors. Sulfonylurea high-persistence herbicides commonly used in cereals such as chlorsulfuron or metsulfuron can damage subsequent annual legumes, including lentil. Due to their persistence, it may be necessary to wait up to four years before sowing lentil, depending on the herbicide, application dose and weather conditions (Phogat et al., 2003; Singh et al., 2009). Soil herbicide persistence depends on many factors, including soil pH, moisture, temperature, soil texture, and soil organic matter (Kumar et al., 2016; Delchev, 2018, 2021).

The purpose of this investigation was to establish the changes in the seed yield and structural elements of yield influenced by some herbicides, herbicide combinations and herbicide tank mixtures in lentil.

Material and methods

The research was conducted during 2019 - 2021 on pellic vertisol soil type. Under investigation was lentil cultivar Ilina (*Lens culinaris* Medik.). It was carried out as a field experiment under the block method, in 4 repetitions; the size of the crop plot was 15 m^2. Total 23 variants were investigated: untreated control, 8 herbicides – Pendistar 40 SC (pendimethalin), Dual gold 960 EC (S-metolachlor), Lentagran VP (pyridate), Challenge 600 SC (aclonifen), Zencor 600 SC (metribuzin), Wish top (quizalofop-P-ethyl), Zetrola (propaquizafop) and Passat 40 (imazamox), as well as combinations and tank mixtures between them.

Soil-applied herbicides were applied during the period after sowing before emergence. Foliar-applied herbicides were applied during 2-3, 4-5 or 6-7 real leaf stage of the lentil. All of the herbicides, herbicide combinations and herbicide tank-mixtures were applied in a working solution of 300 l ha^{-1}. Mixing of foliar-applied herbicides was done in the tank on the sprayer. Due to of low adhesion of the herbicide Passat 40 was used in addition with adjuvant Dash HC – 1 l ha^{-1}.

At lentil maturity all plots were evaluated for seed yield and yield components – beans number per plant, seeds number per bean and 1000 seeds weight and test weight, to evaluate the influence of the herbicides, herbicides combinations and herbicide tank mixtures on lentil seed yield and yield components. It was investigated changes who tested herbicides done on the plant height and first bean height. Analysis of variance was used to study the influence of herbicide treatments on lentil.

Results and discussion

The highest yields of lentil seeds on average for the period are obtained by herbicide combination Pendistar 5 l ha^{-1} + Lentagran 500 + 500 ml ha^{-1} - 129.5 % compared to weedy control, followed by herbicide combination Pendistar 5 l ha^{-1} + Lentagran 1 l ha^{-1} - 128.2 % compared to the control (Table 1). High yields were also obtained by combining Challenge 3 + 1 l ha^{-1} with Wish top 1.25 l ha^{-1} - 127.3 % compared to weedy control, as well as by herbicide combination Challenge 4 l ha^{-1} + Wish top 1.25 l ha^{-1} – 126.8 % compared to the control.

Combining of Challenge with antigraminaceous herbicide Zetrola led to lower seed yields than combining Challenge with antigraminaceous herbicide Wish top. This is due to the lower efficacy of Zetrola against some annual graminaceous weeds, as well as the higher phytotoxicity of these herbicide combinations. It is important to note that the combination of Zetrola with Challenge has initial phytotoxic effect on lentil, resulting in inhibition of plant growth for the first 15 - 20 days after foliar treatment. Subsequently, the lentil overcomes this negative effect and at the end of the growing season in these variants, high values of seed yield are reported.

Combining of foliar-applied herbicide Lentagran with soil-applied herbicide Dual gold led to lower seed yields than combining of Lentagran with soil-applied herbicide Pendistar.

The single use of herbicide Zencor at dose of 350 ml ha^{-1} during period after sowing before the emergence of the lentil resulted in low seed yields due to the low herbicide efficacy of Zencor at this minimum dose. The use of Zencor in doses of 600 ml ha-1 during period after sowing before the emergence + 300 ml ha^{-1} during 3-7 leaf stage reduced seed yield.

Table 1. Influence of different herbicides, herbicide combinations and herbicide tank mixtures on seed yield and structural elements of the yield at lentil (mean 2019 - 2021)

Variants	Seed yield, kg ha^{-1}	Beans per plant, number	Seeds per bean, number	1000 seeds weight, g	Test weight, kg	Plant height, cm	First bean height, cm
Control	1280	73.1	1.53	48.6	79.1	37.3	23.1
Pendistar 5 l ha^{-1}	1446	88.1	1.61	59.9	79.9	40.0	25.4
Dual gold 1.2 l ha^{-1}	1422	87.2	1.60	58.8	79.8	39.7	25.0
Lentagran 1+1 l ha^{-1}	1477	88.6	1.65	60.3	80.0	40.4	26.6
Lentagran 750+500+500 ml ha^{-1}	1492	88.8	1.66	60.4	80.0	40.5	26.7
Pendistar 5 l ha^{-1} + Lentagran 1 l ha^{-1}	1641	91.3	1.80	62.2	80.9	42.3	28.1
Dual gold 1.2 l ha^{-1} + Lentagran 1 l ha^{-1}	1551	90.0	1.72	61.1	80.4	41.4	27.5
Pendistar 5 l ha^{-1} + Lentagran 500+500 ml ha^{-1}	1658	91.8	1.82	62.4	80.9	42.4	28.2
Dual gold 1.2 l ha^{-1} + Lentagran 500+500 ml ha^{-1}	1563	90.1	1.74	61.3	80.5	41.5	27.6
Wish top 1.25 l ha^{-1}	1354	85.2	1.55	57.7	79.2	38.6	24.3
Zetrola 1.6 l ha^{-1}	1344	85.0	1.54	57.6	79.1	38.5	24.2
Challenge 4 l ha^{-1}	1501	89.0	1.67	60.5	80.1	40.6	26.8
Challenge 3+1 l ha^{-1}	1510	89.2	1.69	60.6	80.2	40.8	26.9
Challenge 4 l ha^{-1} + Wish top 1.25 l ha^{-1}	1623	91.0	1.77	61.9	80.8	42.0	28.0
Challenge 4 l ha^{-1} + Zetrola 1.6 l ha^{-1}	1596	90.5	1.75	61.7	80.7	41.8	27.9
Challenge 3+1 l ha^{-1} + Wish top 1.25 l ha^{-1}	1629	91.1	1.79	62.0	80.9	42.2	28.1
Challenge 3+1 l ha^{-1} + Zetrola 1.6 l ha^{-1}	1580	90.3	1.76	61.5	80.6	41.7	27.8
Zencor 900 ml ha^{-1}	1371	86.2	1.57	58.2	79.5	39.2	24.6
Zencor 600+300 ml ha^{-1}	1394	86.6	1.58	58.4	79.6	39.4	24.8
Zencor 350 ml ha^{-1}	1400	86.8	1.59	58.6	79.7	39.6	24.9
Zencor 350 ml ha^{-1} + Wish top 1.25 l ha^{-1}	1459	88.4	1.63	60.1	79.9	40.2	25.5
Zencor 350 ml ha^{-1} + Zetrola 1.6 l ha^{-1}	1450	88.2	1.62	60.0	79.9	40.1	25.4
Passat 1.2 l ha^{-1}	1363	85.7	1.56	58.0	79.4	39.0	24.4
LSD 5%	63	3.1	0.06	2.9	0.03	0.4	0.2
LSD 1%	78	4.5	0.08	3.6	0.05	1.1	0.8
LSD 0.1%	97	5.4	0.11	4.5	0.09	1.9	1.5

The double use of Zencor, although it increased the efficacy of the herbicide against weeds, reduced its selectivity to lentil. The single use of Zencor as soil-applied herbicide at dose

900 ml ha^{-1} controled even more weeds, but had even lower selectivity to lentil, resulting in even lower yields.

Combination of Zencor with foliar-applied herbicides Wish top and Zetrola always led to bigger increase in yield compared to single use of the respective herbicides during the three years of the study.

In treatments with herbicide Passat during the three years of the study, higher seed yields were reported compared to weedy control. Lentil lag behind in their development, the ripening stage was delayed by 4-5 days, but nevertheless seed yields were not significantly reduced, as weed infeatation was significantly lower compared to untreated control.

To explain changes in seed yield were investigated some of the structural elements that determine it. Differences in the efficacy and selectivity of the studied herbicides, herbicide combinations and tank herbicide mixtures led to changes in the values of the indexes number of beans per 1 plant, number of seeds per 1 bean and 1000 seeds weight. The greatest increase in the beans number per plant, seeds number per bean and 1000 seeds weight was obtained by combinations of soil-applied herbicide Pendistar with foliar-applied herbicide Lentagran at doses 500 + 500 ml ha^{-1} and 1 l ha^{-1}, followed by combinations of herbicide Challenge at doses 3 + 1 l ha^{-1} and 4 l ha^{-1} with antigraminaceous herbicide Wish top. Although the test weight has less influence on the yield value, the increase in the test weight was lower. More importantly in lentil, the beans are filled with normally developed, well-fed and ripened seeds.

It is interesting to note that the join use of herbicides Challenge and Zetrola had initial phytotoxic effect on crop, consisting in retaining growth during the first 15-20 days after applying. This phytotoxicity was manifested both in soil application of Challenge, followed by foliar application of Zetrola, as herbicide combination, and also in simultaneous foliar application of both herbicides as tank mixture. Subsequently, the lentil overcomed this negative effect and at the end of vegetation in the variants, high values of this indicator have been obtained. This was due to the good chemical control against existing weeds, which provided better lentil growth.

The plant heights were the highest with combinations of soil-applied herbicide Pendistar with foliar-applied herbicide Lentagran at doses 500 + 500 ml ha^{-1} and 1 l ha^{-1}, followed by combinations of herbicide Challenge at doses 3 + 1 l ha^{-1} and 4 l ha^{-1} with antigraminaceous herbicide Wish top.

In all legumes including the lentil, the first beans height is very important index. First beans height was the lowest with herbicides Zetrola, Wish top and Passat.

The values of this index were the highest by combinations of soil-applied herbicide Pendistar with foliar-applied herbicide Lentagran at doses 500 + 500 ml ha^{-1} and 1 l ha^{-1}, followed by combinations of herbicide Challenge at doses 3 + 1 l ha^{-1} and 4 l ha^{-1} with antigraminaceous herbicide Wish top. Increasing the first bean height with these herbicide combinations is additional positive effect of their use as it reduces harvest losses and downfall of the yield.

Conclusions

High yields were also obtained when combining Challenge 3 + 1 l ha^{-1} with Wish top 1.25 l ha^{-1}, as well as with the herbicide combination Challenge 4 l ha^{-1} + Wish top 1.25 l ha^{-1}. Increase in seed yield was due to the greatest degree of increase in indexes beans number per plant, seeds number per bean and 1000 seeds weight. First beans height was the highest with use of combinations of soil-applied herbicide Pendistar with foliar-applied herbicide Lentagran in doses of 500+500 ml ha^{-1} and 1 l ha^{-1}, followed by combinations of herbicide Challenge in doses of 3+ 1 l ha^{-1} and 4 l ha^{-1} with the herbicide Wish top.

References

Baird J.M., Shirtliffe S.J., Walley F.L. (2009). Optimal seeding rate for organic production of lentil in the northern Great Plains. Canadian Journal of Plant Science, 89: 1089-1097.

Chandrakar D.K., Nagre S.K., Ramsingh D.D., Singh A. P. (2016). Influence of different herbicides on growth, yield and economics of lentil. Indian Journal of Weed Sciences, 48 (2): 182-85.

Chandrakar U.K. (2011). Chemical weed control in lentil (*Lens culinaris* Medikus subsp. *Culanaris*). M. Sc. (Ag) Thesis, IGKV, Raipur (CG).

Delchev G. (2018). Chemical control of weeds and self-sown plants in eight field crops. Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp. 397.

Delchev G. (2021). Efficacy and selectivity of some herbicides in five field crops. Monograph, LAP LAMBERT Academic Publishing, Saarbrücken, Germany, pp 225.

Elkoca E., Kantar F., Zengin H. (2004). Effect of chemical and agronomical weed control treatment on weed density, yield and yield parameters of lentil (*Lens culinaris* L. cv. Erzurum-89). Asian Journal of Plant Sciences, 3 (2): 187-192.

Kumar A., Nandan R., Sinha K., Ghosh D. (2016). Integrated weed management in lentil (*Lens culinaris* Medik) in calcareous alluvial soils of Bihar. Indian Journal of Agronomy, 61 (1): 75-78.

Lhungdim J., Singh Y., Singh R. (2013). Integration of chemical and manual weed management on weed density, yield and production economics of lentil (*Lens culinaris* Medikus) International Journal of Bio-resource and Stress Management, 4 (4): 593-98.

Malik M.R., Haqqani A.M., Habib-ur-Rahman K.K., Ozair C.A., Malik B.A. (2001). Economic implications of pre and post-emergence weed control methods in lentil (*Lens culinaris* Medik.). Journal of Biological Sciences, 1: 113-116.

Manjunath R., Kumar S., Thakral S. (2010). Effect of irrigation and weed management on lentil (*Lens culinaris* Medic.) under different planting techniques. Indian Journal of Weed Science, 42 (1/2): 56-59.

Mojeni H.K., Zadeh H.M., Hosseini N.M., Peighambari S.A. (2005). Efficiency of either single or integrated application of different herbicides on lentil (*Lens culinaris* Medik.) yield and yield components in winter sowing and spring sowing dates. Iranian Journal of Agricultural Sciences, 36 (1): 209-218.

Phogat S., Kumar S., Sangwan N., Hooda R. (2003). Effect of herbicides and cultural practices on weed flora of lentil. Indian Journal of Pulses Research, 16 (2): 119-121.

Sharara F., El-Shahawy T., El-Rokiek K. (2011). Effect of prometryn/benzoic acid combination on weeds, seed yield and yield components of lentil (*Lens culinaris* L.), Electronic Journal of Polish Agricultural Universities, 14 (1): 2-9.

Singh S., Singh I., Gill R., Kumar S., Sarkar A. (2009). Genetic studies for yield and component characters in large seeded exotic lines of lentil. Journal of Food Legumes, 22: 229-232.

Sultan S., Nasir Z. (2007). Intranasal variation in weed communities of lentil fields in Chakwal, Pakistan. Pakistan Journal of Botany, 39 (5): 1471-1479.

Tepe I., Erman M., Yazlk A., Levent R., Ipek K. (2004). Effect of different control methods on weeds, yield components and nodulation in the spring lentil. Turkish Journal of Agriculture and Forestry, 28: 49-56.

STRUČNI RAD

Odredbe Senjskog statuta o važnosti pšenice kao glavne prehrambene namirnice u srednjovjekovnoj Hrvatskoj

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Sažetak
Senjski statut, jedan od bitnijih pravnih spomenika hrvatskog srednjovjekovlja, spominje pšenicu na nekoliko mjesta, gdje uživa određene zakonske iznimke kao glavna prehrambena namirnica srednjovjekovnog čovjeka. Zakonske odredbe iz 14. stoljeća pšenicu, odnosno žito, spominju u četiri odvojena članka i navode kako je prijevoz žita po moru slobodan te da su stanovnici Senja oslobođeni plaćanja maltarine na žito i povrće koje dovoze u grad Senj. Samim time, zakonodavac pšenicu stavlja u poseban položaj, pokazujući kako je, kao poljoprivredni proizvod, bila vrlo cijenjena i bitna u svakodnevnom životu srednjovjekovnih ljudi.

Ključne riječi: Senjski statut, pšenica, Hrvatska, srednji vijek, pravna povijest

Uvod

Početak uzgoja pšenice veže se s područjem Bliskog istoka, tzv. Plodnim polumjesecom, koje se datira između 9. i 7. tisućljeća pr. Kr. Kao početak pripitomljavanja biljke uzima se u pravilu razdoblje oko 8000. godine prije Krista (Bell, 1987.). Tijekom duge povijesti, pšenica se proširila po poznatom svijetu, a pšenično zrno koristilo se prvenstveno za prehranu ljudi. Danas se nije puno toga promijenilo, a upotreba pšenice ostala je ista, budući da se pšenica koristi u proizvodnji kruha, kolača, tjestenine te kao glavni sastojak za fermentaciju pšeničnih piva i nekih drugih alkoholnih pića. Pšenica je također nezamjenjiva u ishrani stoke, a njezina slama se koristi i kao građevinski materijal. Osim u prehrani i građevini, u posljednje vrijeme se koristi i u industriji bio goriva. Do početka 19. stoljeća uzgoj i žetva pšenice nisu se mnogo razlikovali od metoda antičkog svijeta, a poljoprivrednici su žetvu vršili uglavnom ručno (srpom ili kosom).

Pšenica se kao jedna od najvažnijih poljoprivrednih kultura srednjeg vijeka spominje i u najstarijem hrvatskom pravnom spomeniku Vinodolskom zakonu (Haman, 2021.b). Uz pšenicu, hrvatski seljaci uzgajali su i ostale žitarice poput ječama, zobi i raži, dok je uzgoj maslina i vinove loze bio je vrlo produktivan na jadranskoj obali. Ostali usjevi uključivali su mahunarke (grah i grašak), kao i voće i povrće. Proizvodnja žitarica bila je važna i ključna za razvoj društva u većini europskih regija, ne samo u Hrvatskoj. Pšenica je danas, uz kukuruz, najzastupljenija ratarska kultura u biljnoj proizvodnji Hrvatske, ali i svijeta (Iljkić i sur., 2019.).

Materijal i metode

Pravno povijesna metoda korištena je kao osnovna metoda za provedeno istraživanje. Korišten je cjeloviti tekst Senjskog statuta kako bi se detaljno i u potpunosti moglo provesti valjano istraživanje. U svrhu usporedbi pojedinih odredbi, korištena je komparativno-pravna metoda istraživanja, prvenstveno za potrebe usporedbe s Vinodolskim zakonom, kao i najstarijim slavenskim zakonom Zakon sudnij ljudem. Nekoliko drugih knjiga i znanstvenih članaka su konzultirani kako bi se dovršio ovaj članak i oni su navedeni u nastavku.

Rezultati i rasprava

Senjski statut iz 1388. godine predstavlja najstariju pravnu kodifikaciju grada Senja. Obuhvaća 130 članaka i prilog od 38 članaka koji se odnose na senjske plemićke povlastice. Statut je napisan latinicom na pergamentu. Izvorno nije imao naslov, ali je naslov "Statutum Segniae" dodan u naknadnom rukopisu na dnu prve neparne stranice. Sveukupno Statut sadrži 168 članaka, no samo određeni broj tih članaka potječe iz 1388. godine, a ostali su naknadno dodani (Bartulović, 1997.). Lujo Margetić, poznati hrvatski pravni povjesničar, istražio je nastanak Senjskog statuta kako bi utvrdio da prvih 68 članaka datira iz 1388., članci od 69. do 130. od 1390. do 1393., a članci od 131. do 168. doneseni su krajem 1402. ili ubrzo nakon toga (Milović, 2007.).

Izvornik ovog Statuta čuvao se u Arhivu Grada Senja do Drugoga svjetskog rata, kada je nestao u ratnim previranjima. Na sreću, njegov je tekst u potpunosti sačuvan jer ga je 1854. godine objavio Ivan Mažuranić (Mažuranić, 1854.). Postoje dvije inačice teksta Statuta, jedna je napisana na latinskom, a druga na hrvatskom jeziku, ali se u ovom radu razmatra hrvatski tekst Senjskog statuta kao prijepis iz 1701. godine. Ovaj je tekst najvjerojatnije pripremljen za praktičnu uporabu na dvoru ili u nekim sličnim prigodama.

Senjski statut na prvi pogled odaje dojam nesređenosti, no vidljivo je nastojanje sastavljača da se određena građa prikupi na jednom mjestu. Najčešći način prikaza redoslijeda članaka u Statutu je: 1-17 Prava plemića i građana, 18-22 O općinskim vlastima, 23-26 O nekretninama, 27-42 Postupak, 43-68 Kazneno pravo i 69 -130 Razno (Margetić, 2007.).

U Senjskom statutu postoje četiri članka (3., 4., 118. i 120.) koja izrijekom spominju pšenicu, odnosno žito.

§ 3. Nadalje su utvrdili i odredili neka bude i svakako neka senjski plemić ostane slobodan od maltarine na žito i na bilo što drugo svoje i trgovačku robu, koju dovozi u Senj ili izvozi ili dade izvesti na bilo koji način.

(§ 4.) Nadalje su utvrdili i odredili neka svaki senjski plemić bude slobodan i oslobođen i na vratima mitnice i na moru za svoje prihode koje odande prevozi na svojim životinjama kao i za svaku i bilo koju svoju trgovačku robu na koju se plaća desetina ili drugo podavanje, bilo na gradskim vratima, bilo na moru; za bilo koju vrstu žita koje kupuje radi izvoza preko mora, svaki plemić dužan je i treba službenicima gospode platiti odgovarajuće podavanje, osim ako kupuje za potrebe svojih radnika.

(§ 118.) Nadalje, senjski plemići smiju slobodno prenijeti po moru žito, koje daju svojim radnicima u vinogradima i nekretninama, i to za njihovu hranu i za čitavo vrijeme koje tamo borave. Isto se odnosi na senjske građane.

§ 120. Nadalje, rečeni plemići, a također i pučani, izuzeti su i slobodni od svake maltarine na žito ili povrće koje dovode u grad Senj na svojim ili tuđim životinjama; ipak stranac prijevoznik plaća jedan soldin za svoje životinje i to se zove okasina.

Na samom početku imamo posebno isticanje žita za koje plemići nisu dužni plaćati maltarinu koju dovoze u Senj ili izvoze iz njega. Međutim, ono što je bitno je da plemići ne plaćaju maltarinu na žito, ali „i na bilo što drugo svoje i trgovačku robu", što znači da nije bilo potrebe posebno isticati žito u ovom slučaju, ali je zakonodavac to svejedno učinio. Takav stav zakonodavca jasno daje do znanja da je žito u povlaštenom položaju nad ostalim prehrambenim namirnicama, barem izrijekom. U nastavku, već u 4. članku, jasno se definira

da su plemići oslobođeni svih vrsta davanja, od maltarine, do feudalne desetine za trgovinu žitom, ali su dužni pokriti određene administrativne troškove, osim ako je žito kupljeno za potrebe svojh radnika. Ovaj članak nas direktno povezuje sa 118. člankom koji zapravo definira konkretnu uporabu toga žita, a to je upravo prehrana radnika.

U svega nekoliko članaka Senjskoga statuta imamo priliku vidjeti koliko je žito bilo važno u prehrani, a da se prijevoz i kupnja žita, ukoliko se koristilo za prehranu, moglo prevoziti i dostavljati bez ikakvih davanja, kako od strane plemstva, tako i od pučana.

Senjski statut nije jedini srednjovjekovi pravni spomenik koji ističe važnost žita, kao važne namirnice u ljudskoj prehrani. Vinodolski zakon, najvažniji i najstariji poznati hrvatski pisani zakon, također spominje važnost žita/pšenice i propisuje kažnjavanje ukoliko dođe do krađe ili spaljivanja. U članku 8. spominje se velika kazna za krađu žita, neuobičajeno velika, što nam jasno daje do znanja koja je bila važnost žita za običnog pučanina, budući da njegovo preživanjavanje ovisi o dostupnosti žita tijekom godine (Haman, 2021.b). Osim toga, članak 10. bavi se spaljivanjem žita na polju. Iako Vinodolski zakon ne propisuje kaznu za palež, temeljem nekih starijih slavenskih zakona, ponajprije Zakona sudnij ljudem (Dewey and Kleimola, 1977.), najstarijeg slavenskog zakona, možemo vidjeti koliko je palež ozbiljan zločin u srednjem vijeku, budući da Zakon sudnij ljudem propisuje smrtnu kaznu samo za podmetanje požara (Haman, 2021.a). Uzimajući to u obzir, nije teško zaključiti važnost pšenice kao primarne prehrambene namirnice u srednjovjekovnoj Hrvatskoj, ali i u Europi.

Zaključak

U srednjem vijeku, bez doticaja s američkim žitaricama i poljoprivrednim kulturama, pšenica je bila nezamjenjiva u ljudskoj prehrani, prvenstveno u izradi kruha te je tako bila simbol ljudskog postojanja i blagostanja. Deset tisućljeća nakon njezine domestikacije, pšenica je bila i ostala simbol proizvodnje hrane i prehrane čovječanstva u gotovo cijelome svijetu te je održala svoj značaj kroz povijest sve do današnjih dana.

Senjski statut iz 1388. godine predstavlja najstariju pravnu kodifikaciju grada Senja i kao takav uživa posebno mjesto u hrvatskoj pravnoj povijesti. Dok je pšenici, odnosno žitu posvećeno tek četiri članka, kroz njih imamo priliku jasno vidjeti važnost pšenice u prehrani, trgovini, ali i životu ljudi toga vremena. Usporedimo li Senjski statut s Vinodolskim zakonom, možemo jasno reći kako je pšenica bila istinski glavna prehrambena namirnica srednjovjekovne Hrvatske.

Literatura

Bartulović Ž. (1997). *Neka pitanja stvarnih i obveznih prava: Vinodolski zakon (1288), Krčki i Senjski statut (1388): S uvodnim razmatranjem o povijesti otoka Krka, Vinodola i Senja od antike do konca XV. stoljeća*, Rijeka, Hrvatska: Matica Hrvatska - Ogranak Rijeka.

Bell G. D. H. (1987). The history of wheat cultivation. Objavljeno u *Wheat Breeding: Its scientific basis* (F. G. H. Lupton, ed.), Chapman and Hall Ltd, London, UK: 31-49.

Dewey H. W., Kleimola A. M. (1977). *Zakon sudnyj ljudem (Court law for the people)*. Objavljeno u *Michigan Slavic Materials No. 14*. Ann Arbor, USA: Department of Slavic languages and literatures, University of Michigan.

Haman D. (2021a). Distinction between *dolus* and *culpa* with reference to arson in the Zakon Sudnyj Ljudem, the Vinodol Law and the Statute of Senj. Journal De Jure, 22 (1): 142-146.

Haman D. (2021b). The Vinodol Law provisions related to wheat as the most important agricultural crop in the Middle Ages. Objavljeno u *Proceedings of the 56th Croatian and 16th International Symposium on Agriculture*, Rozman V., Antunović Z. (ed.), 420-423.

Vodice, Hrvatska: Fakultet agrobiotehničkih znanosti Osijek, Sveučilište Josipa Jurja Strossmayera u Osijeku.
Iljkić D., Kovačević V., Rastija M., Antunović M., Horvat D., Josipović M., Varga I. (2019). Long term effect of Fertdolomite on soil, maize and wheat status on acid soil of eastern Croatia. Journal of Central European Agriculture, 20 (1): 461-474.
Margetić L. (2007). Senjski statut iz godine 1388. Senjski zbornik 34: 5-160.
Mažuranić I. (1854). Statutum Segniae. Objavljeno u *Arkiv za povjestnicu jugoslavesku I*, Družtvo za jugoslavensku povjestnicu i starine, Zagreb: 155-169.
Milović, Đ. (2007). Kazneno pravo senjskog statuta iz godine 1388. Senjski zbornik 34: 197-243.

The Statute of Senj provisions on the importance of wheat as the main food source in medieval Croatia

Abstract
The Statute of Senj, one of the most important legal monuments of the Croatian Middle Ages, mentions wheat in several places, where it enjoys certain legal exceptions as the main food of medieval man. Legal provisions from the 14th century mention wheat and grain in two separate articles and state that the transport of grain by sea is free and that the inhabitants of Senj are exempt from paying malt on grain and vegetables they bring to the town of Senj. Thus, the legislator puts wheat in a special position, showing how, as an agricultural product, it was highly valued and important in the daily lives of medieval people.

Key words: Statute of Senj, wheat, Croatia, Middle Ages, legal history

IZVORNI ZNANSTVENI RAD

Utjecaj vremenskih prilika i sorte na variranje sadržaja proteina u zrnu pšenice

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Sažetak
Sadržaj proteina u zrnu pšenice je, pored prinosa zrna, najvažnije svojstvo u proizvodnji. Iako nije proteinska kultura, pšenica se smatra značajnim izvorom proteina u prehrani čovjeka jer se konzumira svakodnevno u većim količinama. Cilj istraživanja bio je utvrditi sadržaj proteina u zrnu deset sorata pšenice tijekom višegodišnjeg razdoblja. Poljski pokusi su postavljeni tijekom petogodišnjeg razdoblja (2015.-2019.) na površinama Poljoprivrednog instituta Osijek u Osijeku po slučajnom bloknom rasporedu u tri ponavljanja. Korištene su sorte Žitarka, Srpanjka, Golubica, Lucija, Alka, Ficko, Renata, Katarina, Felix i OS Olimpija. Temeljem provedenog istraživanja sorte su ostvarile visokih 13,03 % proteina u zrnu. Provedenom analizom varijance utvrđena su veća odstupanja između sorti (4,02 %) nego između ispitivanih godina (1,80 %), dok je najveće odstupanje uočeno u interakciji godina x sorta (6,20 %).

Ključne riječi: pšenica, kvaliteta, protein, vremenske prilike, sorta

Uvod

Pšenica (*Triticum aestivum* L.) je jedna od najstarijih i najvažnijih svjetskih usjeva za proizvodnju hrane. Prema zasijanim površinama nalazi se na prvom mjestu, a prema proizvodnji na drugom, odmah iza kukuruza (FAOSTAT, 2021.). Preradom zrna dobiva se kvalitetno brašno koje se koristi u prehrani ljudi za proizvodnju mnogih pekarskih i konditorskih proizvoda. Pšenica se koristi i u hranidbi domaćih životinja, a neizostavna je sirovina u mnogim prerađivačkim industrijama (Kovačević i Rastija, 2014.). Iako nije proteinska kultura, smatra se da je pšenica značajan izvor proteina u prehrani čovjeka jer se njeni proizvodi konzumiraju svakodnevno u većim količinama. Španić (2016.) navodi kako kvalitetu pšenice određuju fizikalna svojstva (hektolitarska masa, masa 1000 zrna, krupnoća, oblik i boja zrna, caklavost i brašnavost zrna), kemijski sastav zrna (sadržaj proteina i ugljkihidrata), zdravstveno stanje zrna i tehnološka svojstva brašna, tijesta i kruha. Nasljeđivanje tih svojstava je regulirano složenim fiziološkim procesima koji se razlikuju između genotipova, a pod utjecajem su okolišnih čimbenika.
Količina i kakvoća proteina zrna pšenice glavni su čimbenici utjecaja na tehnološke karakteristike brašna. Jedinstvena visokoelastična i kohezivna svojstva tijesta u najvećoj su mjeri pod utjecajem glutena čije dvije glavne komponenete glijadini i glutenini, kao rezervni proteini endosperma zrna, čine 80 % ukupnih proteina zrna pšenice (Horvat i sur., 2022.). Međutim, proteini pšeničnog zrna pokazuju visoku kompleksnost i različit međusobni stupanj interakcije zbog čega je njihova karakterizacija teška (Simić i Žilić, 2018.). Ma i sur. (2019.) ih općenito dijele na glutenske i neglutenske, a Wang i sur. (2013.) na topive i netopive frakcije.

Usprkos relativno niskom sadržaju proteina, hranjivu vrijednost pšenice ne bi trebalo podcjenjivati jer na tehnološku kvalitetu brašna, odnosno kvalitet kruha i drugih pekarskih proizvoda u značajnoj mjeri ovisno o količini i kvaliteti proteina. Upravo je prilikom otkupa pšenice sadržaj proteina jedan od najvažnijih parametara kvalitete obzirom na svrstavanje sorti u razrede kvalitete (Narodne novine, 62/2019) i formiranje cijene otkupa.
Cilj provedenog istraživanja bio je utvrditi sadržaj proteina u zrnu deset sorti pšenice tijekom višegodišnjeg razdoblja u različitim vremenskim uvjetima.

Materijal i metode

Tijekom petogodišnjeg razdoblja (2015.-2019.) provedeni su poljski pokusi na površinama Poljoprivrednog instituta Osijek u Osijeku na antropogeniziranom eutrično smeđem tlu. Svake godine sjetva je obavljena u optimalnim rokovima druge dekade listopada sa sjetvenom normom od 330 zrna po m^2. Pokusi su postavljeni po shemi slučajnog bloknog rasporeda u tri ponavljanja pri čemu je veličina parcele iznosila 7,56 m^2. Korištene su visoko prinosne sorte Poljoprivrednog instituta Osijek (Žitarka, Srpanjka, Golubica, Lucija, Alka, Ficko, Renata, Katarina, Felix i OS Olimpija). Obrada tla i predsjetvena priprema bila je uobičajena za proizvodnju ozime pšenice. Ukupna količina primijenjenih hraniva (osnovna gnojidba + prihrane) ovisno o pretkulturi kretala se od 101 do 114 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 i 120 kg ha^{-1} K_2O. Zaštita usjeva kemijskim preparatima je bila obavljena prema potrebi u skladu s preporukama struke. Neposredno nakon žetve uzeti su prosječni uzorci od 1 kg za brzu analizu proteina na uređaju Infratec 1241 (Foss, Sweeden). Svi podatci su obrađeni uz pomoć računalnog softwera, a analiza varijance pomoću statističkog programa SAS Enterprise guide 7.1.
Za analizu vremenskih prilika korišteni su podaci o mjesečnim količinama oborine i mjesečnim srednjim temperaturama zraka tijekom pet vegetacijskih razdoblja pšenice od 2014. do 2019. godine te višegodišnje prosječne vrijednosti s meteorološke postaje Osijek Državnog hidrometeorološkog zavoda Republike Hrvatske.

Rezultati i rasprava

Za nesmetan rast i razvoj te dobru kvalitetu zrna, pšenica ima određene zahtjeve prema vremenskim prilikama. Na nedostatak vlage pšenica je najosjetljivija u fazi vlatanja te u fazi oplodnje, cvatnje, formiranja i nalijevanja zrna (Pospišil, 2010.).

Količine oborina i prosječne temperature zraka prikazane u Tablici 1 pokazuju variranja vremenskih prilika među godinama. U usporedbi s višegodišnjim prosjekom ukupna količina oborina kretala se od -8 % do +25 % pri čemu je jedna vegetacija imala ispodprosječnu (2016./2017.), dvije vegetacije prosječnu (2014./2015. i 2018./2019.) i dvije vegetacije (2017./2018. i 2015./2016.) iznadprosječnu količinu oborina. Međutim, važno je naglasiti i značaj distribucije količine oborina tijekom vegetacije s obzirom na potrebe pšenice.

U pogledu prosječnih temperatura zraka odstupanja su se kretala u pozitivnom pravcu od +5 % do +25 %, odnosno sve vegetacije su bile iznadprosječno tople u usporedbi s višegodišnjim prosjekom.

Kovačević i Rastija (2014.) navode kako kemijski sastav zrna pšenice ovisi o sorti (genotipu), uvjetima uzgoja, vremenskim prilikama, geografskom području, klimi, tipu tla, agrotehnici (prvenstveno gojidbi) i drugo.

Sadržaj proteina je, pored prinosa zrna pšenice, najvažnije svojstvo u proizvodnji. Stoga je cilj svakog proizvođača proizvesti zrno što većeg sadržaja proteina jer se otkupna cijena temelji najviše na ovom parametru. Prosječna vrijednost sadržaja proteina u istraživanju je bila relativno visokih 13,03 % uz statistički značajnu razliku između sorti i godina (Tablica

2.). Prema trenutno važećem Pravilniku o ugovornim odnosima pri otkupu pšenice (NN, 62/2019) pšenica bi u prosjeku pripadala u II. kvalitetnu klasu.

Tablica 1. Količine oborina (mm) i prosječne temperature zraka (°C) tijekom ispitivanog razdoblja i tridesetogodišnji prosjek (1971.-2001.) za meteorološku postaju Osijek

Mjesec/ Vegetacija	X.	XI.	XII.	I.	II.	III.	IV.	V.	VI.	Prosjek/ Ukupno
Oborine (mm)										
2014./2015.	87,9	8,8	66,0	73,7	57,1	50,5	12,9	113,4	17,1	487,4
2015./2016.	142,1	45,1	1,9	67,0	68,3	68,2	39,8	63,1	99,5	595,0
2016./2017.	65,4	57,1	0,5	25,2	74,4	67,6	49,7	50,6	45,4	435,9
2017./2018.	68,7	33,0	51,7	61,7	70,2	83,4	21,0	27,4	126,8	543,9
2018./2019.	12,2	25,2	26,7	42,4	26,8	8,4	68,6	150,8	112,8	473,9
Temperature zraka (°C)										
2014./2015.	13,3	8,3	3,5	2,9	2,5	7,5	12,1	17,8	20,8	9,85
2015./2016.	11,1	7,5	3,2	0,8	6,9	7,5	13,1	16,5	21,0	9,73
2016./2017.	10,4	6,2	-0,1	-5,1	4,2	9,5	11,3	17,5	22,4	8,48
2017./2018.	11,8	6,7	3,6	4,2	0,9	4,6	17,0	20,6	21,7	10,12
2018./2019.	14,4	7,6	1,5	0,5	4,2	9,1	12,8	14,0	23,1	9,69
Višegodišnji prosjek (1971.-2001.)										
Oborine (mm)	55,9	61,5	48,8	41,6	34,5	40,5	51,0	59,2	82,0	475,0
Temperatura (°C)	11,1	5,1	1,3	-0,2	1,8	6,4	11,2	16,7	19,6	8,11

Najveći udio proteina kroz svih pet godina istraživanja je u prosjeku imala sorta OS Olimpija koji je iznosio 15,76 % dok je najmanji prosječni udio imala sorta Alka (11,74 %) što predstavlja razliku od čak 4,02%. Temeljem navednih rezultata i važećeg Pravilnika o ugovornim odnosima pri otkupu pšenice, sorta OS Olimpija s višegodišnjim udjelom proteina od 15,76 % pripada premium klasi, Golubica I. klasi (13,7 %), a sve ostale sorti bi u prosjeku bile II. ili III. klasa kvalitete (od 11,74 % do 13,38 %).

Temeljem interakcije godine i sorte, pojedinačno najveći udio proteina imala je 2015. godine sorta OS Olimpija (16,8 %), a najmanji udio proteina u zrnu pšenice imala je 2016. godine sorta Alka (10,6 %) uz postignutu razliku od čak 6,2 %, što ukazuje na značaj i kompleksnost interakcije.

Iako je udio proteina između ispitivanih godina također bio signifikantan, te razlike su bile slabije izražene i kretale su se od 12,18 % (2017.) do 13,98 % (2015.). Najveća statistička razlika ostvarena je između vegetacije 2017. i 2015. Na sintezu i akumulaciju proteina u zrnu, osim gnojidbe dušikom, najviše utječu vremenske prilike tijekom fenoloških faza klasanja i nalijevanja zrna (svibanj i lipanj). U ovom istraživanju najmanja količina oborina u lipnju je možda povezana s najvećim vrijednostima sadržaja proteina. Temeljem trogodišnjeg ispitivanja u sušnim i uvjetima navodnjavanja Alizadeh (2021.) ističe pozitivnu korelaciju suše i sadržaja proteina.

Vollmer i Mußhoff (2018.) su analizirali 148 800 uzoraka ozime pšenice iz Njemačke u razdoblju od 2004. do 2015. te zaključili da vremenski uvjeti (oborine, temperature i broj sunčanih sati) imaju različit utjecaj na udio proteina u zrnu ovisno o mjesecu koji se promatra. Visoke temperature u travnju i lipnju dovode do pada udjela proteina, dok u svibnju i srpnju dovode do njegovog rasta. Što se tiče oborina, rezultati pokazuju da više oborina u travnju i lipnju povećavaju protein, dok više oborina u srpnju smanjuje njegov

udio u zrnu. Međutim, navedeno se ne može povezati s ovim istraživanjem jer su u nekim slučajevima rezultati kontradiktorni.

Tablica 2. Udio proteina u zrnu ozime pšenice tijekom ispitivanog razdoblja (%)

Sorta	2015.	2016.	2017.	2018.	2019.	Prosjek	Klasa*
Žitarka	13,7	12,9	11,6	13,9	12,8	12,98^{CD**}	II.
Srpanjka	14,5	11,9	11,7	12,1	13,6	12,76^{BC}	II.
Golubica	14,2	12,8	13,2	14,3	14,0	13,7^{E}	I.
Lucija	13,2	11,9	11,7	12,7	11,9	12,28^{AB}	II.
Alka	12,7	10,6	10,7	12,5	12,2	11,74^{A}	III.
Ficko	14,3	12,8	12,3	13,1	14,4	13,38^{DE}	II.
Renata	14,4	12,2	12,1	12,7	13,6	13,0^{AB}	II.
Katarina	12,3	11,1	11,2	12,3	12,3	11,84^{A}	III.
Felix	13,7	12,5	12,4	12,8	12,9	12,86^{CD}	II.
OS Olimpija	16,8	15,4	14,9	15,8	15,9	15,76^{F}	Premium
Prosjek	13,98^{C}	12,41^{A}	12,18^{A}	13,22^{B}	13,36^{B}	13,03	II.

* Temeljem Pravilnika o ugovornim odnosima pri otkupu pšenice

** Vrijednosti u istom stupcu tablice označene različitim slovima statistički značajno se razlikuju

Utjecaj vremenskih prilika u vrijeme žetve na tehnološku kakvoću pšenice s područja Međimurja i Slavonije tijekom tri godine je ispitivao i Horvat-Štefulj (2011.). Rezultati istraživanja ukazuju da je količina padalina u korelaciji s hektolitarskom masom, količinom proteina i s rastezljivosti tijesta. Kod pšenica iz Međimurja utvrđena je značajna korelacija prosječne temperature zraka i količine i kvalitete proteina, aktivnosti α-amilaze i reoloških svojstava tijesta. Kod pšenica iz Slavonije također je utvrđena korelacija između temperature zraka i količine padalina sa sadržajem proteina te reološkim svojstvima tijesta.

Zaključak

Temeljem provedenog petogodišnjeg istraživanja s 10 sorti ozime pšenice prosječan sadržaj proteina iznosio je 13,03 %. Provedenom analizom varijance utvrđena su veća odstupanja između sorti (4,02 %) nego između ispitivanih godina (1,80 %), dok je najveće odstupanje uočeno u interakciji godina x sorta (6,20 %). To je naročito značajno jer na izbor sorte proizvođač može utjecati, dok su vremenske prilike izvan njegove kontrole.

Literatura

Alizadeh M. (2021). Potein profile and seeds storage proteins changes in wheat genotypes under control and drought stress conditions, Bioscience Research, 18 (3): 2164-2169.

Državni hidrometeorološki zavod (2020). Meteorološki podaci, Klimatološko meteorološki sektor, Državna hidrometeorološka stanica Osijek-Klisa, Zagreb.

FAOSTAT (2021). Food And Agriculture Organization of the United Nations, URL: http://www.fao.org/faostat/en/#home, datum pristupa: 16.04.2022.

Horvat D., Dvojković K., Novoselović D., Tucak M., Andrić L., Magdić D., Drezner G. (2022). Response of Wheat Yield and Protein-Related Quality on Late-Season Urea Application, Agronomy, 12:886.

Horvat-Štefulj Z. (2011). Utjecaj klimatskih promjena na tehnološku kakvoću pšenica s područja Međimurja i Slavonije (žetva 2009.-2011.), magistarski rad, Prehrambeno-biotehnološki fakultet Zagreb.

Kovačević V., Rastija M. (2014). Žitarice, Poljoprivredni fakultet u Osijeku, Osijek.

Ma W.J., Yu Z.T., She M.Y., Zhao Y., Islam S. (2019). Wheat gluten protein and its impacts on wheat processing quality, Frontiers of agricultural science and engineering, 6 (3): 279-287.
Narodne novine (2019). Pravilnik o ugovornim odnosima pri otkupu pšenice, Zakon o poljoprivredi (NN 62/2019). Ministarstvo poljoprivrede.
Pospišil A. (2010). Ratarstvo I. dio, Zrinski d.d., Čakovec.
Simić M., Žilić S. (2018). Proteini pšenice sa tehnološkog, nutritivnog i zdravstvenog aspekta. Hrana i ishrana, 59 (2): 68-73.
Španić V. (2016). Pšenica, Poljoprivredni institut Osijek, Osijek.
Vollmera E., Mußhoff O. (2018). Average protein content and its variability in winter wheat: a forecast model based on weather parameters. Earth intercations, 22 (19): 1-24.
Wang J.S., Jia F., Yang X.J., Qi B.J., Qu L.B. (2013). The classification and characteristic of proteins in wheat, Material design, processing and applications, Parts 1-4: 690-693.

Influence of weather conditions and variety on variation of protein content in wheat grain

Abstract
Protein content is, in addition to wheat grain yield, the most important property in production. Although not a protein crop, wheat is considered to be a significant source of protein in the human diet as it is consumed in larger quantities daily. The aim of the study was to determine the protein content of wheat grain over a multi-year period. Field experiments were carried out during a five-year period (2015-2019) on the Agricultural Institute Osijek in a randomize block design with three replications. Varieties Žitarka, Srpanjka, Golubica, Lucija, Alka, Ficko, Renata, Katarina, Felix and OS Olimpija were used. Based on the conducted research, the varieties achieved a high 13.03% of grain protein. The analysis of variance revealed larger deviations between varieties (4.02%) than between the examined years (1.80%), while the largest deviation was observed in the interaction of years x varieties (6.20%).

Key words: wheat, quality, protein, weather conditions, variety

ORIGINAL SCIENTIFIC PAPER

Biopriming: multiple effects on soybean germination metrics

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Abstract

Biopriming is a pre-sowing seed inoculation technique based on beneficial microorganisms. The aim of the study was to estimate biopriming potential of microbial consortium on soybean seeds. Obtained results showed a stimulative effect of biopriming resulting in 8% higher germination percentage. Inoculated seeds were characterised as more vigorous according to vigor I, vigor II and electrical conductivity results. Accelerated aging test decreased germination percentage of both treatments to approximately 66±2%. Inoculated seeds were marked as more vigorous according to vigor I index. Biopriming of soybean seeds resulted in an increase of germination parameters that suggest stimulative effects on seed quality.

Key words: biopriming, germination, soybean, microbial inoculant

Introduction

Seed quality is of crucial importance from the standpoint of seed production, directly affecting plant growth and yield (Miladinov et al., 2018). Numerous methods of seed pretreatment are being applied to improve all aspects of seed quality, prolonge storage and slow seed aging (Miladinov et al., 2018). Among numerous, biopriming, a pre-sowing technique that implies treatment with plant growth-promoting microorganisms (PGPM), gained special attention. This group of soil organisms poses multifarious traits with beneficial effects on seeds, seedlings and plant development. Bioprimed seeds have enhanced germination while bioprimed plants pose a higher level of resistance to environmental stress (Mitra et al., 2021; Sarkar et al., 2021). Seed biopriming can improve the efficiency of nutrient management and increase resistance to phytopathogens by activating the immune system (Devika et al., 2021; Sarkar et al., 2021). The most usual plant growth-promoting rhizobacteria (PGPR) representatives used as biopriming agents are among *Bacillus* sp., *Pseudomonas* sp., *Azotobacter* sp., *Azospirillum* sp., and *Burkholderia* sp. (Mitra et al., 2021). Soybean (*Glycine max* L. Merr.) is one of the world's most valued crops with broad utilization in the food, feed, pharmaceutical and chemical industries (Staniak et al., 2021). It has low mineral fertilization requirements and is involved in nitrogen fixation by the *Bradyrhizobium japonicum*.

The aim of the study was an estimation of biopriming effects of mixed Plant Growth-Promoting Bacteria (PGPB) inoculum on germination parameters of soybean seeds.

Material and methods

The experiment was performed in the Laboratory of Environmental Microbiology at the Faculty of Agriculture (Belgrade, Serbia). Soybean seeds cultivar Wendy (maturity group I, PZO Seed GmbH), and a commercial inoculant BioGnezdo (Biofor system, Serbia) were used in the study.

PGPB inoculant is a consortium consisted of: *Azotobacter chroococcum, Bacillus licheniformis, Bacillus circulans, Bacillus megaterium,* and *Bacillus amiloliquefaciens.*

Soybean seeds were surface sterilized by immersion in 70% ethanol (v/v) for 2 min, followed by 2 min exposure to 2% NaOCl (v/v) and thorough rinsing with sterile deionized water. Seeds were inoculated by immersion into bacterial suspension for 15 min/28 ± 2 °C/120 rpm (KS 260, IKA, Germany). The control seeds were immersed in sterile water.

Germination test was performed on filter paper in glass Petri dishes (Ø 20 cm). Total 100 seeds per treatment were maintained at 25°C for a week. Seeds with radicles of 2 mm or longer were counted, and the number of germinated seeds was recorded daily. The following parameters were calculated:

- The germination percentage (G) was measured on the eighth day using the formula:

G (%) = (total number of germinated seeds/ total seeds) x 100 (ISTA 2009);

- The germination speed (GS) was calculated by formula:

GS = (No of germinated seeds/days of first count) + (No of germinated seeds/days of final count) (Ruan et al., 2002);

- The germination index was calculated by formula: GI = n/d

'n' number of emerged seeds in day d, d is day after planting (Abdul-Baki and Anderson, 1973);

- Vigour index I and vigour index II were calculated by formula:

Vigor I (VI) = (Germination percentage × Seedling length)/100;

Vigor II (VII) = Germination percentage × Seedling dry weight (Abdul-Baki, Anderson 1973);

Electrical conductivity test was performed by immersion of 2x50 seeds in 250 ml of deionized water. Previously, the seeds were weighed with a precision scale of 2 decimal places. After 24 h at 25°C the solutions containing the seeds were slightly stirred and immediately measured by the conductivity meter Eutech cond 6^+ (Thermo Scientific, MA, USA). Obtained results were calculated according to the formula: $\mu S\ cm^{-1}g^{-1}$ = Conductivity ($\mu S\ cm^{-1}$)/Sample weight (g) (Hampton and TeKrony, 1995)

Accelerated aging test was conducted in plastic boxes containing 50 seeds placed 2 cm above water level and exposed to temperature of 41°C and humidity of 95% for 72 h. After that, the germination test was set up. The germination percentage, germination speed, germination index, vigor I and vigor II, seedling length and dry biomass were recorded 8 days after. The test was performed in three replicates. The ability of PGPB inoculant to antagonize *Fusarium graminearum* was assessed using a confrontation assay on potato dextrose agar (Himedia, India) plates in three replicates. Petri dishes were incubated at 25 ± 2°C, for seven days. Statistical analyses of variance were conducted by T-test at the 5% level of probability. All statistical analyses were performed using Statistica 12.0 (StatSoft, Tulsa, OK, USA).

Results and discussion

The presented study was conducted with the aim to estimate biopriming potential of a mixed consortium of selected PGPB. The effects were estimated through standard germination parameters (Table 1). The comparison of germination percentage confirmed the stimulative effect of biopriming on soybean seeds increasing by 8%. This result is in accordance to Miladinov et al. (2018) who reported 1-9% increase in several soybean lines as a result of seed priming. Kerečki et al. (2021) reported 15% increase of germination percentage as a result of soybean seeds biopriming with *Azotobacter chroococcum* F8/2. In our study, inoculated seeds were also characterized with higher germination speed and germination index.

Table 1. Germination parameters of soybean seeds and seedlings length and dry biomass (LSD 5%)

Treatment/Parameters	Control seeds	Inoculated seeds
G (%)	76±2.00[b]	82±1.50[a]
GS	95±2.50[b]	102±2.20[a]
GI	6.67±0.58[b]	10.67±2.08[a]
Vigor I	2.71±1.20[b]	8.28±3.20[a]
Vigor II	654±8.10[b]	713±7.30[a]
Conductivity	5.21±0.48[a]	1.17±0.05[b]
Lenght (cm)	3.57±0.15[b]	10.10±0.13[a]
Dry biomass (g)	8.61±0.15[a]	8.70±0.25[a]

The results showed that inoculated seeds are characterized by higher vigor index. Statistically, significant difference was observed in vigor I which was 3-fold higher while vigor II was increased by 12%. Kerečki et al. (2021) reported an increase of 23% of vigor II as a result of biopriming. The vigor is a good measure of seed quality and viability providing the producer with a more accurate picture of crop performance in real conditions (Marcos-Filho, 2015). There are several ways of vigor index determination and the electrical conductivity test is one of them (Catão and Caixeta, 2019). Reported data showed that seed inoculation led to a fall of this parameter, indicating more vigorous seeds compared to control. Namely, damaged seeds, seeds that are possibly less vigorous, release more exudates to the outside of the cell, resulting in higher electrical conductivity values and *vice versa* (Matera et al., 2019). Nevertheless, all seeds whose electrical conductivity is < 25 $\mu S\ cm^{-1}g^{-1}$ are considered highly vigorous (Milošević and Kobiljski, 2011). The results on seedling´s length and biomass (Table 1) confirmed that inoculated seedlings are significantly longer (almost 3-fold longer) while no differences were recorded in dry biomass. This is in accordance with Kerečki et al. (2021) who reported an increase of 100% of bioprimed seedlings´ length while no differences were recorded in dry biomass. Accelerating aging test is a suitable way for evaluation of vigor index and is commonly used in case of soybean seeds providing promt information regarding storage capacity and field performance (Matera et al., 2019; Santos Suñé et al., 2021). The germination percentage of control and bioprimed seeds decreased from 76 and 82% to 68 and 64%, respectively. Similarly, Rastegar et al. (2011) reported a germination percentage of 65% of soybean seeds after accelerated aging. Also, germination speed and vigor II was similar in both treatments (Table 2). The vigor I results make difference and qualify inoculated seeds as more vigorous compared to control. Matera et al. (2019) claim that seeds characterised as high vigor in this test keep their viability when exposed to severe abiotic conditions.

The seedlings that emerged after the accelerated aging test significantly differ among treataments (Table 2). Inoculated seeds gave 70% longer seedlings with 8% higher dry biomass suggesting a role of biopriming in the alleviation of temperature and humidity stress.

A comparison of results obtained in the standard germination test (Table 1) to those obtained after the accelerated aging test (Table 2) revealed negative effects of accelerated aging on seed vigor.

Table 2. Germination parameters of soybean seeds and seedlings length and dry biomass after accelerated aging test (LSD 5%)

Treatment/Parameters	Control seeds	Inoculated seeds
G (%)	68±1.50[a]	64±2.50[a]
GS	61±1.50[a]	58±1.20[a]
GI	2.67±0.58[a]	7±1.00[b]
Vigor I	2.37±0.20[b]	3.08±0.15[a]
Vigor II	334±6.10[a]	340±4.30[a]
Lenght (cm)	3.48±0.05[b]	5.93±0.06[a]
Dry biomass (g)	4.91±0.10[b]	5.32±0.19[a]

Namely, the germination percentage of control and bioprimed seeds was decreased by 11% and 22%, respectively. Seedlings length and dry biomass also decreased. According to the obtained results, aging treatment resulted in a reduction in germination metrics. This in accordance with Rastegar et al. (2011) who claimed that accelerated aging affects germination percentage, rate, vigor due to biochemical changes in a seed. The additional value of applied consortia was revield by a confrontation test which show high antagonistic activity towards *Fusarium graminearum* whose growth was decreased by 77%. In some areas, *Fusarium graminearum* is regarded as a key pathogen of soybean, producing seed and root rot, as well as pre- and post-emergence damping off (Chiotta et al., 2016). Germination metrics monitored in the study confirm the potential of consortia designed in this study for soybean´s seed quality improvement. The antagonistic potential of applied consortia provides additional services to seeds and seedlings by raising the level of protection against phytopathogens. Nowadays, biopriming is perceived as a revolutionary method based on principles of sustainability which leads to the better establishment, adaptation and health of plants resulting, ultimately, in increased yield (Deshmukh et al., 2020; Chitra and Jijeesh, 2021).

Conclusion

Germination test and accelerated aging test provide insight into seed physiological quality and biopriming effects on the same. According to obtained results it can be concluded that biopriming rise germination percentage, speed and index and bioprimed seeds are more vigorous compared to control. Further, biopriming lower down seeds electrical conductivity suggesting more vigorous seeds compared to control and accelerated aging test showed that inoculated seeds are more vigorous and gave longer seedlings with higher dry biomass. It can be concluded that PGPB can be used as a cost-effective tool for biopriming of soybean seeds.

Acknowledgement

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References

Abdul-Baki A., Anderson J.D. (1973). Vigor determination in soybean seed by multiple criteria. Crop Science. 13: 630-633.

Catão H.C.R.M., Caixeta F. (2019). Electrical conductivity test in soybean seeds with reduced imbibition period. Revista de Ciências Agrárias. 42: 387-393.

Chiotta M.L., Alaniz Zanon M.S., Palazzini J.M., Scandiani M.M., Formento A.N., Barros G.G., Chulze S. N. (2016). Pathogenicity of *Fusarium graminearum* and *F. meridionale* on soybean pod blight and trichothecene accumulation. Plant Pathology 65: 1492-1497.

Chitra P., Jijeesh C.M. (2021). Biopriming of seeds with plant growth promoting bacteria Pseudomonas fluorescens for better germination and seedling vigour of the East Indian sandalwood. New Forests. 52: 1-13.

Deshmukh A.J., Jaiman R.S., Bambharolia R.P., Patil V.A. (2020). Seed biopriming- a review. International Journal of Economic Plants. 7: 038-043.

Devika O.S., Singh S., Sarkar D., Barnwal P., Suman J., Rakshit A. (2021). Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. Frontiers in Sustainable Food Systems. 5: 1-11.

ISTA. (2009). Seed health testing. International rules for seed testing. [ISTA: 2009]. Bassersdorf, Switzerland: International Seed Testing Association.

Hampton J.G., Te Krony D.M. (1995). Handbook of Vigour Test Methods, 3rd Edition, 1-117. Zurich, Switzerland: International Seed Testing Association.

Kerečki S., Jovičić-Petrović J., Kljujev I., Lalević B., Karličić V., Petrović I., Raičević V. (2021). Biopriming: a sustainable support for crop establishment. In *The Proceedings of the XII International Scientific Agricultural Symposium "Agrosym 2021"*, Kovačević D. (ed.), 188-194. Jahorina, Republic of Bosnia and Herzegovina: East Sarajevo: Faculty of Agriculture.

Matera T.C., Pereira L.C., Braccini A.L., Krzyzanowski F.C., Scapim C.A., Piana S.C., Marteli D.C.V., Pereira R.C., Ferri G.C., Suzukawa A.K. (2019). Accelerated aging test and its relationship to physiological potential of soybean seeds. Journal of Seed Science. 41: 301-308.

Marcos-Fliho J. (2015). Seed vigor testing: an overview of the past, present and future perspective. Scientia Agricola. 72: 363-374.

Miladinov Z., Balešević-Tubić S., Đukić V., Ilić A., Čobanović L., Dozet G., Merkulov-Popadić L. (2018). Effect of priming on soybean seed germination parameters. Acta Agriculturae Serbica. 45: 15-26.

Milošević M., Kobiljski B. (2011). Semenarstvo, Milošević M., Kobiljski B. (eds), 1-400. Novi Sad, Republic of Serbia: Institute of field and vegetative crops.

Mitra D., Mondal R., Khoshru B., Shadangi S., Mohapatra P.K.D., Panneerselvam P. (2021). Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. Current Research in Microbial Sciences. 2: 1-5.

Santos Suñé A., da Silva Almeida A., Figueiredo J.C., dos Reis B.B., de Avila Nunes C., Saraiva C.R.C., Kroessin L., Correa W.L., de Tunes L.M.W. (2021). Adequacy of the accelerated aging test for soybean seeds. Colloquium Agrariae. 17: 1-13.

Sarkar D., Singh S., Perihar M., Rakshit A. (2021). Seed bio-priming with microbial inoculants: A tailored approach towards improved crop performance, nutritional security, and agricultural sustainability for smallholder farmers. Current Research in Environmental Sustainability. 3: 1-10.

Staniak M., Stepień-Warda A., Czopek K., Kocira A., Baca E. (2021). Seeds quality and quantity of soybean (*Glycine max* (L.) Merr.) cultivars in response to cold stress. Agronomy. 11: 1-13.

Rastegar Z., Sedghi M., Khomari S. (2011). Effects of Accelerated Aging on Soybean Seed Germination Indexes at Laboratory Conditions. Notulae Scientia Biologicae. 3:126-129.

Ruan S., Xue Q., Thlkowska K. (2002). Effect of seed priming on germination and health of rice (*Oryza sativa* L.) seeds. Seed Science Technology. 30: 451-458.

ORIGINAL SCIENTIFIC PAPER

Yield stability of cotton cultivars

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Abstract
The study was investigated three cotton cultivars grown on soil type typically meadow-cinnamon soil under non-irrigated and irrigated conditions with different dose of nitrogen. The field experiments were conducted during 2018-2020 period. Raw cotton yield stability parameters were determined using the stability variants (σ_i^2 and Si^2) according to Shukla and Kang's stability criterion (YSi). In the case of the Helius cultivar, the criterion for stability is highest when fertilizing with N_{160}. A maximum number of bolls (4.84) were formed in the cultivar Darmi, followed by the cultivar Helius (4.77 pieces) and Isabell. For the variety Helius, when fertilized with N_{240}, the number of boxes increases to 5.86 per plant. The average weight for one boll is 4.68 g. Without fertilization, it is 4.47 g, and for fertilization with N_{80}, N_{160} and N_{240} kg ha^{-1}, it reaches 4.66 g, 4.72 and 4.82 g, respectively.

Key words: cotton, irrigation, fertilization, stability, yield

Introduction

Drip irrigation can result in 30 to 50% savings of water and a 20 to 40% increase in yield (Ravinder et al., 2011). Field studies with eight genotypes grown under both natural moisture and irrigation conditions indicate that irrigation slows vegetative growth (Pettigrew, 2004). Optimizing the irrigation regime for drip irrigation (70–60% of FC) increases the efficiency of 35–103% for drip irrigation compared to furrow irrigation (Ibragimov et al., 2007). Furthermore, Yang et al. (2012) found that single fertilization provides a higher rate of biomass accumulation and is a practical alternative to cotton fertilization. The low N level in the nutrient solution greatly reduces the components of vegetative growth, number of boxes and seed yield. Trends in the impact of drought on crops were also analyzed using various dependencies, models and services to predict crop productivity (Dochin et al., 2018; Kostadinov et al., 2019).
The study aims to investigate the ecological stability of three Bulgarian cotton cultivars with different genetic origins in the conditions of Central Southern Bulgaria.

Material and methods

During 2018-2020 period, field experiments on cotton were conducted in the training and experimental base of the Department of Plant Breeding, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria. The stability parameters of raw cotton yield were determined using the stability variants (i^2, Si^2) according to Shukla (1972) and the stability criterion (YSi) of Kang (1993). The STABLE program of the Lousiana State University Agricultural Center, Baton Rouge, USA, was used to calculate these parameters. The experiment was performed by the method of fractional plots in four replications at basic plot size of 15 m^2. The influence of irrigation, fertilization and year was studied in the three cultivars of cotton Helius, Darmi and Isabell. Nitrogen (NH_4NO_3) fertilization rates were applied once before sowing in dose of N_0, N_{80}, N_{160} and N_{240} kg ha^{-1}. Irrigation was carried out with a drip irrigation system in rate of 15 mm when reaching soil moisture below 75% field capacity (FC) for the layer 0-50 cm. The parameters of the yield's structural elements were established by variants: number of harvested mature bolls per 1 plant and weight per 1 boll (g). Shukla stability variants (σi^2 and Si^2), which take into account linear and nonlinear interactions, respectively, unilaterally evaluate the stability of the variants. These variants, which show lower values, are considered more stable because they interact less with environmental conditions. The negative values of the indicators σi^2 and Si^2 are considered to be 0.

Results and discussion

Based on the interaction of the tested variants x year (Table 1), the stability of the manifestations at each tested level in the three cultivars concerning the yield of raw cotton was assessed.

Table 1. Dispersion analysis for a total yield of unopened cotton (average for 2018-2020)

Source of variation	Degree of freedom	Sum of squares	Mean squares	F
Total	71	441877.5	-	-
A - Tested variants	23	171243.8	7445.4	$^{n.s.}$1.51
B - Years	2	43398.0	21699.0	**97.16
A x B	46	227235.8	4939.9	**22.12
Heterogeneity	23	183315.6	7970.2	**4.17
Residual value	23	43920.2	1909.6	**8.55
Error	138	-	223.3	-

The variants of stability σi^2 and Si^2 according to Shukla, ecovalence Wi according to Wricke and the criterion for stability YSi of Kang were calculated, and the latter is especially valuable (Table 2). Stability criterion (YSi), takes into account the stability and the value of the yield in different variants.

Kang's YSi indicator gives valuable information about the technological value of the variants for simultaneous assessment of yield and stability, based on the reliability of the differences in yield and the variant of interaction with the environment. The value of this criterion is that using non-parametric methods and statistical proof of differences, were obtained a generalized assessment, ranking the options in descending order according to their economic value.

Table 2. Stability parameters of cotton

Irrigation	Cultivar	N	Yield, t/ha	σ_i^2	S_i^2	W_i	YS_i
Non-irrigated	Helius	N_0	1.1853	2203.6**	3905.2**	4454.6	-10
		N_{80}	1.4593	80002.**	1128.0*	15078.7	-5
		N_{160}	1.6727	8692.6**	288.6^{ns}	16348.1	3
		N_{240}	1.4978	2613.1**	13.9^{ns}	5202.3	-2
	Darmi	N_0	1.3556	3940.5**	3351.9**	7635.8	-8
		N_{80}	1.5337	5806.2**	2585.8**	11053.3	-1
		N_{160}	1.6135	4213.3**	1528.0**	8136.1	2
		N_{240}	1.6011	7323.2**	240.6^{ns}	13837.5	1
	Izabell	N_0	1.2313	2528.6**	4170.4**	5047.4	-9
		N_{80}	1.4276	4789.3**	2622.1**	9192.0	-7
		N_{160}	1.4408	3612.6**	2181.5**	7034.8	-6
		N_{240}	1.4744	4786.0**	995.5*	9186.0	-3
Irrigated	Helius	N_0	1.7073	3904.2**	220.4^{ns}	7569.3	6+
		N_8	1.8332	7721.5**	-27.5^{ns}	14567.8	9+
		N_{16}	1.9630	7023.4**	203.4**	13287.9	14+
		N_{24}	1.9151	13109.3**	3124.0**	24445.5	11+
	Darmi	N_0	1.9086	1884.0**	743.5^{ns}	3865.7	10+
		N_{80}	2.0355	3455.3**	2329.7**	6746.4	16+
		N_{160}	2.1791	2531.3**	2650.0**	5053.4	19+
		N_{240}	2.0947	7175.6**	4919.1**	13566.9	18+
	Izabell	N_0	1.7602	1762.3**	1349.4*	3642.5	7+
		N_{80}	2.0374	437.1^{ns}	721.6^{ns}	1213.1	25+
		N_{160}	2.0235	4564.4**	1790.7**	8779.7	15+
		N_{240}	1.9596	6480.4**	2958.1**	1229.3	13+
Average			**1.705**				**4.92**
LSD $_{0.05}$			*11.59*				

Growing cotton plants under drip irrigation conditions significantly increases the number of fruit bolls, proven at $p \leq 0.001\%$. The positive influence of irrigation on the number of bolls is clearly manifested in all three years (Table 3). The influence of the three nitrogen levels in the cultivars Helius and Darmi have been statistically proven, and in the case of the cultivar Isabell, the influence of only the high norm has been highly proved (Table 3). Under irrigated conditions in 2019, a decrease in the number of mature boxes at fertilizer rate N_{240} compared to N_{160} was found, associated with the non-cracking of some of the formed bolls. McConnell et al. (1995) also indicate that high nitrogen levels in the soil and high nitrogen fertilizer rates can reduce the number of cracked boxes due to excessive vegetative growth.

Table 3. Number of bolls per 1 plant of three cultivars of cotton (average for 2018-2020)

Irrigation	Cultivar	Fertilization	2018	2019	2020	Average, number	% to non-irrigated, Helius, N_0	% to N_0
Non-Irrigated	Helius	N_0	4.00	3.18	2.78	3.32	100.0	100.0
		N_{80}	4.25	4.12	2.85	3.74	112.6	112.6
		N_{160}	4.80	5.38	3.17	4.45	134.0	134.0
		N_{240}	5.25	5.45	3.14	4.61	138.8	138.8
		Average	**4.58**	**4.53**	**2.98**	**4.03**	-	-
	Darmi	N_0	3.75	3.88	2.88	3.50	105.4	100.0
		N_{80}	4.40	4.30	2.95	3.88	116.9	110.8
		N_{160}	5.00	5.23	3.28	4.50	135.5	128.6
		N_{240}	5.25	5.75	3.48	4.83	145.5	138.0
		Average	**4.60**	**4.79**	**3.15**	**4.18**	-	-
	Izabell	N_0	3.75	3.10	2.70	3.18	95.8	100.0
		N_{80}	4.40	3.53	2.95	3.63	109.3	114.2
		N_{160}	4.50	3.42	3.16	3.69	111.1	116.0
		N_{240}	4.65	3.93	3.40	3.99	120.2	125.5
		Average	**4.33**	**3.50**	**3.04**	**3.62**	-	-
Average non-irrigated			**4.50**	**4.27**	**3.06**	**3.94**	-	-
Irrigated	Helius	N_0	3.95	4.30	6.98	5.08	153.0	100.0
		N_{80}	4.50	4.50	7.23	5.41	163.0	106.5
		N_{160}	4.90	5.00	7.28	5.73	172.6	112.8
		N_{240}	5.25	4.92	7.40	5.86	176.5	115.4
		Average	**4.65**	**4.68**	**7.22**	**5.52**	-	-
	Darmi	N_0	3.95	4.32	6.53	4.93	148.5	100.0
		N_{80}	4.65	4.58	7.35	5.53	166.6	112.2
		N_{160}	5.05	4.92	7.33	5.77	173.8	117.0
		N_{240}	5.35	4.65	7.33	5.78	174.1	117.2
		Average	**4.75**	**4.62**	**7.13**	**5.50**	-	-
	Izabell	N_0	4.25	4.03	5.83	4.70	141.6	100.0
		N_{80}	4.60	4.48	5.90	4.99	150.3	106.2
		N_{160}	4.70	6.60	6.00	5.77	173.8	122.8
		N_{240}	4.80	4.93	6.50	5.41	163.0	115.1
		Average	**4.59**	**5.01**	**6.06**	**5.22**	-	-
Average irrigated			**4.66**	**4.77**	**6.80**	**5.41**	-	-
Average			**4.58**	**4.52**	**4.93**	**4.68**	-	
LSD 5.0 %			*0.24*	*1.40*	*0.64*	*1.83*		
LSD 1.0 %			*0.32*	*1.87*	*0.85*	*2.44*		
LSD 0.1 %			*0.41*	*2.42*	*1.10*	*3.19*		

In 2018, the influence of all three factors, as well as their interaction, is significant as the nitrogen fertilization factor plays a decisive role in the changes in the mass of the boll (52.1%). In 2020, irrigation is a decisive factor for the size of the boll, but the influence of N fertilization, as well as the interaction Irrigation x Cultivar are significant. The weight of one boll on average (4.68 g) for the study is good (Table 3). Significant differences in the

size of the boll during the experimental years were not found. When grown with irrigation, the weight of the boll reaches an average of 4.80 g, which is 5.3% above the size without irrigation.

Table 4. Weight of one boll (g) of three cultivars of cotton (2018-2020)

Irrigation	Cultivar	Fertilization	2018	2019	2020	Average	% to non-irrigated, Helius, N_0	% to N_0
Non-irrigated	Helius	N_0	4.03	4.56	4.23	4.27	100.0	100.0
		N_{80}	4.67	4.37	4.53	4.52	105.8	105.8
		N_{160}	4.88	4.50	4.46	4.61	108.0	108.0
		N_{240}	5.00	4.61	4.57	4.73	110.8	110.8
		Average	**4.65**	**4.51**	**4.45**	**4.54**	**-**	**-**
	Darmi	N_0	3.95	4.62	4.21	4.26	99.8	100.0
		N_{80}	4.35	4.49	4.32	4.39	102.8	103.0
		N_{160}	4.60	4.66	4.37	4.54	106.3	106.6
		N_{240}	5.05	4.90	4.47	4.81	112.6	112.9
		Average	**4.49**	**4.67**	**4.34**	**4.50**	**-**	**-**
	Izabell	N_0	4.36	4.90	4.01	4.42	103.5	100.0
		N_{80}	4.62	4.95	4.56	4.71	110.3	106.6
		N_{160}	4.98	4.97	4.35	4.77	111.7	107.9
		N_{240}	5.19	4.53	4.23	4.37	102.3	98.9
		Average	**4.79**	**4.84**	**4.29**	**4.64**	-	**-**
Average non-irrigated			**4.64**	**4.67**	**4.36**	**4.56**	**-**	**-**
Irrigated	Helius	N_0	4.37	4.56	4.77	4.57	107.0	100.0
		N_{80}	4.95	4.64	4.85	4.81	112.6	105.2
		N_{160}	5.07	4.52	4.87	4.82	112.9	105.5
		N_{240}	5.12	5.03	5.05	5.07	118.7	110.9
		Average	**4.88**	**4.69**	**4.88**	**4.82**	-	**-**
	Darmi	N_0	4.33	4.53	4.93	4.60	107.7	100.0
		N_{80}	4.52	4.83	5.04	4.80	112.4	104.3
		N_{160}	4.61	4.69	5.24	4.85	113.6	105.4
		N_{240}	5.21	4.65	5.16	5.01	117.3	108.9
		Average	**4.67**	**4.65**	**5.09**	**4.80**	-	**-**
	Izabell	N_0	4.58	4.60	4.94	4.71	110.3	100.0
		N_{80}	4.63	4.63	5.02	4.76	111.5	101.1
		N_{160}	4.65	4.41	5.18	4.75	111.2	100.8
		N_{240}	4.80	4.73	5.21	4.91	115,0	104.2
		Average	**4.67**	**4.59**	**5.09**	**4.78**	**-**	**-**
Average irrigated			**4.74**	**4.64**	**5.02**	**4.80**	**-**	**-**
Average			**4.69**	**4.66**	**4.69**	**4.68**	**-**	**-**
LSD 5.0 %			*0.11*	*0.80*	*0.39*	*0.47*		
LSD 1.0 %			*0.15*	*1.06*	*0.52*	*0.63*		
LSD 0.1 %			*0.19*	*1.37*	*0.68*	*0.82*		

In case of nitrogen deficiency the weight of the boll is on average 4.47 g, and in case of fertilization with N_{80}, N_{160} and N_{240} kg/ha it reaches average values of 4.66 g, 4.72 g and 4.86 g. This is 4.2-8.7% above the unfertilized control. Self-nitrogen fertilization stimulates the growth of the boll (Table 4). The presence of nitrogen affects the remobilization of biomass in cans and affects the growth of cans. Chen et al. (2018) found that the application of nitrogen gradually improves the development of the bolls and leads to higher weight. Sawan et al. (2006) also reported that the mass of the cans increased with an increase in the

nitrogen rate from 95 to 143 kg ha^{-1}. The data prove the usefulness of nitrogen fertilization to increase the reproductive development of cotton plants.

Conclusions

According to Kang's generalized YSi stability criterion, irrigated variants are technologically valuable. The highest score was given to N_{80} fertilization of Isabell cultivar, followed by N_{160} and N_{240} fertilization of Darmi cultivar. The rating is also high for the cultivar Darmi with N_{80} and Isabell with N_{160}. In the case of the Helius cultivar, the criterion for stability is highest when fertilizing with N_{160}. These variants combine high values of raw cotton yield and high stability of this indicator in different years. From the point of view of the technology for growing cotton, the fertilization with N_{240} in the cultivar Helius and in the cultivar Isabell is also highly valued.

References

Chen Y., Li Y., Zhou M., Rui Q., Cai Z., Zhang X., Chen D. (2018). Evaluation and optimization of Ultra-High Performance Concrete (UHPC) subjected to harsh ocean environment: Towards an application of Layered Double Hydroxides. Construction and Building Materials. 177: 51-62.

Dochin K., Kuneva V., Ivanova A., Iliev I. (2018). Current state of phytoplankton in Batak reservoir (Southwestern Bulgaria). Bulgarian Journal of Agriculture Science. 24 (4): 686-697.

Ibragimov N., Evett S., Esanbekov Y., Kamilov B., Mirzaev L. (2007). Water use efficiency of irrigated cotton in Uzbekistan under drip and furrow irrigation. Agricultural Water Management. 90 (1–2): 112-120.

Kang M.S. (1993). Simultaneous selection for yield and stability in crop performance trial. Agronomy Journal. 85: 754-757.

Kostadinov K., Filipov S., Valcheva D., Kuneva V. (2019). Influence of biological fertilization on vegetative behavior and productivity of greenhouse eggplant. Scientific Papers, Series B. Horticulture, 63 (1): 297-305.

McConnell J.S., Glover R.E., Vories E.D., Baker W.H., Frizzell B.S., Bourland F.M. (1995). Nitrogen fertilization and plant development of cotton as determined by nodes above white flower. Journal of Plant Nutrition. 18: 1027103.

Pettigrew W.T. (2004). Moisture Deficit Effects on Cotton Lint Yield, Yield Components, and Boll Distribution. Agronomy Journal. 96 (2): 377383.

Ravinder R. A., Majumdar, G.G., Reddy, A.R. (2011). Validation of farm pond size for irrigation during drought. Indian Journal of Agronomy. 56 (4): 356364.

Sawan Z. M., Mahmoud M. H., El-Guibali A. H. (2006). Response of yield, yield components, and fiber properties of Egyptian cotton (Gossypium barbadense L.) to nitrogen fertilization and foliar-applied potassium andmepiquat chloride. Journal of Cotton Science. 10: 224–234.

Shukla G.K. (1972). Some statistical aspects of partitioning genotype – environmental components of variability. Heredity. 29: 237-245.

Yang G., Tang H., Tong J., Nie Y., Zhang X. (2012). Effect of fertilization frequency on cotton yield and biomass accumulation. Field Crops Research. 125: 161-166.

ORIGINAL SCIENTIFIC PAPER

Influence of different inoculants on soybean (*Glycine max* L. Merr.) yield and quality

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Abstract
Soybean is one of the most prevalently grown and used oilseeds worldwide. About 85% of the world's soybeans are processed, or "crushed," annually into soybean cake, meal and oil. The aim of this study was to investigate the effect of four different microbiological inoculants for seed treatment on soybean grain yield and quality. Soybean seed inoculation with RizoliqTop inoculant which contains simbiotic bacteria of the genus *Bradyrhizobium* increased yield for 18.6% and plant density for the 36.5% compared to the variant with non-simbiotic bacteria inoculation i.e. control treatment.

Key words: soybean, microbiological inoculants, non-simbiotic bacteria, yield, quality

Introduction

Soybean, *Glycine max* (L.) Merrill, is an annual self-pollinated diploid legume (subfamily *Fabaceae*). An erect and productive crop, it is believed to have been domesticated for food from its viny wild relative, *Glycine soja* Sieb & Zucc. in eastern China more than three thousand years ago (Hymowitz and Bernard, 1991). Soybean is one of the most prevalently grown and used oilseed. Today, the world's top producers of soybean are the United States, Brazil, Argentina, China and India (Faostat, 2022). About 85% of the world's soybeans are processed into soybean meal, cake and oil. Approximately 98% of the soybean meal that is crushed is further processed into animal feed with the balance used to make soy flour and proteins (Oil and Grain News, 2022). In addition to mineral fertilization, inoculation or bacterization of soybean seeds with various microbiological preparations is recommended in order to better supply the plant with nutrients, mostly nitrogen. The ability of soybean to fix N_2 from the air through symbiosis and leave it for the next crop makes it a desirable part of crop rotation in agricultural systems (Flajšman et al., 2019). Worldwide, numerous authors in their research stated the positive effects of soybean seed inoculation on plant growth and development and soybean grain yield and quality (Ulzen et al., 2016; Marinković et al., 2018; Igiehon and Babalola, 2021).
The aim of this study was to investigate the effect of four different microbiological inoculants for seed treatment on soybean grain yield and quality.

Material and methods

Two field experiments with four various inoculants for seed treatment were set up in the spring of 2021 on the production areas of Center for Seed and Seedlings (Osijek) and Faculty of agrobiotechnical sciences Osijek (Tenja). According to laboratory analyzes the soil chemical properties for these two locations were different regarding soil pH, available phosphorus and potassium. However, both were poor in organic matter content (Table 1).

Table 1. Soil chemical properties (0-30 cm depth)

Location	pH (KCl)	Organic matter	AL-P_2O_5	AL-K_2O
		%	mg 100 g^{-1} soil	mg 100 g^{-1} soil
Osijek	5.84	1.52	32.50	27.80
Tenja	7.31	1.86	85.02	50.49

The field trials were set up according to a random block design in three replications. The area of the basic plot was 10 m^2. Mineral fertilizers of the formulation 7:20:30, urea and monoammonium phosphate (MAP) were added (Table 2).

Table 2. Mineral fertilizers added into the soil (kg ha^{-1})

Location	NPK - fertilizer	Urea	MAP
	7:20:30	46%	15:51:0
Osijek	400	100	0
Tenja	0	70	150

Pre-sowing inoculation / bacterization of soybean seeds with four different microbiological inoculants was performed as follows: 1. control (C), 2. bacterization of seeds with non-symbiotic bacteria (N), 3. bacterization of seeds with RizoliqTop (Rizobacter, Argentina), which contains bacteria of the genus *Bradyrhizobium* (R), 4. bacterization of seeds with HiCoat®Super (BASF, Germany), which also contains bacteria from the genus *Bradyrhizobium* (H) and 5. inoculation of seeds with mycorrhizal fungi (M).
Soybean sowing was carried out in the first half of May 2021 with a Wintersteiger self-propelled seed drill at a row spacing of 25 cm with a medium-early soybean variety Sunce of the Agricultural Institute Osijek. The sowing unit was 130 kg ha^{-1}. Herbicides Sencor SC 600 (Bayer AG, Germany) and Dual Gold 960 EC (Syngenta Crop Protection AG, Switzerland) were used in Tenja, while herbicides Corum® (BASF, Germany) and Focus® Ultra (BASF, Germany) were used at Osijek location. The plant density of 1 m^2 area was determined before harvest time in October and soybean harvest of each basic plot (10 m^2) was done with a Wintersteiger combine in the second half of October. The yield was expressed on the basis of 13% grain moisture. Oil and protein content was determined in the laboratory with a Infratec 1241 Grain Analyzer device (Foss, Denmark), which works on the principle of NIT technology (Near Infrared Transmission). Total soybean utility was determined based on the sum of oil content and protein content values.
Data were statistically processed by analysis of variance (ANOVA) using the software Microsoft Excel and SAS 9.4 while statistically significant differences between treatments were determined by F-test using LSD values at the level of 0.05 and 0.01.

Results and discussion

There were no statistically significant differences found between the analyzed parameters were found at the field trial situated in Osijek. Highest soybean yield (3.44 t ha^{-1}) was achieved on the variant where seed inocultation was carried out with RizoliqTop (R) and was for 18.6% higher than the control variant (C). Obtained results are in accordance with the statements of numerous authors about positive effects of seed inoculation with *Bradyrhizobium japonicum* on yield and quality as well as on plant growth and development of soybean plants (Shiri-Janagard et al., 2012; Leggett et al., 2017). Also, the highest oil content (21.10%) and total seed utility (62.87%) were achieved at the same variant R. Plant density was highest on variant with mycorrhizal fungi inoculation (48.7 plants m^{-2}) while

highest protein content (42.70%) was achieved on variant H where seed inoculation was carried out with HiCoat®Super (Table 3)

Table 3. Influence of different inoculants on soybean plant density, seed yield and quality

Location	Variants	Plant density (m^2)	Yield (t ha^{-1})	Oil content (%)	Protein content (%)	Total Utility (%)
OSIJEK	C	38.3	2.90	20.43	42.23	62.67
	N	37.7	2.90	21.00	41.80	62.80
	R	45.0	3.44	21.10	41.77	62.87
	H	37.0	2.99	20.00	42.70	62.70
	M	48.7	2.94	20.47	42.17	62.63
	LSD 0.05	n.s.	n.s.	n.s.	n.s.	n.s.
TENJA	C	59.7	5.12	21.63	40.93	62.57
	N	51.3	5.19	21.20	41.40	62.60
	R	70.0	4.87	21.63	41.10	62.73
	H	57.7	5.18	21.47	41.13	62.60
	M	61.7	4.72	21.70	40.83	62.53
	LSD 0.05	15.508	n.s.	n.s.	n.s.	n.s.
Mean Osijek		41.33	3.03	20.60	42.13	62.73
Mean Tenja		60.01	5.01	21.53	41.08	62.61
LSD 0.01		10.234	0.4702	0.6211	0.6995	n.s.

Variants: 1. Control (C), 2. Bacterization of seeds with non-symbiotic bacteria (N), 3. Bacterization of seeds with RizoliqTop (R), 4. Bacterization of seeds with HiCoat®Super (H), 5. Inoculation of seeds with mycorrhizal fungi (M).

Highest soybean grain yields at Tenja-Klisa location were obtained with non-simbiotic bacteria (5.19 t ha^{-1}) and HiCoat®Super (5.18 t ha^{-1}) inoculation. Compared to the control, these two variants resulted in an yield increase of about 1.36%. The inoculation with RizoliqTop (4.87 t ha^{-1}) and mycorrhizal fungi (4.72 t ha^{-1}) resulted in an yield decrease of about 4.89% and 7.81% compared to control variant. Yield response to inoculation is unpredictable and, while there are reports about environments that are more likely to respond to inoculation, few are scientifically supported (Leggett et al., 2017). Statistically significant differences (0.05) were found in plant density between variant R and N. Plant density was 36.5% higher in variant R compared to N variant but did not result in higher yield regarding those two variants. Oil content (21.70%) was highest on variant with mycorrhizal fungi inoculation while highest protein content (41.40%) was achieved on variant N where seed inoculation was carried out with non-simbiotic bacteria (Table 3). The assumption is that non-symbiotic bacteria influenced an increase in nutrient uptake by the plant and thus the increase in protein content in soybeans, which is in accordance with the results of research carried out by other authors (Antunović et al., 2012; Yin et al., 2016).

Statistically significant differences were found between the two locations in almost all observed parameteres although the same cultivar was sown. This can be mostly explained through determined soil chemical properties of these two locations (Table 1) and the statement of Vratarić and Sudarić (2000) that differences in soybean yield between individual cultivars are conditioned by differences in the genetic basis, and that within a cultivar, yield differences are conditioned by environmental influences.

Although there were differences in plant density, no correlation was found between plant density and grain yield at individual sites (Figure 1). However, if locations were excluded, then the correlation between these two parameters is positive and very significant (r = 0.69).

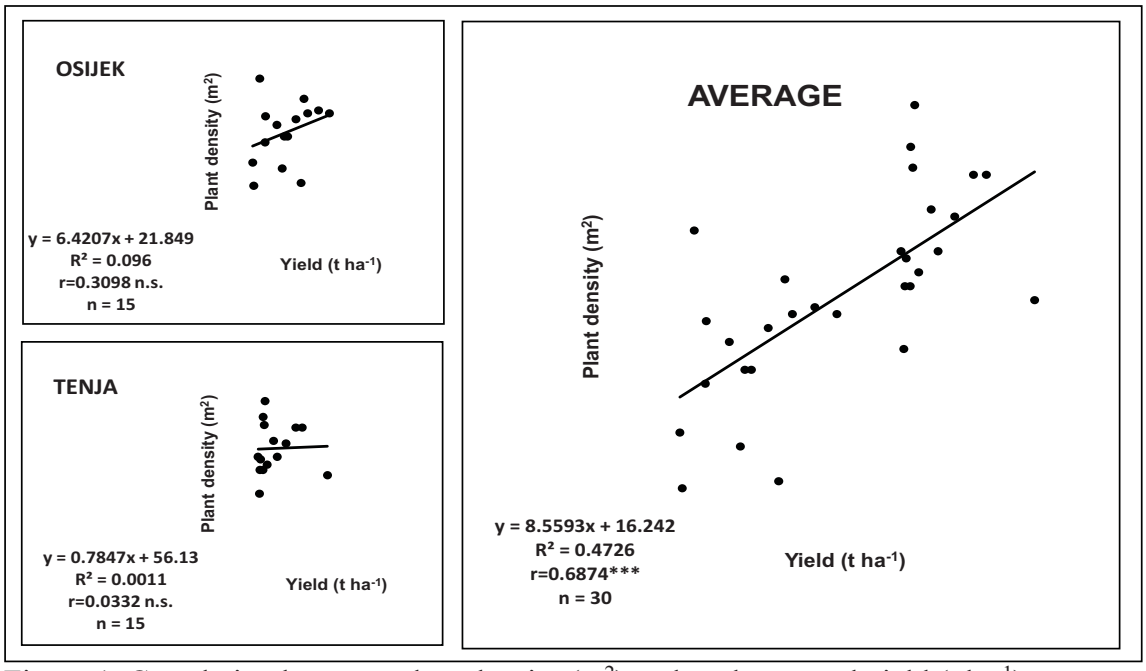

Figure 1. Correlation between plant density (m^2) and soybean seed yield (t ha^{-1})

According to obtained results of oil and protein content in seed, a very strong negative correlation of the mentioned parameters was determined at individual locations and also at the level of both experiments (Figure 2). Thus, the correlation coefficient between oil and protein content at the Osijek location was r = -0.9391, at the Tenja location r = -0.5736, while at the level of both localities it was r = -0.9384. The obtained results are in accordance with the results of other authors (Chung et al., 2003; Popovic et al., 2013).

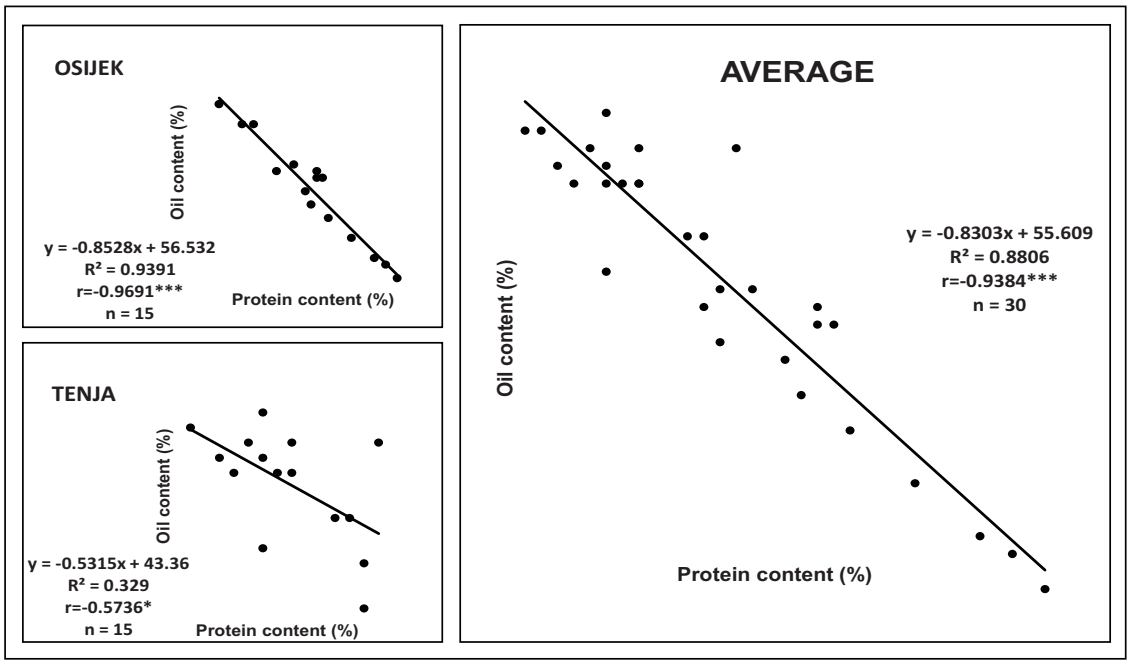

Figure 2. Correlation between oil content (%) and protein content (%) in soybean seed

Conclusions

Soybean seed inoculation with RizoliqTop inoculant which contains bacteria of the genus *Bradyrhizobium* resulted in a 18.6% increase in yield compared to the control variant at Osijek location and in a 36.5% increase in plant density compared to the variant with non-

simbiotic bacteria inoculation at Tenja location, although this increase did not result in higher yield compared to other variants. Obtained results do not indicate positive effects of soybean seed inoculation which points to the fact that the effect of seed inoculation is unpredictable and that more long-term researches are needed to establish the effects of seed inoculation.

References

Antunović M., Rastija M., Sudarić A., Varga I., Jović J. (2012). Response of soybean to phosphorus fertilization under drought stress conditions. Növénytermelés. 61 (2): 117-120.

Chung J., Barka H. L., Staswick P. E., Le D. J., Gregan P. B., Shoemaker R. C., Specht J. E. (2003.). The seed protein, oil and yield QTL on soybean linkage group I. Crop Sciences. 43: 1053-1067.

Hymowitz T., Bernard R. L. (1991). Origin of the soybean and germpiasm introduction and development in North America, in Use of Plant Introductions in Cultivar Development Part I, Shands, H. L. and Wiesner, L. E., eds., Crop Sci. Soc. of Amer., Madison, WI, 147.

FAOSTAT (2022). https://www.fao.org/faostat/en/#data [23.05.2022]

Flajšman M., Šantavec I., Kolmanič A., Kocjan Ačko D. (2019). Bacterial seed inoculation and row spacing affect the nutritional composition and agronomic performance of soybean. International Journal of Plant Production, 13: 183−192.

Igiehon O. N., Babalola O. O. (2021). Rhizobium and Mycorrhizal Fungal Species Improved Soybean Yield Under Drought Stress Conditions. Current Microbiology. 78 (4): 1615-1627.

Leggett M., Diaz-Zorita M., Koivunen M., Bowman R., Pesek R., Stevenson C., Leister T. (2017). Soybean Response to Inoculation with Bradyrhizobium japonicum in the United States and Argentina. Agronomy Journal. 109:1031–1038.

Marinković J., Bjelić D., Tintor B., Miladinović J., Dukić V., Dordević V. (2018). Effects of soybean co-inoculation with plant growth promotingrhizobacteria in field trial. Rom Biotechnol Lett. 23: 13401–13408.

Oilseed & Grain news. (2022). Available online: https://www.oilseedandgrain.com/soy-facts [20.01.2022].

Popovic V., Miladinovic J., Tatic M., Djekic V., Dozet G., Đurić V., Grahovac N. (2013). Stability of soybean yield and quality components. African Journal of Agricultural Research. 8 (45): 5651-5658.

Shiri-Janagard M., Raei Y., Gasemi-Golezani G., Aliasgarzad N. (2012). Influence of Bradyrhizobium japonicum and phosphate solubiliz-ing bacteria on soybean yield at different levels of nitrogen and phosphorus. International journal of Agronomy and Plant Production. 3: 544–549.

Ulzen J., Abaidoo R. C., Mensah N. E., Masso C., AbdelGadir A. H. (2016). Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in Northern Ghana. Frontiers in plant science. 7: 1770.

Vratarić M., Sudarić A. (2000.). Soja Glycine max (L.) Merr. Poljoprivredni institut Osijek, Osijek.

Yin X., Bellaloui N., McClure A. M.,Tyler D. D., Mengistu A. (2016). Phosphorusfertilization differentially influences fatty acids, protein, and oil in soybean. American Journal of Plant Sciences. 7 (14): 1975-1992.

REVIEW ARTICLE

Potential of legume sprouts as functional food

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Summary
Sprouts are considered a natural functional food that can be enriched with phytochemicals by manipulating growth, adding essential micronutrients. In the human diet, there is a growing interest in adapting one's eating habits in order to maintain and improve the health of those interested in using seedlings in the diet. The main advantage is that the seeds used for sprouts have not been previously treated with pesticides and the production of sprouts is usually very simple and fast. Recently, sprouts use has increased in the human diet in every aspect, not just in the vegan diet.

Key words: sprouts, diet, legumes, benefits

Introduction

Functional food is one that, in addition to its basic nutritional functions, provides health benefits, reducing the risk of chronic degenerative diseases, such as cancer and diabetes, among others. Sprouts of different plant seeds are well recognised as a potencial functional food. Health benefits of sprouts are antioxidant activity, cytotoxic, antidiabetic, antiviral, antiatherosclerosis, hypocholesterolemic and anti-obesity activity. In recent years, it has become increasingly popular to add germinated cereals to food (Pasko et al., 2015). Now the knowledge about the harms and benefits of germinated cereals is quite fragmented, so some people agree that they are a real source of vitamins and minerals, but others think that in can cause serious damage to the body. Only in one they converge - before you directly enter the diet seedling, people suffering from chronic diseases, you should consult with your doctor. Gu et al. (2017) found that sprouting in darkness was a useful method for increasing the number of isoflavone aglycones, B soyasaponins, phytosterols, inositol metabolites, antioxidants, and amino acids associated with nutritional and/or taste qualities of soybean products. According to USDA (2021), 100 g of broccoli sprouts (*Brassica oleracea* var. *italica*) contains 36 kcal energy, 3.57 g of protein, 107 mg of calcium, Ca, 1.43 mg of iron, Fe. The beneficial properties of broccoli sprouts are mainly attributed to the reduction of oxidative stress by activating antioxidants (Wu et al., 2004). Sprouts are commonly grown in the dark under high relative humidity. They are harvested when the cotyledons are still under-developed and true leaves have not begun to emerge, usually after 3–5 days from seed hydration. The entire plant (root, seed, and shoot) is consumed (Ebert, 2022).

Soybean sprouts

Sprouts develop from seeds during the germination process. They are a major source of proteins, vitamins, minerals because they are used in the initial growth phase of the plant, while their concentration is very high. During the process of germination, the amount of anti-nutritive substances (trypsin inhibitor, photoacid, tannin) decreases thus improving the phytochemical properties (Márton et al., 2010). That is why sprouts are a functional food product that has a positive impact on the human body and health maintenance. According to the USDA (2021), 100 grams of sprouts of golden beans (*Vigna radiata* L.) or soybean

(*Glycine max* L. Merr.) have a nutritional value of 30 cal and contain 90,4 g of water and much minerals (Table 1).
In addition, as with other foods of plant origin, sprouts do not contain cholesterol, which can eventually cause primarily atherosclerosis, and then other heart and circulatory diseases, thereby increasing the risk of cardiovascular diseases (Zempelas and Magriplis, 2019).

Table 1. Nutritional composition of 100 g of soybean or golden beans (*Source:* USDA, 2021)

Group	Contains	Minerals	Contains	Vitamins	Contains
Proteins	3.04 g	Calcium, Ca	13.0 mg	Ascorbic acid (C)	13.2 mg
Fat	5.94 g	Iron, Fe	0.91 mg	Pyridoxine (B6)	0.088 mg
Fibers	1.80 g	Magnesium Mg	21.0 mg	E	0.1 mg
Carbohydrates	5.94 g	Phosphorus, P	54.0 mg	K	33 µg
		Potassium, K	149 mg	Thiamine (B1)	0.084 mg
		Sodium, Na	6 mg	Riboflavin (B2)	0.124 mg
		Zink, Zn	0.41 mg	Niacin (B3)	0.749 mg
		Copper, Cu	0.164 mg	Folate	61 µg
		Selenium, Se	0.6 µg	Choline (B4)	14.4 mg

It is interesting to point out that folic in sprouts are important vitamins of the B group. Folic is a form found primarily in food and in human organisms, in 90% of cases in the form of 5-methyltetrahydrofolate (5-MTHF), and folic acid is the basic ingredient of food supplements used to supplement vitamin B9 intake. It is recommended that the average adult needs are 400 µg/day, and in pregnant and breastfeeding women 600 µg/day and 500 µg/day, while the intake of this vitamin with food does not reach half of those values. Germination of these seeds caused a decrease in total protein, fat and carbohydrate contents with increased germination time in all legume seed flours while non-protein nitrogen increased. Germination resulted in a significant decrease in the antinutritional factors of all germinated legume seed flours. Levels of trypsin inhibitors and tannins decreased in the first 72 hr then increased gradually in the last 120 hr but remained lower than the controls. Reduction in phytic acid and hemagglutinin activities increased with increased germination time. Germination significantly improved in vitro protein digestibility (El-Adawy et. al., 2003). Gu et al. (2012) monitored the metabolic profile of soybean sprouts for 0, 1, 2, 3, and 4 days. Their data shows that sprouting is a useful processing step to improve soybean nutritional quality, and metabolomic analysis is useful in understanding nutritional change during sprouting. Metabolic analysis of non-acrylic soybean seed based on rice seeds and germs with different periods of germination showed that the profiles of primary derivatives associated with energy production and secondary metabolites with different health benefits were changed significantly during germination.

Lens sprouts

The lens sprouts according to USDA (2021) contain in 100 grams sprouts: 67.34 g water, 106 kcal energy, 8.96 g protein, 0.55 g fat, 16.5 mg vitamin C, 100 g folate. The lens germination process increases phenol content by as much as 122%. Phenol compounds are a group of plant antioxidants that can provide anticarcinogens, anti-inflammatory, and anti-allergic properties (Singh et al., 2017). Due to increased antioxidant capacity, lens sprouts may reduce LDL (poor) cholesterol (Huang et al., 2008). Unlike kidney bean sprouts, lens sprouts can also be enjoyed boiled or raw.

Pea sprouts

Pea sprouts (*Pisum sativum* L.) are characteristic of their slightly sweet taste. Both green and yellow peas can sprout. According to USDA (2021), 100 grams of pea traps contain 62.27 g

water, 124 kcal energy, 8.8 g protein, 0.68 g fat, 10.4 mg vitamin C, 144 g folate. Pea sprouts contain almost twice the amount of folate (B9) as raw peas. The lack of this vitamin may result in congenital damage (Czeizel et al., 2013). Pea sprouts are more gentle than most sprouts. They are well combined with leafy greenery in salads, but they can also be fried.

Chickpews sprouts

Chickpea sprouts (*Cicer arietinum* L.) also called garbanzo bean or Bengal gram are easily produced and take about 2 days to sprout, which is relatively fast. They contain more proteins than other sprouts and are full of nutrients. According to USDA (2021), 100 sprouts of chickpea contain 7.68 g water, 378 kcal energy, 20.47 g protein, 6.04 g fat, 4 mg vitamin C, 557 g folate. It is interesting to note that germination drastically increases total isoflavone content in chickpeas by more than 100 times. Isoflavones are a phytoestrogen – a herbal compound imitating the role of estrogen hormone (Wu et al., 2012). Germinating succulents can be eaten raw as a fast and nutritious snack or mixed to produce raw hummus.

Golden bean sprouts

Golden beans or green soy sprouts (*Vigna radiata L.*) originate from the beans of mung, which are mostly grown in Eastern Asia but are also popular in many western restaurants and shops. They have an extremely low-calorie count, according to USDA (2021), 100 grams of lightning bean sprouts contain 90.4 g water, 30 kcal energy, 3.04 g protein, 0.18 g fat, 13.2 mg vitamin C, 61 ug folate. Germination increases the flavonoid and male beans content by up to 7 and 24 times. In return, this enhances their antioxidant properties (Guo et al., 2012). Moreover, some investigations connect these sprouts with potential anti-carcinogenic benefits by fighting harmful free radicals (Sawa et al., 1999). Similarly, a study in human cells treated with this extract revealed a toxic effect on cancer cells - without damage to healthy cells (Hafidh et al., 2012). The golden beans are the basic ingredient of Asian cuisine and are therefore perfect for dishes such as roasted rice and spring pastries.

Adzuki bean sprouts

Adzuki bean sprouts (*Vigna angularis* Willd. Ohwi & H. Ohashi) are small red beans grown in Eastern Asia and very similar to lightning beans. According to USDA (2021), adzuki bean sprouts in 100 grams contain 13.4 g water, 329 kcal energy, 19.9 g protein, 0.53 g fat, 622 g folate. As with most sprouted beans, the beans of adzuki, which grow and increase the proportion of phenol antioxidants by 25%. The most prominent phenolic compound in these sprouts is synaptic acid (Khang et al., 2016). Synaptic acid has many health-promoting properties, including improved blood sugar monitoring and anti-inflammatory, antibacterial, and anti-carcinogenic effects. Adzuki beans sprouts have a nutty flavor and can be added raw to lettuces, coatings, and smoothies. It can be cooked in soup as well.

Alfafla sprouts

Alfalfa sprouts (*Medicago sativa* L.) in 100 grams contain (USDA, 2021): 30.6 g of water, 7.59 kcal of energy, 1.23 g of protein, 2.71 mg of vitamin C, 11.9 g of folate, 10.1 g of vitamin K. Alfalfa sprouts contain high vitamins of K. Although it benefits most people, they may be dangerous for others. High doses of vitamin K may cause blood thinners such as warfarin to be less effective. It is therefore important that people taking these medicines avoid major changes in their vitamin K intake (Mousa, 2010).

Gaweł at al. (2017) and associates tested the amount of protein in the human diet. Research results have shown that the alfalfa sprouts concentrate can be a food supplement that helps to combat malnutrition and various digestive tract problems, increases the immune system's resistance, improves hemoglobin content in the blood helps to recover from severe cancer-

related disorders, and supplements the treatment of certain diseases such as tuberculosis, HIV, and malaria.

Mung bean sprouts

Mung bean (*Vigna radiata* L.) becoming popular functional food in promoting good health and prevent cancer and melanogenesis (Hou et al., 2019). The sprouts of Mung beans or green soybeans come from mung beans, which are mainly grown in East Asia, but are also popular in many Western restaurants and shops. They have an extremely low number of calories, according to the USDA (2021), 100 grams of mung bean sprouts contain: 90.4 g of water, 30 kcal of energy, 3.04 g of protein, 0.18 g of fat, 13.2 mg of vitamin C, 61 ug folate. Germination increases the content of flavonoids up from 7 and 24 times. In turn, this enhances their antioxidant properties (Guo et al., 2012). Moreover, some research links these germs to potential anticancer benefits by fighting harmful free radicals (Sawa et al., 1999). Similarly, a study on human cells treated with this extract found a toxic effect on cancer cells - without damaging healthy cells (Hafidh et al., 2012). The popularity of mung bean is related to its specifc growth characteristics, such as relative drought tolerance and short growth cycle (70–90 days) (Hou et al., 2019.). Mungbean has been reported to be suitable for children as well keeping in view of its lesser flatulence and hypoallergic properties (Sehrawat et al., 2020.)

Conclusion

Legumes are valued worldwide as a sustainable and inexpensive meat alternative and are considered to be one of the most important food sources. Legume sprouts are rich in few important micronutrients, including potassium, folate, iron, magnesium and zinc and they are an important source of protein. They are among the only plant foods that provide significant amounts of amino acids. Some reasearches have shown that most of the bioactive compounds in legumes possess antioxidant properties,which play a role in the preventation of some cancers, osteoporosis, heart diseases and other degenerative diseases.

References

Czeizel A., Dudás I., Vereczkey A., Bánhidy F. (2013). Folate Deficiency and Folic Acid Supplementation: The Prevention of Neural-Tube Defects and Congenital Heart Defects. Nutrients *5(11), 4760–4775.*

Ebert A, (2022). Sprouts and Microgreens—Novel Food Sources for Healthy Diets 11(4), 571; https://doi.org/10.3390/plants11040571

El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A., & El-Beltagy, A. E. (2003). Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. Plant Foods for Human Nutrition, 58(3), 1–13.

Gaweł, E., Grzelak, M., Janyszek, M. (2017). Lucerne (*Medicago sativa* L.) in the human diet—Case reports and short reports. Journal of Herbal Medicine, 10, 8–16.

Gu, E.-J., Kim, D. W., Jang, G.-J., Song, S. H., Lee, J. I., Lee, S. B., Kim, B. M., Cho, Y., Lee, H.-J, Kim, H.-J. (2017). Mass-based metabolomic analysis of soybean sprouts during germination. Food Chemistry, 217, 311–319.

Guo X., Li, T., Tang, K., Liu, R. H. (2012). Effect of Germination on Phytochemical Profiles and Antioxidant Activity of Mung Bean Sprouts (*Vigna radiata L.*). Journal of Agricultural and Food Chemistry, 60(44), 11050–11055.

Hafidh, R. R., Abdulamir, A. S., Bakar, F. A., Jalilian, F. A., Abas, F., Sekawi, Z. (2012). Novel molecular, cytotoxical, and immunological study on promising and selective anticancer activity of Mung bean sprouts. BMC Complementary and Alternative Medicine, 12(1).

Hou D, Yousaf L, Xue Y, Hu J, Wu J, Hu X, Feng N, Shen Q (2019). Mung bean (*Vigna radiata* L.): bioactive polyphenols, polysaccharides, peptides, and health benefts. Nutrients 11(6):1238

Huang H., Mai W., Liu D., Hao Y., Tao J., Dong Y. (2008). The oxidation ratio of LDL: A Predictor for Coronary Artery Disease. Disease Markers, 24(6), 341-349.

Khang D. T., Dung T. N., Elzaawely A. A., Xuan T. D. (2016). Phenolic profiles and antioxidant activity of germinated legumes. *Foods*, *5*(2), 27.

Márton M., Mándoki Zs., Csapó-Kiss Zs, Csapó, J. (2011). The role of sprouts in human nutrition. A review. Acta Universty.Sapientiae, Alimentaria, 3: 81-117.

Mousa S.A. (2010). Antithrombotic effects of naturally derived products on coagulation and platelet function. in anticoagulants, antiplatelets, and thrombolytics (pp. 229-240). Humana Press, Totowa, NJ

Pasko P., Gdula-Argasinska J., Podporska-Carroll J., Quilty B., Wietecha-Posluszny R., Tyszka-Czochara M., Zagrodzki P. (2015). Influence of selenium supplementation on fatty acids profile and biological activity of four edible amaranth sprouts as new kind of functional food. Journal of food science and technology, 52(8), 4724-4736.

Sawa T., Nakao M., Akaike T., Ono K., Maeda H. (1999). Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: Implications for the anti-tumor-promoter effect of vegetables Journal of agricultural and food chemistry, 47(2), 397–402.

Sehrawat D, Yadav M, Kumar S, Upadhyay S.K., Singh M, Sharma A.K. (2020). Review on health promoting biological activities of mungbean: A potent functional food of medicinal importance, Vol. 20, Supplement 2, 2020 pp. 2969-2975

Singh B., Singh J. P., Kaur A., Singh N. (2017). Phenolic composition and antioxidant potential of grain legume seeds: A review. Food Research International, 101, 1–16.

USDA, 2021. U.S. Department of Agriculture. https://www.usda.gov/ (accesed on 22th December 2021)

Wu L., Noyan M.H., Facci M. (2004). Dietary approach to attenuate oxidative stress, hypertension, and inflammation in the cardiovascular system. Proc Natl Acad Sci, 101 (18): 7094–7099.

Zempelas A., Magriplis E. (2019). New Insights into Cholesterol Functions: A Friend or an Enemy? Nutrients, 11(7): 1645. doi: 10.3390/nu11071645

Potencijal klica mahunarki kao funkcionalne hrane

Sažetak

Klice se smatraju prirodnom funkcionalnom hranom koja se može obogatiti fitokemikalijama i dodavanjem esencijalnih mikronutrijenata. S obzirom da raste interes za promjenom prehrambenih navika u svrhu održavanja i poboljšanja zdravlja uzgoj klica ima veliku važnost u prehrani. Glavna prednost je što sjeme koje se koristi za klice nije prethodno tretirano pesticidima te je proizvodnja klica obično vrlo jednostavna i brza. U posljednje vrijeme je generalno povećana upotreba klica u ljudskoj prehrani, a ne odnosi se samo na vegansku prehranu.

Ključne riječi: klice, prehrana, mahunarke, dobrobiti

ORIGINAL SCIENTIFIC PAPER

Towards the use of undervalued African truffle and pearl millet to modulate glucose release in crackers

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Abstract
Indigenous and underutilised food resources can play a role in nutrition. Pearl millet, an underutilised cereal, has slowly digestible starch. However, it hardly features in many diets possibly due to its limited diversified use in high value convenient food products. Some mushrooms also improve postprandial glycaemic responses. This study investigated the effect of compositing Kalahari truffle (KT) with fermented pearl millet and bread wheat flour on the nutrient and *in vitro* starch digestibility of the crackers. Fermented whole grain pearl millet flour was mixed with bread wheat flour (1:1). KT powder was added to comprise 5, 10 and 15% of the composite flours. KT incorporation significantly increased the crackers' protein and significantly reduced their *in vitro* starch digestibility.

Key words: mushroom, diabetes, glycemic, indigenous, digestibility

Introduction

Generally, cereal grains are responsible for the huge part of human carbohydrates intake, thus the alteration of carbohydrate especially starch quality in cereal products has a potential positive effect on nutrition. In-vitro starch digestibility assist in the description of the carbohydrate component of a meal (Englyst and Englyst, 2005). Starch that is hydrolysed within 20 minutes is regarded as rapidly digestible starch, whereas starch that is digested between 20 and 120 minutes is called slowly digestible starch, beyond 120 minutes, it is regarded as resistant starch (Englyst et al., 1992). Rice, barley, maize, wheat, sorghum, oat, rye and millets are classified as major cereals in terms of their contribution to people's diets (Adebiyi et al., 2018). Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is cultivated in India, West and Central Africa, southern Africa, Brazil and the United States of America (Taylor, 2016). It is a staple food for over half of the Namibian human population. Pearl millet is gluten free, making it one of the low-cost alternative cereal for people suffering celiac disease (Dias-Martins et al., 2018). Additional health benefits include the management of blood glucose levels, as well as anti-cancer and prebiotic properties (Dias-Martins et al., 2018). On the other hand, millets contain less than the recommended lysine content required by adults (only 17% of the 1.93 g) (Taylor, 2016). This impediment may be overcome by incorporating mushrooms in pearl millet products. Edible mushrooms are known to be an excellent source of protein, with exceptional amounts of essential amino acids (Islam et al., 2016). Additionally, mushrooms contain β-glucans which are known to positively influence postprandial glycaemic response in humans (Ng et al., 2017).

Namibia is home to indigenous and or endemic species of edible mushrooms such as Kalahari truffles (*Kalaharituber pfeilii*), *Termitomyces schimperi*, *Termitomyces sagittiformis*, and Ganoderma mushroom species, which are barely utilised commercially. With an increased consumer interest in functional foods and biscuits and crackers can be excellent products for functional ingredients due to their popular consumption (Pasqualone et al., 2015). Crackers are significant in the baking industry due to their low moisture and

sugar contents which contribute to a better shelf life and they also offer variety in taste, texture and aroma (Reddy et al., 2019). Crackers belong to a group of crisp, chemically leavened or fermented bakery products that may contain up to 30% fat (Reddy et al., 2019). Crackers can either be fermented (soda, saltine and cream crackers) or chemically leavened (snack crackers) (Reddy et al., 2019). The aim of this study was to develop mushroom incorporated pearl millet-wheat crackers and evaluate the mushroom's influence on the resultant crackers' nutrients content and starch digestibility.

Materials and Methods

Pearl millet grains were purchased from subsistence farmers around Omuthiya gwIipundi in Oshikoto region. Grains were cleaned to remove foreign objects and fermented at a ratio of 1:2 (w/v) for 24 hours at 37 ºC as outlined by Onweluzo and Nwabugwu (2009). Fermented grain was dried at 40 ºC in an oven (Scientific, Model 222, South Africa) for 48 hours before milling using a hammer mill (Trapp TRF 4000, Metalúrgica Trapp Ltda. Brazil) and passed through a 250 µm screen. Bread wheat flour was purchased from a local retail shop in Windhoek, Namibia. Kalahari truffles were purchased from informal vendors at Omuthiya gwIipundi Informal (open) Market in Oshikoto region. Truffles were sliced and dried at 40 °C for 48 hours. The mushroom was milled using a blender (Waring Commercial 7011HS 2-Speed Food Blender) before passing through a 250 µm screen.

Composite flours were prepared by mixing equal amounts of wheat and pearl millet flours to make a pearl millet-wheat composite flour (control). The composite crackers were formulated with 51.4% pearl millet-wheat flour (1:1) for control sample. The pearl millet wheat flour was then replaced by 5%, 10% and 15% Kalahari truffle flours for the other samples. The ingredients at constant inputs in all samples were baking powder (0.9%), sugar (1.4%), salt (1.7%), vegetable oil (17.8%) and water (26.8%). The dough was rested for 20 minutes followed by sheeting and cutting. The crackers were baked at 180 °C in a conventional oven (Macadams Convecta 7, Cape Town, South Africa) for 8 minutes.

Crackers were ground using a blender (Waring Commercial 7011HS 2-Speed Food Blender) and passed through a 500 µm screen. Moisture, ash, crude fat, crude protein and crude fibre contents were determined following AACC methods 44-15.02 A, 08-01, 30-10.01, 46-30.01 and 32-10.01 respectively. Total starch was determined using the Megazyme Total Starch Assay Kit. *In vitro* starch digestibility was determined using a modified Englyst method (Englyst et al., 1992). About 100 mg of dry sample was suspended in 2 mL 0.1 M sodium acetate buffer (pH 5.2) and incubated at 37 °C for 5 minutes before the addition of 100 µL of diluted α-amylase and 100 µL of diluted amyloglucosidase. Samples were incubated in a shaking water bath at 37 °C for 120 minutes. 50 µL aliquots were pipetted into 2 mL micro-centrifuge tubes containing 400 µL of cold ethanol after 20, 60 and 120 minutes of digestion. Glucose released at the specified time intervals was determined using the Megazyme D-Glucose Assay Procedure (GOPOD).

The experiment (baking) was done twice. All analyses were done in duplicates and results represent the average of duplicates, presented as means ± standard deviation. Data obtained was subjected to an analysis of variance (ANOVA) and Duncan's least significant differences (LSD) test using SPSS Statistics Software, Version 25 (IBM, USA).

Results and discussion

Results for the chemical characteristics of mushroom supplemented pearl millet-wheat crackers are presented in Table 1. Kalahari truffle crackers' moisture content decreased as the percentage of Kalahari truffle powder increased, except at 5% mushroom inclusion. These results are also consistent with the findings of Ng et al., (2017). In contrast, Gadallah and Ashoush, (2016) reported that the moisture content of biscuits fortified with desert truffle powder increased as the truffle powder percentage was increased. The fibre in the

mushrooms' powders absorbs water and that could be the reason for the inverse relationship between moisture content and mushroom incorporation (Ng et al., 2017).

Table 1. Chemical characteristics of mushroom supplemented pearl millet-wheat crackers.

Crackers	Moisture (%)	Ash (%)	Protein	Total Starch (%)	Crude Fibre	Crude Fat
Control	3.44 ±0.01^{d}	3.77 ±0.02^{a}	8.72 ±0.26^{a}	58.99 ±3.03^{b}	0.93 ±0.19^{a}	25.50 ±0.47^{a}
5% KTC	4.27 ±0.00^{f}	3.83 ±0.00^{a}	9.13 ±0.09^{b}	50.04 ±3.13^{a}	1.06 ±0.18^{ab}	25.79 ±1.23^{a}
10% KTC	3.18 ±0.04^{c}	3.96 ±0.05^{b}	9.67 ±0.14^{c}	48.97 ±4.77^{a}	1.51 ±0.12^{abc}	26.11 ±0.97^{a}
15% KTC	2.22 ±0.01^{a}	4.19 ±0.00^{c}	10.55 ±0.47^{d}	48.62 ±2.56^{a}	1.68 ±0.29^{c}	26.21 ±0.29^{a}

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different ($p > 0.05$). Values were adjusted to dry weight conditions. KTC = Kalahari truffle cracker

The control cracker had the least ash content (3.77%). There was a gradual increase in the cracker's ash content with every increase in Kalahari truffle incorporation since the truffle had a high ash content (5.61%). This is consistent with the findings of Farzana and Mohajan (2015) who found that the ash content of mushroom fortified biscuits increased (from 1.50% to 1.76%) with every increase in the percentage of soy flour. These findings suggest that the addition of Kalahari truffle can potentially be used in improving the mineral content of cereal based baked products. Kalahari truffle incorporation significantly increased the crackers' protein content. This could have been due to the mushrooms' high protein content than that of wheat or pearl millet. The results are in agreement with the trend of protein content found in composite biscuits by Kolawole et al. (2018). Kalahari truffle incorporation significantly reduced the cracker's starch content (59% to 49%). The decrease in the mushroom incorporated cracker's starch content could be attributed to the Kalahari truffle low starch content. The decrease in starch content is consistent with the findings of Farzana and Mohajan (2015) who recorded a reduction (from 65.62% to 56.38%) in the starch content of mushroom fortified biscuits. Similarly, Ng et al. (2017) also reported a decrease in the starch content of mushroom fortified biscuits as the mushroom percentage increased. Gadallah and Ashoush (2016) also reported similar findings on biscuits fortified with desert truffle powder. Kalahari truffle incorporation of up to 15% significantly increased the crackers' crude fibre content from 0.93 to 1.68%. A similar trend was reported by Kolawole et al. (2018). The crackers' fat content was between 25.02 and 26.21%. There was no significant difference observed between the control and all Kalahari truffle crackers.

In vitro starch digestibility results are presented in Table 2. Compared to the mushroom powder enriched crackers, the control cracker had the highest digestible starch at both G20 (16.3%), G60 (26.6%) and G120 (37.7%), respectively. It was observed that starch digestibility significantly ($p < 0.05$) decreased as Kalahari truffle powder incorporation increased. This could be a result of the low starch contents of Kalahari truffle powders and/or the effect of mushroom powder on the digestibility of starch. These results are consistent with the findings of Ng et al. (2017), who reported a similar trend in mushroom fortified biscuits. Similarly Brennan et al. (2013) recorded a decline in the amount of glucose released from mushroom enriched extruded snacks.

Table 2 *In vitro* starch digestibility of Kalahari truffle enriched crackers (%).

Crackers	G20	G60	G120
Control	16.32 ±0.55[c]	26.63 ±1.08[b]	37.71 ±1.05[d]
5% KTC	15.89 ±0.39[bc]	25.84 ±1.40[ab]	33.75 ±0.34[c]
10% KTC	15.09 ±0.04[b]	23.54 ±2.92[a]	32.78 ±1.88[bc]
15% KTC	14.16 ±0.42[a]	23.67 ±1.82[a]	30.11 ±0.86[a]

*Values are means (n = 4) ± standard deviation, values with the same letter in a column are not significantly different ($p > 0.05$). KTC = Kalahari truffle cracker. G20 = glucose measured at 20 min of hydrolysis; G60 = glucose measured at 60 min of hydrolysis; G120 = glucose measured at 120 min of hydrolysis

The decline in the release of glucose could be due to the increase in mushroom percentage, which could have in turn resulted in an increase in the crackers' dietary fibre content. Dietary fibre is especially important as it improves glucose metabolism and insulin response in patient suffering from Type 2 diabetes, by facilitating the gradual hydrolysis of carbohydrates and glucose release (Brennan, 2005). Wan Rosli et al. (2012) reported an increase in the amount of dietary fibre as the mushroom percentage increased in butter biscuits. These authors reported that butter biscuits formulated with 4% and 6% mushroom powder had high amounts of β-glucans, 0.72% and 0.79% respectively. Mushroom dietary fibre has been of interest lately, as it has been found to contain β-glucans which have positive effects on blood glucose modulation (Cheung, 2013). The soluble fraction of β-glucans is apparently responsible for their ability to lower blood glucose levels (De Paula et al., 2017). These *in vitro* starch digestibility results showed that the compositing of Kalahari truffle powder into crackers can significantly reduce the amount of glucose released. Therefore, the incorporation of the Kalahari truffle in pearl millet-wheat crackers can potentially be beneficial to individuals with the Type 2 diabetes.

Conclusions

Incorporating Kalahari truffle in pearl millet-wheat crackers increased the protein content of resultant crackers. Furthermore, Kalahari truffle can be used in the development of low *in vitro* starch digestibility foods. Pending large consumer acceptance assessments, this study can be a foundation to the development of value-added Kalahari truffles and pearl millet healthful products.

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References

Adebiyi J. A., Obadina A. O., Adebo O. A., Kayitesi E. (2018). Fermented and malted millet products in Africa: Expedition from traditional/ethnic foods to industrial value-added products. Critical Reviews in Food Science and Nutrition. 58: 463–474.

Brennan C. S. (2005). Dietary fibre, glycaemic response, and diabetes. Molecular Nutrition and Food Research. 49: 560–570.

Brennan M. A., Derbyshire E., Tiwari B. K., Brennan C. S. (2013). Integration of β-Glucan Fibre Rich Fractions from Barley and Mushrooms to Form Healthy Extruded Snacks. Plant Foods for Human Nutrition. 68: 78–82.

Cheung P. C. K. (2013). Mini-review on edible mushrooms as source of dietary fiber : Preparation and health benefits. Food Science and Human Wellness. 2: 162–166.

De Paula R., Abdel-Aal E. S. M., Messia M. C., Rabalski I., Marconi E. (2017). Effect of

processing on the beta-glucan physicochemical properties in barley and semolina pasta. Journal of Cereal Science. 75: 124–131.

Dias-Martins A. M., Letícia K., Pessanha F., Pacheco S., Avelino J., Rodrigues S., Wanderlei C., Carvalho, P. (2018). Potential use of pearl millet (*Pennisetum glaucum* (L .) R . Br .) in Brazil : Food security, processing , health bene fi ts and nutritional products. Food Research International. 109:175–186.

Englyst H. N., Kingman S. M., Cummings J.(1992). Classification and measurement of nutritionally important starch fractions.pdf. European Journal of Clinical Nutrition. 46: S33–S50.

Englyst K. N., and Englyst H. N. (2005). Carbohydrate bioavailability. British Journal of Nutrition 94: 1–11.

Farzana T., and Mohajan S. (2015). Effect of incorporation of soy flour to wheat flour on nutritional and sensory quality of biscuits fortified with mushroom. Food Science and Nutrition. 3: 363–369.

Gadallah G. E. M., and Ashoush S. I. (2016). Value Addition on Nutritional and Sensory Properties of Biscuit Using Desert Truffle (*Terfezia claveryi*) Powder. Food and Nutrition Sciences. 07: 1171–1181.

Islam T., Yu X., Xu B. (2016). Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushrooms commonly consumed in China. LWT - Food Science and Technology. 72: 423–431.

Kolawole F. L., Akinwande B. A., Ade-Omowaye B. I. O. (2018). Physicochemical properties of novel cookies produced from orange-fleshed sweet potato cookies enriched with sclerotium of edible mushroom (*Pleurotus tuberregium*). Journal of the Saudi Society of Agricultural Sciences. 19: 1–5.

Ng S. H., Robert S. D., Ahmad W. A. N. W., Ishak W. R. W. (2017). Incorporation of dietary fibre-rich oyster mushroom (Pleurotus sajor-caju) powder improves postprandial glycaemic response by interfering with starch granule structure and starch digestibility of biscuit. Food Chemistry. 227: 358–368.

Onweluzo J. C., and Nwabugwu C. C. (2009). Fermentation of millet (Pennisetum americanum) and Pigeon Pea (Cajanus cajan) seeds for flour production: Effects on composition and selected functional properties. Pakistan Journal of Nutrition. 8: 737–744.

Pasqualone A., Bianco A. M., Paradiso V. M., Summo C., Gambacorta G., Caponio F., Blanco A. (2015). Production and characterization of functional biscuits obtained from purple wheat. Food Chemistry. 180: 64–70.

Reddy P. A., Pranusha G., Rao G. L., Reddy A. N. (2019). Development and Analysis of Sorghum Crackers. International Journal of Scientific Research in Science and Technology. 6: 26–31.

Taylor J.R.N. (2016). Millet Pearl: Overview. *In Encyclopedia of Food Grains, Volume 1 2nd ed*, Wrigley C., Corke H., Seetharaman K., Faubion J., (eds.), (190–198). Oxford, United Kingdom: Academic Press, Elsevier.

Wan Rosli W. I., Nurhanan A. R., Aishah M. S. (2012). Effect of partial replacement of wheat flour with oyster mushroom (*Pleurotus sajor-caju*) powder on nutritional composition and sensory properties of butter biscuit. Sains Malaysiana. 41: 1565–1570.

IZVORNI ZNANSTVENI RAD

Utjecaj vremena primjene krutog stajskog gnoja na kvalitetu krme poluprirodnog travnjaka

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Sažetak
Cilj istraživanja bio je utvrditi utjecaj proljetne i jesenske gnojidbe poluprirodnog travnjaka krutim stajskim gnojem (KSG) u količini od 30 t ha^{-1} na kvalitetu krme nakon prve i desete godine provedbe istraživanja.
Između proljetne i jesenske primjene 30 t ha^{-1} KSG nisu utvrđene statistički značajne razlike u sadržaju sirovih proteina (P>0,05), neutralnih detergent vlakana (P>0,05) i kiselih detergent vlakana (P>0,05) krme poluprirodnog travnjaka nakon prve niti nakon desete godine istraživanja.
Zaključeno je da primjena 30 t ha^{-1} KSG nužno ne utječe na kvalitetu krme poluprirodnog travnjaka, ali omogućava kruženje organske tvari u prirodi, a time potiče održivu poljoprivrednu proizvodnja.

Ključne riječi: poluprirodni travnjak, kruti stajski gnoj, kvaliteta krme

Uvod

Poluprirodni travnjaci su značajan, ali nedovoljno iskorišten krmi resurs koji zauzima 40,59 % ukupno korištene poljoprivredne površine Republike Hrvatske (DZS, 2018.). Proizvodni potencijal poluprirodnih travnjaka se može povećati primjenom nekih od agrotehničkih zahvata u proizvodnji krme, poput gnojidbe (Štybnarova i sur, 2014.). Naime, utvrđena je pozitivna korelacija između plodnosti tla i hranidbene vrijednosti krme (Schulte i Herlily, 2007.). Gnojidbom se može povećati prinos krme (Simić i sur., 2016.), sadržaj sirovih proteina (SP) u krmi, udio lista, brzina probave stanične stjenke, konzumacija suhe tvari (ST), opskrbljenost životinja amino-kiselinama (McCartney i sur., 2004.), a time ukupna hranidbena vrijednost krme u hranidbi životinja (Vranić i sur., 2004., 2017.; Knežević i sur., 2009.). U ranijim je istraživanjima gnojidba travnjaka s 30 t ha^{-1} krutog stajskog gnoja (KSG) rezultirala većim prinosom ST travnjaka i većim sadržajem SP u usporedbi s primjenom 0 i 15 t ha^{-1} KSG (Patel i sur., 2008.). Stajski gnoj povećava sadržaj organske tvari u tlu pa gnojidba travnjaka s KSG u sušnim vegetacijskim sezonama ima izraženiji pozitivan utjecaj na prinos i hranidbenu vrijednost krme u odnosu na vegetacijske sezone s dovoljno padalina (Julianna i sur., 2013.). Naime, vegetacijska sezona može dovesti do značajnih razlika u kvaliteti iste biljne vrste košene u približno istoj fazi fenološke zrelosti (Buxton i Casler, 1993) te varijabilnog utjecaja gnojidbe na kvalitetu krme (Coleman i sur., 2004.). U nekim istraživanjima, gnojidba prirodnih travnjaka s 30 t ha^{-1} KSG je povećala prinos po hektaru, ali nije utjecala na kemijski sastav proizvedene krme (Simić i sur., 2016.). Hipoteza istraživanja je bila da će vrijeme primjene 30 t ha^{-1} KSG na poluprirodnom travnjaku tijekom 10 godina, u proljeće ili jesen, utjecati na sadržaj SP, sadržaj vlakana

ekstrahiranih s neutralnim detergentom (NDF) i vlaknana ekstrahiranim s kiselim detergentom (ADF).
Cilj istraživanja bio je utvrditi sadržaj SP, NDV i KDV krme poluprirodnog travnjaka nakon prve i nakon desete godine primjene KSG ovisno o vremenu primjene KSG.

Materijali i metode

Istraživanje je provedeno na pokusnim površinama pokušališta Sveučilišta u Zagrebu Agronomskog fakulteta Centar za travnjaštvo.

Gnojidbeni tretmani

Na poluprirodnom travnjaku zajednice *Arhenatheretum medioeuropaeum ruderale* provedeno je istraživanje vremena primjene KSG u razdoblju od deset godina (2004. – 2014.). Pokus je bio postavljen po slučajnom bloknom rasporedu s četiri ponavljanja. Istraživani su gnojidbeni tretmani primjene 30 t ha^{-1}KSG svako proljeće i svaku jesen od 2003. godine do 2014. godine. KSG je ručno ravnomjerno raspoređen grabljama po parcelama površine 12 m². Pokusne parcele su pokošene samohodnom oscilatornom kosilicom 2004. i 2014 godine. u početku metličanja dominantnih trava na visinu od 5 cm od razine tla. Kao obračunska parcela uzeta je površina od 1,2×6 m jer je košnja obavljena po sredini svake pokusne parcele na način da je kosilicom zahvaćena traka (zahvat kosilice) širine 1,2 m. Pokošena biljna masa je izvagana, te je uzet uzorak zelene mase (oko 500 g) koji je sušen na temperaturi od 60°C do konstantne mase radi utvrđivanja sadržaja ST. Osušeni uzorci su samljeveni korištenjem mlina čekićara na veličinu čestica od 1 mm.

Kemijske analize

U uzorcima krme poluprirodnog travnjaka utvrđen je sadržaj N metodom po Kjeldahlu (ISO 5983) korištenjem jedinice za razaranje te automatske jedinice za destilaciju i titraciju uzoraka (Gerhardt). Udio SP je u uzorcima dobiven množenjem udjela N s faktorom 6,25. Sadržaj NDV i KDV je utvrđen metodom po Van Soestu i sur. (1991.).

Statistička obrada rezultata istraživanja

Rezultati istraživanja su obrađeni u statističkom programu SAS, verzija 9.1.3. (SAS Institut, 1997.) pomoću MIXED procedure.

Rezultati i rasprava

Osnovni kemijski parametri hranidbene vrijednosti voluminozne krme obuhvaćaju sadržaj SP, NDV i KDV. Poželjno je da biljna masa ima što veći sadržaj SP koji se u supotnom moraju dodavati obroku iz koncentriranih krmiva kako bi se zadovoljile hranidbene potrebe životinja. S odgađanjem roka košnje biljne mase opada sadržaj SP i raste sadržaj vlakana. Sadržaj SP u biljnoj masi najčešće varira od 80-120 g kg^{-1} ST, sadržaj NDV od 450-550 g kg^{-1} ST, a sadržaj KDV od 300-400 g kg^{-1} ST (Chamberlain i Wilkinson, 1996).
Prosječan sadržaj SP, NDV i KDV za 2004 i 2014 godinu istraživanja po gnojidbenim tretmanima je prikazan u tablici 1.
Primjena 30 t ha^{-1} KSG u jesen povećava prinos ST krme poluprirodnog travnjaka u odnosu na proljetnu gnojidbu (Vranić i sur., 2021.) ili u odnosu na kontrolni tretman bez gnojidbe (Simić i sur., 2016.) ali utjecaj gnojidbe poluprirodnog travnjaka s 30 t ha^{-1} KSG na kemijski sastav krme može biti varijabilan (Coleman i sur., 2004.) i više ovisan o čimbenicima okoline (Simić i sur., 2016.). Najznačajniji utjecaj na kvalitetu krme ima fenološka zrelost tratine u trenutku košnje, zatim temperatura okoline, vlaga, jačina sunčevog zračenja i sadržaj hraniva u tlu (Buxton and Fales, 1994.). Utvrđeno je da razlike u mikroklimatskim čimbenicima između vegetacijskih sezona utječu na kemijski sastav proizvedene krme košene u približno istoj fazi zrelosti (Buxton i Casler, 1993.).

Tablica 1. Utjecaj vremena primjene 30 t ha^{-1} KSG na kemijski sastav krme poluprirodnog travnjaka za 2004. i 2014. godinu (u g kg^{-1} ST)

	Kemijski parametar					
	SP		NDF		ADF	
	g kg^{-1} ST					
Godina	Proljeće	Jesen	Proljeće	Jesen	Proljeće	Jesen
2004	128,17	125,12	499,73	495,29	352,58	346,96
2014	111,25	110,82	583,40	617,51	364,14	374,93
Signifikantnost						
Vrijeme primjene	NS		NS		NS	
Godina	*		**		**	
Vrijeme primjene×godina	NS		NS		NS	

SP - sirovi proteini; NDV- neutralna detergent vlakna; KDV- kisela detergent vlakna

U ovom istraživanju je vidljiva tendencija većeg sadržaja SP i nižeg sadržaja vlakana kod jesenske u odnosu na proljetnu gnojidbu, ali nisu utvrđene statistički značajne razlike (P>0,05) u sadržaju SP, NDV i KDV krme poluprirodnog travnjaka između proljetne i jesenske primjene 30 t ha^{-1} KSG nakon prve niti nakon desete godine istraživanja (Tablica 1). Navedeno je očekivno jer u ranijem istraživanju nisu utvrđene razlike u kemijskom sastavu prirodnog travnjaka, nakon prve i nakon druge godine istraživanja između tretmana jesenske primjene 30 t ha^{-1} KSG i kontrolnog tretmana bez primjene gnojiva (Simić i sur., 2016.).

Međutim, utvrđeni rezultati nisu u suglasju s ranijim istraživanjima u kojima se navodi veća učinkovitost primjene N gnojiva u rano proljeće prije početka vegetacije kada je iskorištenje hraniva iz tla efikasnije u odnosu na jesensku ili zimsku gnojidbu (Smith, 1991.; Wilson i sur, 2009.) zbog manjih gubitaka N nastalih ispiranjem dušika tijekom zime (Smith i Unwin, 1983.).

Prosječne vrijednosti kemijskog sastava krme po godinama istraživanja, neovisno o vremenu primjene KSG (Tablica 1) pokazuju statistički značajno niži sadržaj SP (P<0,05), te veći sadržaj NDV (P<0,01) i KDV (P<0,01) u krmi poluprirodnog travnjaka nakon desete godine provedbe istraživanja u odnosu na prvu godinu. Vrijednosti sadržaja SP u odnosu na sadržaj NDV i KDV u voluminoznoj krmi su obrnuto proporcionalne (Phipps i sur., 2000.) što je vidljivo i iz prikazanih rezultata istraživanja.

Radi nekih od prednosti korištenja organskih gnojiva u usporedbi s mineralnim gnojivima, poput povećanja sadržaja organske tvari u tlu, povećanja vodnog i vodozračnog kapaciteta tla (FAO, 2017.) te promicanja održivosti poljoprivrednih ekosustava (Zheng i sur., 2010.), organska gnojiva se preporuča koristiti u sustavima poljoprivredne proizvodnje, posebno u ekološkom uzgoju (Lampkin, 1990.).

Zaključak

Zaključeno je da vrijeme primjene 30 t ha^{-1} KSG (proljeće ili jesen) ne utječe na kemijski sastav krme poluprirodnog travnjaka, ali razlike mogu biti između vegetacijskih sezona. Primjenom KSG na travnjacima se omogućava kruženje organske tvari u prirodi, a time potiče održiva poljoprivredna proizvodnja.

Literatura

Bašić F., Herceg N. (2010). Temelji uzgoja bilja, Synopsis, Zagreb.

Buxton D.R., Casler M.D. (1993). Environmental and genetic effects on cell wall composition anddigestibility. In: H.G. Jung et al. (Editors), Forage cell wall structure and digestibility. American Society ofAgronomy, Madison, WI, pp. 685-714.

Buxton D.R., Fales S.L. (1994). Plant environment and quality. In: G.C. Fahey, Jr. et al. (Editors), Foragequality, evaluation, and utilization. American Society of Agronomy, Madison, WI, 155-199.

Chamberlain A.T., Wilkinson J.M. (1996). Feeding the Dairy Cow. Chalcombe Publications, PainShall, Ln2 3LT, UK.

Coleman S.W., Moore J.E., Wilson J.W. (2004). Quality and utilization. In: Moser, L.E., et al. (Eds.), Warm Season Grasses. ASA, CSSA, SSSA, Madison, WI, pp. 267–308.

DZS (Državni Zavod za statistiku) (2018). Statistički ljetopis Republike Hrvatske. Državni zavod za statistiku Republike Hrvatske: 588 str.

FAO (Food and Agriculture Organization) (2017). Livestock Solutions for Climate Change. http://www.fao.org/3/a-i8098e.pdf, Accessed date: 9 February 2019.

ISO (International Standard Organization) (1979). Animal Feedstuffs, ISO 5983., International Organization for Standardization, Geneva, Switzerland.

Julianna T., Pál P., Gábor T. (2013). First results of farmyard manure on hilly grassland. Animal Welfare, Ethology and Housing Systems, 9 (1): 61-75.

Knežević M., Vranić M., Perčulija G., Kutnjak H., Matić I., Teskera M.(2009). Effect of the maturity stage of grass at harvesting on the chemical composition of grass clover silage. Mljekarstvo, vol. 59 (1): 49-55.

Lamkin N. (1990). Management of manures, slurry and organic residues. In Organic Farming, pp. 86-124. Organic Farming Books; Ipswich.

McCartney D.H., Bittman S., Nuttall W.F. (2004). The influence of harvest management and fertilizer application on seasonal yield, crude protein concentration and N offtake of grasses in northeast Saskatchewan. Canadian Journal of Plant Science, 84 (1): 205-212.

Patel M.R., Sadhu A.C., Patel N.N., Patel R.M., Patel J.C. (2008). Effect of farm yard manure and nitrogen levels on forage yield and quality of bajra napier hybrid (Pennisetum purpureum). Research on crops, 9 (3): 561-562.

Phipps R.H., Sutton J.D., Beever D.E., Jones A.K. (2000). The effect of crop maturity on the nutritional value of maize silage for lactating dairy cows. 3. Forage intake and milk production. Animal Science, 71, 401-409.

SAS Institute (1997). SAS/STAT software. Changes and enhancements trough release 6.12. SAS Inst.,Cary,NC.

Schulte R.P.O., Herlily M. (2007). Quantifying responses to phosphorus in Irish grasslands:interactions of soil and fertiliser with yield and P concentration. European Journal of Agronomy, 144–153

Simić A., Stojanoivić B., Vučković S., Matrković J., Božičković A., Bijelić Z., Mandić V. (2016). Application of farm yard manure in grassland production. Agrofor International Journal, 2 (1): 20-27.

Smith K.A. (1991). Application of manures and alurries to growing crops. ADAS Eastern Region Soil Science Internal Note, SS/91/8.

Smith K.A., Unwin R.J. (1983). Fertiliser value of organic manures in the UK. The Fertiliser Society Proceedings, No.221, London, The Fertiliser Society.

Štybnarova M., Mićova P., Fijala K., Karabcova H., Latal O., Pozdišek J. (2014). Effect of organic fertilizers on botanical composition of grassland, herbage yield and quality, Agrowyzkum Rapotin Ltd., Vyzkumniku 267, 788 13 Vikyrovice, Czech Republic.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991). Method for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Vranić M., Knežević M., Perčulija G., Grbeša D., Leto J., Bošnjak K., Rupić I. (2004). Kvaliteta voluminozne krme na obiteljskim poljoprivrednim gospodarstvima u Republici Hrvatskoj:Kvaliteta travne silaže na obiteljskim poljoprivrednim gospodarstvima. Mljekarstvo, 54, 3; 165-176

Vranić M., Bošnjak K., Leto J., Lukšić B., Glavanović, S. (2017). Procjena prinosa suhe tvari krme poluprirodnog travnjaka NIR spektroskopijom. Stočarstvo : časopis za unapređenje stočarstva. 70, 1; 3-12.

Vranić M., Bošnjak K., Novak A., Lisjak I., Trubelja A., Županović M., Kiš G. (2021). Primjena krutog stajskog gnoja na travnjacima. Stočarstvo:časopis za unapređenje stočarstva, 75, 1-2; 22-32.

Wilson C., Undi M., Tenuta M., Wittenberg K.M., Flaten D., Krause D.O., Entz M.H., Holley R., Ominski K.H. (2009). Pasture productivity, cattle productivity and metabolic status following fertilization of a grassland with liquid hog manure: A three – year study, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2.

Zheng Y.H., Li Z.F., Feng S.F., Lucas M., Wu G.L., Li Y. (2010). Biomass energy utilization in rural areas may contribute to alleviating energy crisis and global warming: a case study in a typical agro-village of Shandong, China. Renew. Sust. Energ. Rev. 14, 3132–3139.

The effect of term of farm yard manure application on the forage quality of seminatural grassland

Abstract

The aim of the study was to determine the effect of 10-year application of 30 t ha^{-1} every spring or every autumn on the forage quality of semi-natural grassland. The objective of the research was to determine the effect of spring and autumn fertilization of semi-natural grassland with farm yard manure (FYM) in the amount of 30 t ha^{-1} on the forage quality after the first and after the 10th year of the study. No statistically significant differences in crude protein (CP) (P>0.05), neutral detergent fiber (NDF) (P>0.05) and acid detergent fibre (ADF) (P>05) in forage after the first neither after the 10th year of the study were found between spring and autumn FYM application. It was concluded that the application of 30 t ha^{-1} FYM does not necessarily affect the quality of fodder of semi-natural grassland, but allows the circulation of organic matter in nature, and thus encourages sustainable agricultural production

Key words: semi-natural grassland, farm yard manure, forage quality

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Ribarstvo, lovstvo i pčelarstvo

06

Fisheries, Game Management and Beekeeping

ORIGINAL SCIENTIFIC PAPER

Condition of common sole (*Solea solea* L.) in the fishing season in Istrian waters of the Adriatic sea

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Abstract
To determine the condition of the common sole (*Solea solea*, Linnaeus, 1758) in Istrian waters during the fishing season research was conducted from November 2020 to January 2021. In 20 fish sexed monthly sex ratios were 1.22:1 in November, 1.86:1 in December, and 1.50:1 in January, in favour of males. Average values of length-weight ratio were the highest in November, and the lowest in January. Positive allometric growth was recorded with coefficient b values of approximately 3.6. Both sexes exhibited the lowest values of gonadosomatic index in November, and the highest in December. Hepatosomatic index in males was declining from November to January. In females, the highest values of HSI were observed in January, and the lowest in December. High values of GSI indicate that sampled months fall into the spawning season.

Key words: common sole, Adriatic sea, Fulton`s condition factor, allometric growth, hepatosomatic index, gonadosomatic index

Introduction

The common sole (*Solea solea* Linnaeus, 1758) is one of the most commercially important species of the Mediterranean and Black Sea which provides about 15% of the overall worldwide landings (FAO, 2021). In the Adriatic Sea in 2019 total landing of common sole were 1993 metric tons which corresponds to 40% of total landings (FAO, 2021). According to Rogers (1992) the species was reported to be the most abundant in the central and northern parts of the Adriatic due to low depths and wide occurrence of soft bottoms. High-density aggregations are recorded in certain regions such as the western coast of Istria in autumn and winter (Piccinetti and Giovanardi, 1984) when it represents a target species for beam trawlers and small-scale fisheries (Matić-Skoko and Stagličić, 2020). In the small-scale fisheries, which rank second in landings in Croatia, sole gillnets and trammel nets are mostly used when targeting common sole (Matić-Skoko and Stagličić, 2020). The goal of this paper is to determine the condition state of the common sole and to comprehend the physiological changes in form of reserve expenditures and gonadal growth during the fishing season in Istrian waters and to compare them to other investigated populations.

Material and methods

Samples were collected in Istrian waters (Croatia) using trammel nets with a mesh size of 42 mm from November 2020 to January 2021. Samples of *S. solea* were identified in total catch, 40 individuals per each month were picked randomly and measured in total length and

weight, out of which 20 were collected for dissection and further analyses. Liver and gonads were separated and weighed. The sex of the fish was determined macroscopically by examining the shape and colour of the gonads.

Weight–length relations were computed using power regression and the derived formula had the shape: W=a·Lb, where W = mass, L = length and a, b = regression coefficients (Le Cren, 1951). Coefficient a determines the shape of the fish, and coefficient b, the type of growth. If b<3 fish progress faster in length (negative allometric growth), b=3 growth is equal in weight and length (isometric growth), and if b >3 fish progress faster in weight (positive allometric growth). Fulton's condition factor (CF) was calculated using the formula: CF = W/L3 x 100, where W = mass, L = total length (Ricker, 1975). Gonadosomatic index (GSI) was calculated using the formula: GSI = (Mass of the gonads/Mass of the fish) x 100 (de Vlaming et al., 1982). Hepatosomatic index (HSI) was calculated using the formula: HSI = (Mass of the liver/Mass of the fish) x 100 (Wootton et al., 1978).
All the data were analysed using Microsoft Excel 2016. Differences in observed characteristics between sexes were determined using t-test, and differences in monthly proportions of males and females in the sample were determined using chi-squared test.

Results and discussion

Monthly sex ratios were as follows: 1.22:1 in November 2020, 1.86:1 in December 2020 and 1.5:1 in January 2021, always in favour of males. Statistically significant differences were not found using chi-squared test ($p<0.05$). Although not statistically significant, this result differs from the findings of Piccinetti and Giovanardi (1984) who indicated that sex ratio is always roughly 1:1.
The average recorded length and mass values were at their highest in November 2020 for all groups following a slightly decreasing trend throughout the sampling period with the lowest values recorded in January 2021. Average weight and length values were always higher in females (Table 1).

Table 1. Average monthly values of total length and mass of *Solea solea* for all individuals and both sexes separately in November and December 2020 and January 2021 (n = number of individuals, mean = average value, SD = standard deviation)

Species	Month	Sex	n	Total length TL (cm)	Body weight W (g)
				Mean ± SD	Mean ± SD
Solea solea	November	All	40	29.86 ± 1.03	246.4 ± 30.66
		M	11	28.35 ± 2.17	197.0 ± 50.57
		F	9	29.96 ± 1.37	265.0 ± 47.94
	December	All	40	28.93 ± 2.61	217.55 ± 88.78
		M	13	27.25 ± 1.99	173.63 ± 46.10
		F	7	29.57 ± 2.03	248.33 ± 55.57
	January	All	40	28.43 ± 1.09	180.85 ± 32.20
		M	12	26.84 ± 1.35	175.57 ± 39.76
		F	8	27.97 ± 0.71	185.67 ± 18.15

Positive allometric growth was recorded throughout the sampling period with coefficient b values of approximately 3.6 (Fig. 1). High recorded values of coefficient b are due to correspondence of sampling and spawning period. Prior to spawning fish increase food consumption and allocate available energy to gonadal growth (Wooton, 2011). This results in an increase in body weight relative to length which causes an increase in the b value of the weight-length ratio (Ricker, 1975). Contrary to the findings in this paper, Bolognini et

al. (2013) determined b coefficient to be 3.057 for 5401 individuals of *S. solea* sampled between January 1999 and December 2009 in the Italian waters of central and northern Adriatic. Differences in coefficient b in western and eastern coasts of the Adriatic may be due to difference in sampling period, but can also be attributed to the spatial distribution of adults and juveniles. Landings of *S. solea* in Italian waters consist of 80% juvenile specimens (Grati et al., 2013) which do not undergo physiological changes during the spawning period and therefore show lower b coefficient values. Seasonal data on b coefficient values are non-existing for the Adriatic.

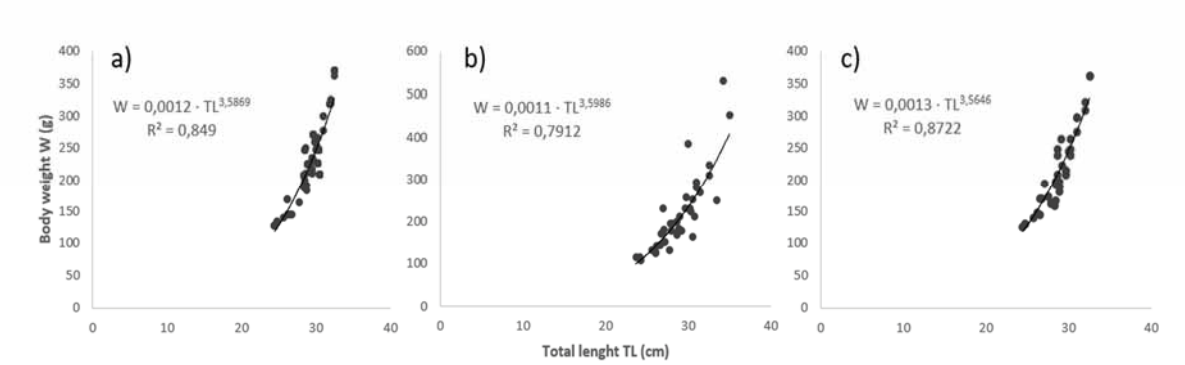

Figure 1. Weight-length relations of *Solea solea* in a) November 2020, b) December 2020 and c) January 2021

Mean values of Fulton's condition factor for 40 monthly measured individuals ranged from 0.86 ± 0.16 g/cm^3 in December 2020 to 0.9 ± 0.09 g/cm^3 in November 2020. In females CF decreased from 0,98 ± 0.07 g/cm^3 in November 2020 to 0.88 ± 0.08 g/cm^3 in January 2021. In males it ranged from 0.84 ± 0.12 in December 2020 to 0.9 ± 0.07 in January 2021 (Fig. 2). Statistically significant differences between males and females were found using t-test in November ($t=3.93$, $p<0.05$) and December ($t=2.14$, $p<0.05$). Data regarding Fulton`s condition factor of spawning individuals in Adriatic are scarce and previous research focused mainly on juveniles (Bolognini et al., 2013).

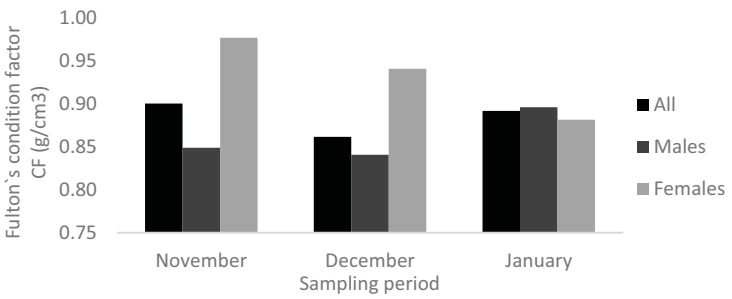

Figure 2. Average monthly values of Fulton`s condition factor of *Solea solea* during the sampling period

According to Pauly (1994) geographical differences in growth are caused by differences in temperature that affect distinct metabolism and oxygen consumption of the two sexes. This can cause variations in food intake (Lozán, 1992) and allocation of surplus energy towards somatic growth or reproduction (Rijnsdorp, 1993) which contributes to the life cycle variability of the same species in different areas. *S. solea* are batch spawners (Murua and Saborido-Rey, 2003) and there are noticeable differences in spawning periods observed by different authors. In the northern Atlantic spawning occurs in spring and in the Mediterranean during the winter (Quéro et al., 1986). High values of GSI recorded in females in this paper indicate that sampled months fall into the spawning season in the northern

Adriatic, which agrees with Bolognini et al. (2013). All examined fish ranging from 24.2 cm to 35 cm had developed gonads. Both sexes exhibited lowest values of gonadosomatic index in November 2020 (0.10 ± 0.04 for males, and 9.25 ± 2.42 for females) and highest in December 2020 (0.23 ± 0.07 for males, and 13.02 ± 1.27 for females) (Fig. 3.a). It is noticeable that GSI values of females were extremely high compared to GSI values of males, all statistically significant and determined using t-test ($p<0.05$), which agrees with Teixeira and Cabral (2010). In order to determine the exact extent of spawning season further research with a longer sampling period is needed.

Values of hepatosomatic index (HSI) in males were declining from November 2020 (1.05 ± 0.36) to January 2021 (0.85 ± 0.16). In females, the highest values of HSI were observed in January 2021 (1.69 ± 0.43) and the lowest in December 2020 (1.35 ± 0.17) (Fig. 3.b). Monthly differences in HSI between sexes were determined using t-test and are all statistically significant ($p<0.05$). Monthly HSI values in males exhibited reverse trends compared to GSI. This suggests that energy stored in the liver is being mobilized due to lower food intake during the spawning season (Wooton, 2011).

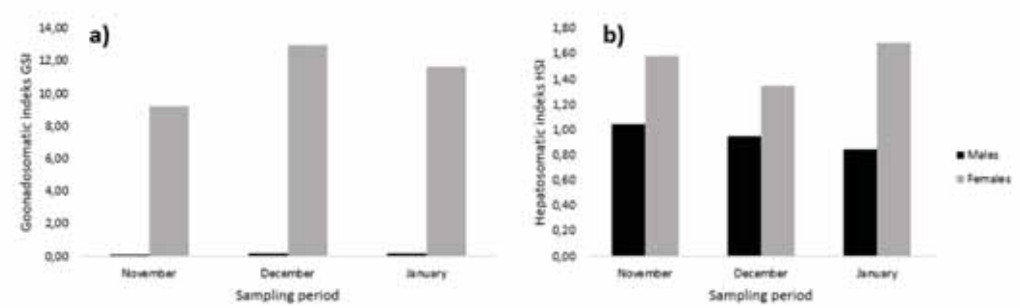

Figure 3. a) GSI and b) HSI of male and female *Solea solea* during the sampling period

Conclusions

Positive allometric growth of common sole was recorded in the coastal waters of Istria throughout November, December and January with coefficient b values of approximately 3.6. Sex ratio ranged from 1.22 to 1.86, in favour of males. High recorded values of GSI in females indicate that sampled months fall into the spawning season. Reverse trends of monthly HSI values in males compared to GSI suggest that energy stored in the liver is being mobilized due to lower food intake during the spawning season.

Acknowledgement

The authors would like to thank the fishermen Daniel Milevoj, Daniele Turkovich and Bojan Iveša. This research was financed by Measure I.3 “Partnership between scientists and fishermen” from the Operational Programme for Maritime Affairs and Fisheries of the Republic of Croatia for the programming period 2014-2020 financed by the European Maritime and Fisheries Fund.

References

Bolognini L., Domenichetti F., Grati F., Polidori P., Scarcella G., Fabi G. (2013). Weight-Length Relationships for 20 Fish Species in the Adriatic Sea. Turkish Journal of Fisheries and Aquatic Sciences. 13: 563-568.

De Vlaming V., Grossman G., Chapman F. (1982). On the use of the gonosomatic index. Comparative Biochemistry and Physiology Part A: Physiology. 73(1): 31–39.

FAO (2021). Fishstat plus. Available at: http://www.fao.org/fishery/statistics/software/fishstat.

Grati F., Scarcella G., Polidori P., Domenichetti F., Bolognini L., Gramolini R., Vasapollo C., Giovanardi B., Raicevich S., Celić I., Vrgoč N., Isajlovic I., Jenič A., Marčeta B., Fabi G. (2013). Multi-annual investigation of the spatial distributions of juvenile and adult sole (*Solea solea* L.) in the Adriatic Sea (northern Mediterranean). Journal of Sea Research. 84: 122–132.

Le Cren E.D. (1951). The length-weight relationship and seasonal cycle in gonad weight and condition factor in perch (*Perca fluviatilis*). Journal of Animal Ecology. 20: 201-219.

Lozán J.L. (1992). Sexual differences in food intake, digestive tract size, and growth performance of the dab, *Limanda limanda* L. Netherlands Journal of Sea Research. 29: 223-227.

Matić-Skoko S., Stagličić N. (2020). How Is Fisheries Management Perceived by Croatian Small-Scale Fishers: Should I Stay or Should I Go?. In: Small-Scale Fisheries in Europe: Status, Resilience and Governance, Pascual-Fernández J., Pita C., Bavinck M. (eds.) Series, vol 23. Springer, MARE Publication Series.

Murua H., Saborido-Rey F. (2003). Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fishery Science. 33: 23-31.

Pauly D. (1994). A framework for latitudinal comparisons of flatfish recruitment. Netherlands Journal of Sea Research. 32: 107–118.

Piccinetti C., Giovanardi O. (1984). Données biologiques sur Solea vulgaris Quensel en Adriatique. FAO, Fisheries Report. 290: 117-121.

Quéro J.C., Desoutter M., Lagardère F. (1986). Soleidae. In: Fishes of the North-eastern Atlantic and the Mediterranean, Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J., Tortonese E. (eds.) UNESCO, Paris. 3: 1308-1324.

Ricker W.E. (1975). Computation and interpretation of biological statistics of fish populations. Bulletin Fisheries Research Board of Canada. 191pp.

Rijnsdorp A.D. (1993). Selection differentials in male and female North Sea plaice and changes in maturation and fecundity. In: Stokes T.K., McGlade J.M., Law R. (eds) The exploitation of evolving resources. Springer: 19–36.

Rogers S.I. (1992). Environmental factors affecting the distribution of sole (*Solea solea* L.) within a nursery area. Netherlands Journal of Sea Research. 29: 153–161.

Teixeira C.M., Cabral H.N. (2010). Comparative analysis of the diet, growth and reproduction of the soles, *Solea solea* and *Solea senegalensis*, occurring in sympatry along the Portuguese coast. Journal of the Marine Biological Association of the United Kingdom. 90(05): 995–1003.

Wootton, R. J. (2011). Energy utilization in growth. Growth: Environmental Effects. In: Encyclopedia of Fish Physiology, Farrell A. (ed.), Academic Press. 1629–1635.

Wootton R.J., Evans G.W., Mills L. (1978). Annual cycle in female Three-spined sticklebacks (*Gasterosteus aculeatus* L.) from an upland and lowland population. Journal of Fish Biology. 12(4): 331–343.

Kondicijsko stanje lista (*Solea solea* L.) tijekom ribolovne sezone u istarskom akvatoriju Jadranskog mora

Sažetak

Cilj rada bio je utvrditi kondicijsko stanje lista (*Solea solea* Linnaeus, 1758) u istarskom akvaktoriju istraživanjem provedenim od studenog 2020. do siječnja 2021. godine. Zabilježeni omjer spolova bio je 1,22:1 u studenom, 1,86:1 u prosincu i 1,50:1 u siječnju, u korist mužjaka. Prosječne vrijednosti odnosa mase i duljine bile su najveće u studenom, a najniže u siječnju. Zabilježen je pozitivan alometrijski rast s vrijednostima koeficijenta b od približno 3,6. Oba spola su imala najniže vrijednosti gonadosomatskog indeksa u studenom, a najviše u prosincu. Hepatosomatski indeks kod mužjaka opadao je od studenog do siječnja. U ženki su najveće vrijednosti HSI zabilježene u siječnju, a najniže u prosincu. Visoke vrijednosti GSI ukazuju na to da uzorkovani mjeseci spadaju u sezonu mrijesta.

Ključne riječi: list, Jadransko more, Fultonov factor kondicije, alometrijski rast, hepatosomatski indeks, gonadosometski indeks

IZVORNI ZNANSTVENI RAD

Dužinsko-maseni odnosi i kondicija srdele (*Sardina pilchardus*) i europskog inćuna (*Engraulis encrasicouls*) sa područja srednjeg Jadrana

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Sažetak
U radu su prikazani dužinsko-maseni odnosi i kondicija srdele (*Sardina pilchardus*) i europskog inćuna (*Engraulis encrasicolus*) sa područja srednjeg Jadrana (Dugi otok). Tijekom travnja 2021. godine iz ukupnog ulova okružujućom mrežom plivaricom izuzeto je 100 uzoraka srdele i 110 uzoraka inćuna. Dužinsko-maseni odnosi pokazali su pozitivan alometrijski rast obje istraživane vrste (srdela $W= 0,004 \times L^{3,167}$, $R^2=0,861$), (inćun $W=0,002 \times L^{3,405}$, $R^2=0.936$). Prosječni Fultonov faktor kondicije analiziranih jedinki srdela iznosio je CF= 0,699 ± 0,043, dok je CF inćuna bio 0,647 ± 0,037. Rezultati dužinsko-masenog odnosa i kondicije srdele mogu se povezati s njenim završetkom mrijesta. Također, dobivene vrijednosti istih pokazatelja kod inćuna upućuju na pripremu populacije za nadolazeći mrijest.

Ključne riječi: dužinsko-maseni odnosi, faktor kondicije, Jadransko more

Uvod

U Jadranskom moru, najsjevernijem plitkom poluzatvorenom bazenu na Mediteranu, ribarstvo i ribolov su općenito dobro razvijeni i predstavljaju važnu granu gospodarstva (Zorica i sur., 2020.). Među pelagičnim vrstama riba na području sjevernog i srednjeg Jadrana dominiraju srdela (*Sardina pilchardus* Walbaum, 1792.) i inćun (*Engraulis encrasicolus* Linnaeus, 1758.) koji, gledajući s ekološkog stajališta, imaju velik utjecaj na prehrambeni lanac kroz prijenos energije s najniže na najvišu trofičku razinu (Domenella i sur., 2016.). Ekonomska važnost navedenih vrsta izražena je i podacima iz 2019. g., gdje je od ukupnog ulova morskih organizama (63 883 t) udio plave ribe iznosio 90,8 % odnosno 58 010 t, a najveći udio odnosio se na ulov srdele koji je činio 77,8 % tj. 45 134 t te na ulov inćuna sa zabilježenih 7 995 t (DZS, 2020.). Prijašnjim istraživanjima u različitim dijelovima Jadranskog mora zabilježene su varijacije u biomasi, ali i vrijednostima bioloških parametara sitnih pelagičnih riba (Mustać i sur., 2020.). Na opće stanje riba utječu čimbenici morskog okoliša poput saliniteta, temperature, količine hrane, te lokacija i fiziološki ciklus riba, što se može prikazati vrijednostima kondicijskog faktora (FC) i dužinsko-masenim odnosima (DM) (Allaya i sur., 2016.). S ciljem očuvanja i dugoročno održivog iskorištavanja ovog biološkog i gospodarsko važnog resursa, neprestano se ističe važnost istraživanja populacija sitne plave ribe tj. srdele i inćuna (Šoštarić i sur., 2016.). Cilj rada jest prikazati dužinsko-masene odnose i kondiciju srdele i europskog inćuna na lokaciji srednjeg Jadrana. Dobivenim podacima doprinijet će se daljnjim istraživanjima i upravljanju populacijama srdele i inćuna u Jadranskom moru.

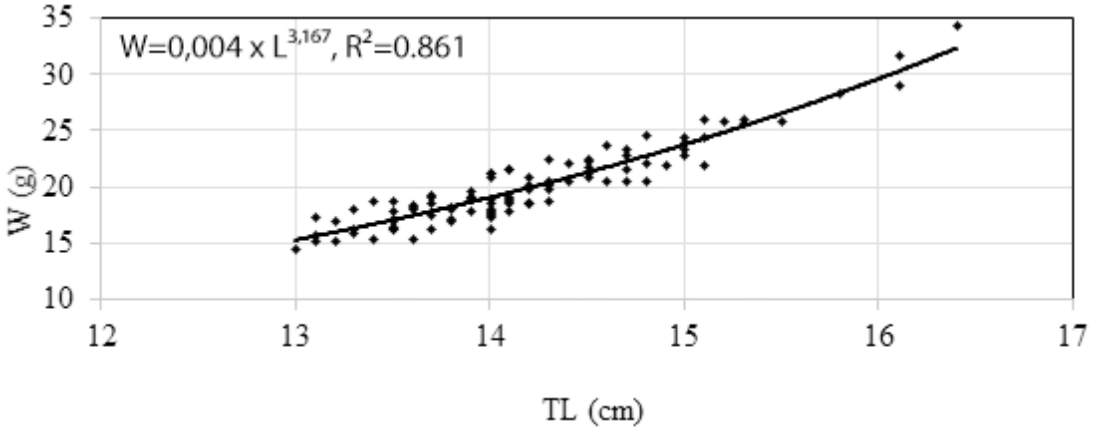
Materijal i metode

Ribolov srdele i inćuna provodio se ribarskim brodom Galo (Ribarski obrt Mišlov d.o.o.), odnosno mrežom plivaricom kod sjevernog dijela Dugog otoka na području srednjeg Jadrana tijekom travnja 2021. godine. Za potrebe ovog istraživanja od ukupnog ulova izuzeto je 100 uzoraka srdele i 110 uzoraka inćuna. Uzorci su skladišteni na -18°C do analize te je nakon odmrzavanja uz pomoć milimetarskog papira i pomične mjerke određena je totalna dužina tijela (TL) a uz pomoć vage s preciznošću od 0.1g i masa (W) ribe. Uz pomoć računalnog programa (SPSS ver. 19), izračunati su DM odnosi metodom regresije prema formuli $W = a L^b$. Oznaka W predstavlja masu u gramima (g), L totalnu dužinu tijela izraženu u cm, dok su a i b konstante. Premda se koeficijent b kreće oko 3, može doći do znatnih odstupanja koja daju informacije o prirodi rasta ribe. U slučaju izometrijskog rasta, kada ribe napreduju podjednako u masi i dužini, b je jednak broju 3 (b= 3). Ako je $b > 3$, to znači da ribe napreduju više u masi i upućuje na pozitivni alometrijski rast, dok se negativni alometrijski rast manifestira u slučaju kada je $b< 3$ i ribe tada dobivaju više na dužini (Treer i Piria, 2019.). Eksponent b je omjer logaritma rasta u odnosu dužine i mase. Fultonov faktor kondicije (CF) izračunat je uz pomoć formule $CF = W L^{-3} 100$, gdje oznaka W također predstavlja oznaku za masu a L za totalnu dužinu tijela ribe.

Rezultati i rasprava

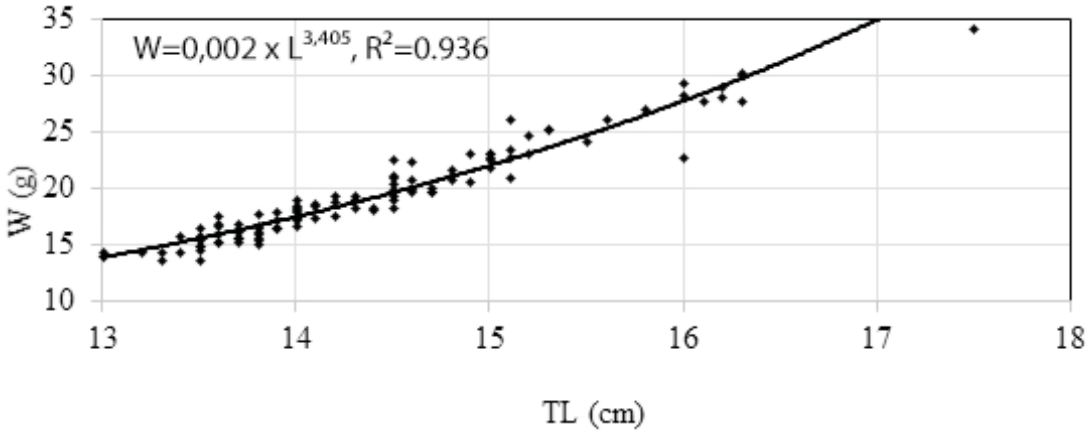
Srednja vrijednost totalne dužine (TL) srdele iznosila je 14,19 ± 0,69 cm varirajući od minimalno 13,0 do maksimalno 16,4 cm, dok je ukupna masa varirala od 14,56 do 34,31 g s prosječnom vrijednošću 20,17 ± 3,53 g. Rezultati se podudaraju s istraživanjem Zorice i sur. (2019.) u kojem je zabilježena srednja vrijednost TL i W srdele iznosila 13,9 ± 0,03 cm odnosno 20,31 ± 0,16 g. Manje vrijednosti TL i W može se uočiti u odnosu na rezultate istraživanja Mustać i Sinovčić (2010.) u kojima je izmjereno 16,03 ± 0,88 cm i 31,49 ± 5,32 g, kao i porast TL u odnosu na jedinke iz Izmirskog zaljeva u Turskoj, gdje je TL srdele iznosila 12,1 cm (Şenbahar i sur., 2020.). Şenbahar i sur. (2020.) navode kako je sezonski ribolovni pritisak vjerojatno razlog niske TL srdele. Analiza DM odnosa analiziranih jedinki srdela ukazala je na pozitivan alometrijski rast (Grafikon 1) pri čemu je koeficijent regresije b iznosio 3,167, a koeficijent determinacije (R^2) 0,861 ($p<0,001$). Pozitivan alometrijski rast srdele utvrđen je i u istraživanju kojeg su proveli Mustać i sur. (2020.) u razdoblju od lipnja 2015. do kolovoza 2016. godine na ukupno 2453 uzoraka srdele i 860 uzoraka inćuna. Ovisnost mase o dužini s naznakom pozitivnog alometrijskog rasta utvrđena je i kod jedinki s područja sjevernog i srednjeg Jadrana uzorkovanih u periodu između travnja i lipnja. 2015. g. (Šoštarić i sur., 2016.). Prosječna vrijednost CF iznosila je 0,699 ± 0,043, te su zamjećene nešto niže vrijednosti u odnosu na ranija istraživanja. Kondicija jedinki iz istočnog dijela Jadranskog mora uzorkovanih u razdoblju od lipnja 2015. do kolovoza 2016. godine, iznosila je CF=0,718 ± 0,043 (Mustać i sur., 2020.). Temeljem istraživanja kojeg su provele Mustać i Sinovčić (2010.) na ukupno 1219 prikupljenih uzoraka srdele sa dvije lokacije zadarskog područja u razdoblju od ožujka 2004. do veljače 2005. godine, primjećuje se prisutnost bolje kondicije jedinki izvan reproduktivne faze za razliku od onih u sezoni mrijesta. Također su zabilježili i veće vrijednosti kondicijskog faktora kod srdela iz obalnih voda (Virsko more) CF= 0,820 od onih iz otvorenog mora (Dugi otok) CF=0,732. Veća gustoća naseljenosti obalnih područja u odnosu na otvoreno more, različite varijacije kopnenih inputa (zbog padalina, poljoprivrede i sl.) te velik broj industrijskih objekata u obalnim područjima imaju značajan utjecaj na brojnost i raznolikost zooplanktonskih organizama. Navedeni čimbenici utječu na količinu anorganskih hranjivih tvari i mnoga druga fizikalno-kemijska svojstva vodenog stupca, što zauzvrat može utjecati na primarne proizvođače, a time i na veću raspodjelu i brojnost zooplanktona (Terbiyik i Polat, 2015.). Uz slanost, isti autori su utvrdili i korelaciju između ukupne brojnosti zooplanktona i obilja fitoplanktona na određenoj

lokaciji. S obzirom da je zooplankton glavni izvor hrane srdelama, može se pretpostaviti kako je, uz netom završen mrijest, manje obilje hranjiva na istraživanoj lokaciji otvorenog mora bio još jedan od razloga nešto nižih vrijednosti kondicijskog faktora.



Grafikon 1. Prikaz dužinsko-masenih odnosa srdele na istraživanoj lokaciji (Dugi otok) (n= 100)

Srednja vrijednost totalnih dužina (TL) inćuna iznosila je TL=14,37 ± 0,87 cm varirajući od 13,0 –17,5 cm, dok je ukupna masa (W) varirala od 13,65 do 34,2 g s prosječnom vrijednošću W=19,51 ± 4,31 g. Dobivene vrijednosti TL i W su u skladu s rezultatima istraživanja kojeg su proveli Mustać i sur. (2020.) na jedinkama inćuna iz istočnog dijela Jadranskog mora. Znatno niže vrijednosti TL i W zabilježene su kod jedinki inćuna iz Izmirskog zaljeva u Turskoj (TL=11,4 cm) (Şenbahar i sur., 2020.), kao i kod inćuna uzorkovanih iz Crnog mora (TL=11,66 cm; W=10,04 g) tijekom ribolovne sezone 2010/ 2011 (Sağlam i Sağlam, 2013.). Rezultati DM odnosa upućuju na pozitivan alometrijski rast s utvrđenim koeficijent regresije b=3,405 i koeficijentom determinacije R^2=0,936 ($p<0,001$) (Grafikon 2). Dobiveni rezultati su u skladu s vrijednostima istraživanja Mustać i sur. (2020.) na jedinkama inćuna iz istočnog dijela Jadranskog mora (b= 3,201) uzorkovanih od lipnja 2015. do kolovoza 2016. godine, te kod jedinki iz Novigradskog mora uzorkovanih sa ušća rijeke Zrmanje (b=3,211) u razdoblju od siječnja do prosinca 2003.g. (Sinovčić i Zorica, 2006.). Prosječna vrijednost Fultonovog faktora kondicije (CF) iznosila je 0,647 ± 0,037. Kondicija inćuna može se usporediti sa dobivenim vrijednostima iz istraživanja Mustać i sur. (2020.) sa područja istočnog Jadrana gdje je utvrđena srednja vrijednost Fultonovog faktora kondicije iznosila CF= 0,635 ± 0,02.



Grafikon 2. Prikaz dužinsko-masenih odnosa inćuna na istraživanoj lokaciji (Dugi otok) (n= 110)

Zaključak

Utvrđeni dužinsko-maseni odnosi ukazali su na pozitivan alometrijski rast obje istraživane vrste. Rezultati DM odnosa i vrijednosti CF odgovaraju vremenu uzorkovanja na kraju hladnijih mjeseci kada dolazi do mrijesta srdele, nakon čega slijedi njezin uobičajeni pad kondicije. Rezultati CF za populaciju inćuna u ovom istraživanju se povezuju s pripremom vrste za mrijest koji se, za razliku od srdele, odvija tijekom toplijih mjeseci u godini. Usporedbom rezultata s ranije provedenim istraživanjima drugih autora utvrđene su sličnosti među dobivenim vrijednostima za obje vrste, premda je uočeno da se kod njih radi o većem broju analiziranih uzoraka, ali i duljem periodu prikupljanja u odnosu na broj uzoraka i vrijeme uzorkovanja ovog rada.

Napomena

Istraživanje je izvedeno u okviru mjere I.3. „Partnerstva između znanstvenika i ribara“ za razdoblje 2017. – 2020. (KLASA: 324-01/20-01/1310; URBROJ: 525-13/0755-20-2).

Literatura

Allaya, H., Ben Faleh, A., Rebaya, M., Zrelli, S., Hajjej, G., Hattour, A., Quignard, J., and Trabelsi, M. (2016). Identification of Atlantic chub mackerel *Scomber colias* population through the analysis of body shape in Tunisian waters. Cahiers de Biologie Marine. 57: 195–207.

Domenella, E., Donato, F., La Mesa, M. (2016). Age and growth of early life stages of European pilchard (*Sardina pilchardus*) from the western Adriatic Sea. Acta Adriatica. 57 (1): 39-48.

Državni zavod za statistiku Republike Hrvatske (2020). Priopćenje, Ribarstvo u 2019. Br. 1.4.2.

Mustać, B., Sinovčić, G. (2010). Reproduction, length-weight relationship and condition of sardine, *Sardina pilchardus* (Walbaum, 1792), in the eastern Middle Adriatic Sea (Croatia). Periodicum biologorum. 112(2): 133-138.

Mustać, B., Zoja Cukar, G. i Vidović, A. (2020). Comparison of growth parametres between sardine *Sardina pilchardus* (Walbaum, 1792) and anchovy *Engraulis encrasicolus* (Linnaeus, 1758) from the Eastern Adriatic Sea. Pomorski zbornik, Special edition (3): 325-333.

Sağlam, N., Sağlam, C. (2013). Age, growth and mortality of anchovy *Engraulis encrasicolus* in the south-eastern region of the Black Sea during the 2010–2011 fishing season. Journal of the Marine Biological Association of the United Kingdom. 93(8): 2247-2255.

Şenbahar, A. M., Güleç, Ö., Tosunoğlu, Z., Özaydın, O. (2020). Length-weight relationship of the most landed pelagic fish species European pilchard (*Sardina pilchardus* Walbaum, 1792) and European anchovy (*Engraulis encrasicolus* Linnaeus, 1758) in the Izmir Bay (Aegean Sea, Turkey) purse seine fishery. Marine Science and Technology Bulletin. 9 (1): 32-37.

Sinovčić, G., Zorica, B. (2006). Reproductive cycle and minimal length at sexual maturity of *Engraulis encrasicolus* (L.) in the Zrmanja River estuary (Adriatic Sea, Croatia). Estuarine Coastal and Shelf Science. 69: 439- 448.

Šoštarić, S., Tomljanović, T., Matulić, D., Aničić, I., Treer, T. (2016). Morfološke karakteristike populacija srdela, *Sardina pilchardus* (Walbaum, 1792) u Jadranskom moru. Zbornik radova 51. hrvatskog i 11. međunarodnog simpozija agronoma, Sveučilište u Zagrebu Agronomski fakultet, 15. - 18. veljače 2016., Opatija, Hrvatska, str. 279-283.

Terbiyik Kurt, T., Polat, S. (2015). Zooplankton abundance, biomass, and size structure in the coastal waters of the northeastern Mediterranean Sea. Turkish Journal of Zoology. 39: 378-387.

Zorica, B., Čikeš Keč, V., Vrgoč, N., Isajlović, I., Piccinetti, C., Mandić, M., Pešić, A. (2020). A review of reproduction biology and spawning/ nursery grounds of the most important Adriatic commercial fish species in the last two decades. Acta Adriatica. 61 (1): 89-99.

Length-weight relationship and condition of sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicouls*) from middle Adriatic Sea

Abstract

In this study, the length-weight relationship and condition of sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) from the middle part of the Adriatic Sea (Dugi otok) are presented. From the total purse seine catches, 100 samples of sardine and 110 samples of anchovy were collected in April 2021. Length-weight relations showed positive allometric growth for both species (sardines W = 0.004 x L3.167, R^2 = 0.861), (anchovies W = 0.002 x L3.405, R^2 = 0.936). The average value of Fulton's condition factor for sardines was CF = 0.699 ± 0.043 and for anchovies CF = 0.647 ± 0.037. The obtained values of length-weight relationship and condition of sardine can be related to the end of spawning. The results of the same parameters in anchovies may indicate that the population is preparing for the upcoming spawning.

Key words: length-weight relationship, condition factor, Adriatic Sea

PRELIMINARY COMMUNICATION

Influence of the hunting method on meat quality of wild boar (*Sus scrofa Linnaeus*) - a peliminary study

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Abstract
The aim of this study was to investigate differences in meat quality parameters of animals hunted by two conventionally used methods: dog-driven hunting and selective/individual hunting. For this purpose meat samples from 21 animals were collected and technological meat quality traits (pH, CIE L*, a*, b*, drip loss, cooking loss, defrosting loss, Warner-Bratzler Shear Force) were determined in laboratory. The results of the study showed that hunting method influences drip loss, cooking loss and instrumental tenderness, indicating that dog-driven hunting is more stressful for the animal and can cause serious deterioration of different meat quality traits.

Key words: wild boar, hunting method, meat quality

Introduction

Wild boar (*Sus scrofa Linnaeus*) is one of the most numerous and most spread wild animals in Europe. It is a generalist omnivore that migrates in search of food (Sales and Kotrba, 2013). In Slavonia, wild boar usually lingers on agricultural land because of the availability of crops, while in autumn and winter it usually scavenges for wild growing forest products. Because of the population number control, agricultural products damage control, and prevention of African swine fever, hunting season on wild boar is open throughout the whole year. When it comes to hunting of the wild boar, two conventional methods are used: hunting using the dogs and selective/individual hunting. Dog-driven hunting is performed with dogs chasing the wild animal from one side of the wood to another (where hunters wait for the game), while selective/individual hunting is performed by shooting the game from the hunting lodge. In Croatia dog-driven hunting is practised from 1. October until the end of January, while selective/individual hunting is opened throughout the whole year. It is generally considered that dog-driven hunting is very stressful for the wild animal, opposite to selective/individual hunting, when the game is usually shot by one direct gunshot without prior chasing, due to which there is no time to discharge a big concentration of stress hormones to animal's bloodstream. Today it is widely accepted that every change during life of an animal will result in stress and changes that occur in the animal body due to stress are also present in the *post mortem* carcass (Ovničević et al., 2013). It is presumed that meat of individuals with a higher concentration of stress hormones (cortisol and creatin-phosphokinase) will have a different pH value than normal meat (Kovačević, 2001). These alterations of pH values can cause serious deterioration in meat quality (from technological to nutritional) and impact significantly its shelf-life and microbiological properties, thus leading to problems in the safety of the meat and its products. A lot is known on influence of pre-slaughter stress on meat quality of domestic pig, however, studies on the effect of different stress factors on meat quality of wild animals are scarce.

Therefore, the aim of this study was to determine differences in technological meat quality traits of wild boars hunted by two divergent hunting methods: dog-driven and selective/individual.

Material and methods

In total, 21 adult (18±4.5 months of age in average, as estimated from tooth eruption and wear according to Boitani and Matei; 1992) animals were included in the study, out of which 11 animals were hunted by dog-driven hunting and 10 were hunted selectively. The animals were shot by one single shot to head or neck in the area of Slatina and Gornji Miholjac (Virovitičko-podravska County) in period from October 2020 to January 2021, when the weather conditions were good (light or no rain) and without snow cover. Initial pH (pH_i) was taken within the 4 hours *post mortem* in *musculus longissimus thoracis* (LT) and *musculus semimembranosus* (MS) muscle as proposed by Viganò et al. (2019). Final pH (pH_u) was measured 24h after death. The pH measurements were determined using HI99163 (Hanna Instruments, USA). Light reflectance scores for CIE L*a*b*, chroma (saturation; C*) and hue angle (h°) of the LT samples (the average of three measurements) were determined by a Minolta CR-410 colourimeter (Minolta Camera Co. Ltd., Japan) with a D65 light source and 10-degree standard observer after allowing 15 min of blooming. Water drip was measured by the EZ_Drip method (Christensen, 2003) after 48h of cooling the samples at 4° C. Instrumental tenderness was determined on 2.54 cm thick LL samples that were frozen for two weeks, defrosted at 4° C for 24h and cooked in a water bath until 70° C in the internal core of the sample was reached. The samples were cooled at 4° C overnight, after which at least six subsamples of 1.27 cm in diameter were extracted. Tenderness was determined as the average of maximal strength in Newtons (N) needed for cutting the samples using a Warner-Bratzler blade attached to the TA.XTplus device (Stable Micro Systems Products, London, UK). Cooking and defrosting losses were determined on samples for instrumental tenderness evaluation by weighing the samples before freezing, after defrosting, and after cooking the samples, and presented as percentages (%).

The obtained data were analysed by one-way ANOVA of Dell Statistica (2015), where the Tuckey HSD test was used for the determination of significance between the investigated groups.

Results and discussion

Differences in meat quality traits of animals hunted using two conventional methods is presented in Table 1. The investigations on histochemical properties of wild boar muscles showed that their MS and LT muscles are mostly comprised of IIB (oxidative glycolytic) fibres (Ruusunen and Puolanne, 2004), in which *post mortem* glycolysis occurs until muscle pH is lowered to pH 5.9–5.5 (England et al., 2014). This high percentage of oxidative fibres observed in wild boars is probably a consequence of the adaptation to scavenging for food, but also alertness to predators in nature (Sales and Kotrba, 2013), due to which prolonged locomotion and high oxidation activity are required (Oshima et al., 2009). High percentage of oxidative fibres will result in higher lactate production, thus leading to lower pH values in meat, which was also observed for MS and LT muscles in both investigated groups (Table 1). According to thresholds for DFD (dark, firm, dry) and a normal meat for wild ungulates proposed by Viganò et al. (2019.) in which $pH_i>6.2$ is coinsidered as DFD (dark, firm, dry), $5.8>pH_i>6.2$ as intermediate DFD, and a $pH_i<5.8$ as good quality, it can be observed from Table 1 that meat samples from both groups were categorised as „good quality“. Furthermore, no significant differences were found between meat samples of animals hunted by dog-driven hunting and by selective/individual hunt, confirming the thesis that wild boar is quite resistant to stress and is able to maintain existing glycogen levels even during

extremely stressful situations. Our results are in agreement with those of Cifuni et al. (2014), who also did not determine significant differences in pH values of meat from wild boars hunted by these two methods. Although in their study pH was measured after thawing the meat samples after three months of frozen storage, the obtained values were very similar to pH_u measured 24h *post mortem* in our study. In their study on changes in domestic pig meat properties during frozen storage, Medić et al. (2018) showed that during 3 months of frozen storage pH does not change significantly from values determined after 24h *post mortem*. Thus, it can be assumed that pH values 24h *post mortem* would not be significantly different than those determined after 3 months of frozen storage. The values of the final pH were however somewhat lower than those determined in Dinarides part of Croatia (Tomljanović et al., 2022).

Table 1. Differences (LS mean ± standard error) between meat quality traits of wild boars hunted by two conventional hunting methods

Trait	Hunting method		Significance (P)
	Dog-driven (N=11)	Selective/idividual (N=10)	
pH_i, MS	5.55±0.45	5.60±0.46	0.8303
pH_i, LT	5.49±0.50	5.59±0.41	0.4723
pH_u, LT	5.51±0.48	5.49±0.17	0.1803
L*	49.19±5.01	47.82±5.38	0.5523
a*	16.63±2.60	15.80±2.15	0.4372
b*	3.80±3.01	4.39±3.10	0.6648
h°	0.21±0.13	0.26±0.18	0.4081
C*	17.20±3.25	16.65±2.26	0.6580
Drip loss, %	10.11^a±3.64	4.73^b±1.89	**0.0005**
Defrosting loss, %	6.66±1.54	7.54±2.25	0.3048
Cooking loss, %	37.00^a±2.05	31.57^b±4.11	**0.0010**
WBSF, N	68.55^a±8.15	58.50^b±13.98	**0.0053**

MS-*m. semimembranosus*; LT-*l. thoracis*; pH_i-initial pH; pH_u-ultimate pH; h°-hue angle; C*-chroma; WBSF-Warner-Bratzler Shear Force

Among all meat quality traits colour is one of the most important from consumer's perception. The colour of wild boar meat is darker than one from the domestic pig (Marsico et al., 2007), with more pronounced redness (a*; Marchiori and de Felício, 2003). Although meat from animals hunted using dogs was slightly paler than those from animals hunted

selective/individual hunting, no significant differences were observed between groups for this trait. Contrary to the results of our study, Cifuni et al. (2014) reported a significant influence of hunting method on L* values, with meat from wild boars hunted by dogs having lighter meat than meat from animals hunted by selective/individual hunting. The authors also observed significant differences in b* values between two wild boar groups, which was not determined in our study. Additionally, Tomljanović et al. (2022) and Amici et al. (2015), both found lower values for L*, regardles of the hunting method used.

Chroma (C*) and hue angle (h°) determine colour intensity, where higher levels of these parameters indicate a more intensive red colour and less colour discolouration during time (Komlenić, 2021). As can be observed from Table 1, meat from both investigated groups had high values of both a* and C*, and low h° values.

The investigated groups differed significantly in drip loss and cooking loss, with higher values for both traits observed in meat from wild boars hunted by dog-driven hunting (Table 1). In domestic pork, an unacceptably high drip loss is observed in carcasses in which pH declines very fast, resulting in ultimate or near ultimate pH while the muscle is still warm (Huff Lonergan, 2009). The major biochemical cause for this is a rapid accumulation of lactic acid in muscles caused by high stress prior to death, and dog-driven hunting involves a stressful and frequently long-lasting fight of the animal.

Animal groups significantly differed in instrumental tenderness, with meat from animals hunted by dog-driven hunting having higher WBSF values than animals hunted by selective/individual hunting (Table 1). The observed difference can be attributed to a higher amount of drip, but also higher cooking loss determined in animals hunted by dog-driven hunting. Contrary to the results of this study, Cifuni et al. (2014) found no significant differences in WBSF values of meat from animals hunted by these two conventionally used hunting methods.

Conclusions

Results of this preliminary study showed significant differences in drip loss, cooking loss and WBSF values of wild boar meat according to the hunting method used. The obtained results indicate that dog-driven hunting can significantly deteriorate meat quality traits of the wild boar. It should be noted, however, that there was a limited number of animals used in this study, so the observed results should be confirmed on a larger sample combined with other stress biomarkers, such as blood cortisol and the rate of glycolysis.

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References

Amici, A., Cifuni, G. F., Contò, M., Esposito, L., Failla, S. (2015). Hunting area affects chemical and physical characteristics and fatty acid composition of wild boar (*Sus scrofa*) meat. Rendiconti Lincei. 26(3): 527-534.

Boitani, L., Mattei, L. (1992). Aging wild boar by toot eruption. In: Proceedings of International Conference Ongule´s/Ungulates: 4419-421, Paris-Toulouse, France.

Christensen, L. B. (2003). Drip loss sampling in porcine *m. longissimus dorsi*. Meat Science. 63(4): 469–477.

Cifuni, G. F., Amici, A., Conto, M., Viola, P., Failla, S. (2014). Effects of the hunting method on meat quality from fallow deer and wild boar and preliminary studies for predicting

lipid oxidation using visible reflectance spectra. European Journal of Wildlife Research. 60: 519–526.

England, E. M., Matarneh, S. K., Oliver, E. M., Apaoblaza, A., Scheffler, T. L., Shi, H., Gerrard, D. E. (2016). Excess glycogen does not resolve high ultimate pH of oxidative muscle. Meat Science. 114: 95-102.

Huff-Lonergan (2009). Fresh meat water-holding capacity. In: Improving the sensory and nutritional quality of fresh meat. Kerry, J. (ed.). Elsevier.

Komlenić, M. (2021). Kakvoća sirovine i Baranjskog kulena (OZP) iz različitih rroizvodnih lanaca. Doktorska disertacija. Fakultet agrobiotehničkih znanosti Osijek.

Kovačević, D. (2001): Kemija i tehnologija mesa i ribe. Sveučilište J. J. Strossmayera Prehrambeno tehnološki fakultet, Osijek.

Marchiori, A. F., de Felício, P. E. (2003). Quality of wild boar meat and commercial pork. Scientia Agricola. 60: 1–5.

Marsico, G., Rasulo, A., Dimatteo, S., Tarricone, S., Pinto, F., Ragni, M. (2007). Pig, F1 (wild boar×pig) and wild boar meat quality. Italian Journal of Animal Science 6. (Supplement 1): 701–703.

Medić, H., Kušec, I. D., Pleadin, J., Kozačinski, L., Njari, B., Hengl, B., Kušec, G. (2018): The impact of frozen storage duration on physical, chemical and microbiological properties of pork. Meat Science. 140: 119-127.

Ovničević, D., Đurkin, I., Segedi, Lj., Lukić, B., Kušec, G. (2013). Transportni stres i kakvoća svinjskog mesa. Meso: prvi hrvatski časopis o mesu. 5: 291-299.

Oshima, I., Iwamoto, H., Nakamura, Y. -N., Takayama, K., Ono, Y., Murakami, T., Shiba, N., Tabata, S., Nishimura, S. (2009). Comparative study of the histochemical properties, collagen content and architecture of the skeletal muscles of wild boar crossbred pigs and commercial hybrid pigs. Meat Science. 81: 382–390.

Ruusunen, M., Puolanne, E. (2004). Histochemical properties of fibre types in muscles of wild and domestic pigs and the effect of growth rate on muscle fibre properties. Meat Science. 67: 533–539.

Sales, J., Kotrba, R. (2013). Meat from wild boar (Sus scrofa L.): A review. Meat Science. 94: 187–201.

Tomljanović, K., Grubešić, M., Medić, H., Potočnik, H., Topolovčan, T., Kelava Ugarković, N., Marušić Radovčić, N. (2022). The Impact of Premortality Stress on Some Quality Parameters of Roe Deer, Wild Boar, and Red Deer Meat. Foods. 11(9):1275.

Viganò, R., Demartini, E., Riccardi, F., Corradini, A., Besozzi, M., Lanfranchi, P., Chiappini, P. L., Cottini, A., Gaviglio, A. (2019). Quality parameters of hunted game meat: Sensory analysis and pH monitoring. Italian journal of food safety. 8(1): 7724.

IZVORNI ZNANSTVENI RAD

Mogućnosti uzgoja mikroalge *Isochrysis galbana* u otpadnoj vodi iz akvakulturnih uzgojnih sustava

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Sažetak
Sve veća pažnja posvećuje se mogućnosti iskorištavanja otpadnih voda iz akvakulturnih uzgojnih sustava. Atraktivnu opciju predstavlja uzgoj mikroalgi, koje pored dosadašnje tradicionalne primjene u mrijestilištima, postaju sve traženija sirovina u farmaceutskoj, kozmetičkoj, prehrambenoj i industriji biogoriva. U ovom je radu istražena podobnost otpadne vode saliniteta 25 psu iz recirkulacijskog akvakulturnog proizvodnog sustava za uzgoj mikroalge *Isochrysis galbana.* Podaci su uspoređeni s rastom uzgojnih populacija ove vrste u steriliziranoj moskoj vodi s dodatkom komercijalnog uzgojnog medija F/2 na dva raziličita saliniteta (25 psu i 33 psu). Rezultati pokazuju da su sastav i količina nutrijenata u otpadnoj vodi dostatni za uspješan uzgoj mikroalge *I. galbana* te da sniženi salinitet povoljno utječe na rast ove mikroalge u otpadnoj vodi i u komercijalnom uzgojnom mediju.

Ključne riječi: akvakulturne otpadne vode, salinitet, bigoriva, fitoplankton

Uvod

Tijekom posljednjih 50 godina uloženi su značajni napori da se iz otpadnih voda akvakulturnih proizvodnih sustava uklone hranjive soli kako bi se izbjegla eutrofikacija vodnih recipijenata (Crab i sur., 2007.). Istraživane su brojne biološke i kemijske metode koje bi bile prikladne za ovaj proces, kao što su: (1) biološki procesi za uklanjanje dušika, nitrifikacija i denitrifikacija te (2) kemijski procesi koji uključuju kemijsku precipitaciju fosfora (Ebeling i sur. 2003.). Jedna od novijih alternativa je biološki tretman pomoću kopnenih ili vodenih biljaka, uključujući kulture mikroalgi. Zbog brze stope rasta, značajnog prinosa biljne biomase i doprinosa očuvanju okoliša, njihovo se korištenje danas smatra jednom od najefikasnijih metoda za biološko pročišćavanje otpadnih voda (Lananan i sur., 2014.). Dobivena algalna biomasa može se koristiti kao sirovina za proizvodnju široke palete proizvoda različitih industrija (Gong i Bassi, 2016.; Sathasivam i sur., 2019.).
Mikroalge su jednostanični planktonski biljni organizmi koji slobodno plutaju u vodenoj sredini. Zbog svoje visoke nutritivne vrijednosti, posebno visokog udjela nezasićenih masnih kiselina (PUFA), osnovna su živa hrana u mrijestilištima morskih organizama te su u posljednjih nekoliko desetljeća provođeni pokusi pri kojima je testiran uzogoj više stotina vrsta kako bi se istražila njihova podobnost za korištenje u akvakulturi. Osnovni parametri koji određuju rast uzgojnih populacija mirkoalgi su sastav nutijenata i minerala te temperatura i salinitet uzgojne sredine. Salinitet uveliko utječe na rast algi djelujući izravno na osmoregulacijske mehanizme unutar stanica pa je od iznimne je važnosti pronaći optimalne vrijednosti za pojedine uzgajane vrste algi (Cañavate i sur., 2020.; Hotos i Avramidou, 1995.).

U posljednje vrijeme mikroalge pobuđuju sve veću pažnju i u drugim gospodarskim granama, posebno u farmaceustskoj, prehrambenoj industriji te proizvodnji biogoriva (Zuorro i sur., 2021.). Biogoriva su sve poželjaniji izvor energije zbog smanjenog utjecaja na okoliš i sve izraženije energetske krize te se i tehnologija njihove proizvodnje ubrzano razvija. Dijele se sukladno vrsti sirovine i stupnju razvitka tehnologije proizvodnje u četiri generacije (Chen i sur., 2015.; Moravvej i sur., 2019.). Bigoriva treće generacije zasnivaju se na proizvodnji od standardnih vrsta algi, dok se razvitak četvrte generacije zasniva na genetski modificiranim mikroalgama i još uvijek je u samim povojima (Moravvej i sur., 2019.). Najpoznatiji predstavnici koji se masovno uzgajaju i koriste u proizvodnji biogoriva su alge skupine Haptohyta. Alge roda *Isochrysis* karakteristične su po tome što nemaju staničnu stijenku pa je ekstrakcija lipida jednostavnija (Sun i sur., 2017.; Cohen, 1986.).
Cilj ovog rada bio je istražiti efikasnost pročišćavanja otpadne vode iz zatvorenog recirkulacijskog sustava za proizvodnju morske ribe pri salinitetu od 25 psu, s obzirom na smanjenje koncentracije osnovnih hranjivih soli u odnosu na komercijalni medij, upotrebom mikroalge *Isochrysis galbana.* Time je ujedno istražena i podobnost otpadne vode kao medija za masovni uzgoj ove mikroalge.

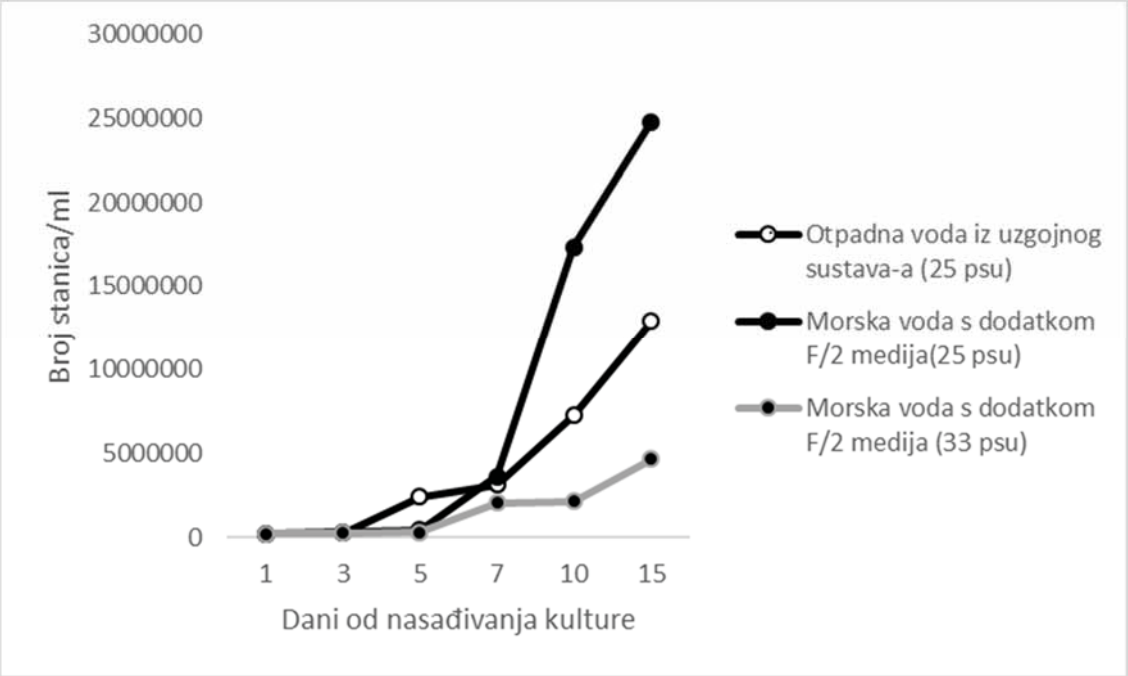
Materijal i metode

Laboratorijsko istraživanje podijeljeno je u dva pokusa. U prvom su pokusu alge uzgajane u 0.5 l morske vode na dva saliniteta (33 i 25 psu) kako bi se s obzirom na različite literaturne podatke istražila pogodnost uzgoja na salinitetu otpadne vode upotrebljene u prvom pokusu. Pokus je proveden u duplikatu. Morska voda je razrijeđena destiliranom vodm do 33, odnosno 25 psu te sterilizirana natrijevim hipokloritom koji je potom neutraliziran natrijevim tiosulfatom (Gavrilović i sur., 2021.). U steriliziranu vodu dodan je komercijalni Guillard (F/2) medij prema uputama proizvođača te je nasađena mikroalga *I. galbana.* Nasadna gustoća bila je jednaka na oba saliniteta i iznosila je 200.000 stanica/ml, kao i uprvom pokusu.
U drugom pokusu je kao medij za uzgoj mikroalge *I. galbana* korištena otpadna voda saliniteta 25 psu iz recirkulacijskog uzgojnog sustava za proizvodnju ribe, koja je najprije profiltrirana kroz mehanički zrnati filtar, a zatim sterilizirana. Također su fotometrom YSI 9300 izmjerene koncentracije nutrijeneta, dušikovih i fosfornih spojeva, kako bi se usporedile s vrijednostima na kraju uzgojnog ciklusa algi. U po 0,5 l otpadne vode, u dvije tikvice, nasađeno je 200.000 stanica/ml.
Kako bi se izbjegao utjecaj drugih čimbenika osim saliniteta na rast populacije algi, tikvice su držane u jednakim uvjetima, pod umjetnim svjetlom jačine 2000 luxa na temperaturi od 22˚C (Gavrilović i sur., 2021.) te spojene na isti aerator. Rast uzgojne populacije algi u oba pokusa praćen je brojanjem stanica 3., 5., 7., 10. i 15. dana od početka inkubacije. Brojanje je obavljano mikroskopski pomoću hemocitometra, pri čemu su prije svakog brojanja alge imobilizirane 4%-tnim formaldehidom. Neposredno prije brojanja na hemocitometar je položeno pokrovno stakalce te je u žljeb hemocitometra pomoću Pasretove sterilne pipete dodana kap kulture. Stanice su brojane u osam rubnih i dva nasumična polja. Ovaj je postupak radi veće preciznosti ponavljan za svaku kulturu tri puta te je izračunat prosjek. Koncentracija algi u uzgojnom volumenu izračunata je prema formuli: n/10x250000=br stanica/ml kulture (Gavrilović i sur., 2021.).

Rezultati i rasprava

Uzgojna populacija *I. galbana* rasla je u Guillard-ovom (F/2) mediju, na dva različita saliniteta (25 psu i 33 psu) s najvećom postignutom gustoćom stanica u manjem salinitetu (25 psu) (slika 1). Brown i sur. (1993.) i Renaud i sur. (1994.) utvrdili su također najbrži rast ove vrste mikroalge na 25 psu, dok su Alkhamis i Qin (2013.) utvrdili da *I. galbana* raste na

širokom rasponu saliniteta od 10 do 65 psu, ali je optimalni bio 35 psu. Odgovarajuća kontrola saliniteta u masovnoj proizvodnji algi ključ je u pronalasku riješenja koja se odnose na optimizaciju rasta algi u procese kvalitetne ekstrakcije lipida (Fong Sing i sur., 2016.). Rast uzgojne populacije *I. galbana* u otpadnoj vodi RAS-a pokazuje sličan trend kao i rast u komercijalnom mediju koji se koristi u uzgoju mikroalgi u akvakulturi (slika 1). Stopa rasta *I. galbana* u otpadnoj vodi je manja od rasta u komercijalnom mediju na 25 psu, i veća od onog na 33 psu. Ti podaci govore o optimalnoj razini relevantnih čimbenika rasta u otpadnoj vodi iz RAS-a i njenoj podobnosti za proizvodnju mikroalgi.



Slika 1. Rast populacije *I. galbana* u različitim uzgojnim uvjetima

U tablici 1. prikazane su koncentracije releventnih nutijenata prije i nakon završenog pokusa. Koncentracije sva četiri ispitivana nutrijenata smanjene su za više od 50%: N-NO_3 za 62 %, N-NO_2 za 56 %, N-NH_4 za 67 % i P-PO_4 za 57 %.

Tablica 1. Koncentracije nutrijenata u otpadnoj vodi RAS-a prije i nakon pokusa

Nutrijenti	**Koncentracija na početku pokusa, mg l^{-1}**	**Koncentracija na kraju pokusa, mg l^{-1}**
N – NO_3	185	70,8
N – NO_2	1,05	0,46
N – NH_4	2,84	0,94
P – PO_4	8,63	3,72

Do sličnog zaključka dolaze i Hawrot-Paw i sur. (2020.) koristeći otpadne vode iz ribogojilišta za proizvodnju mikroalgi. U njihovom pokusu sadržaj ukupnog dušika i ukupnog fosfora u otpadnim vodama otvorenog uzgojnog sustava smanjen je za 88%, odnosno preko 99%. Uz to, sadržaj N-NO_3 smanjen je za 88,6%, N-NO_2 za 74,3%, a otopljenih ortofosfata za 99%. Manje postotke dobivene u ovom pokusu moguće je objasniti većom koncentracijom hranjivih soli u otpadnoj vodi RAS-a u isporedbi s onom u otvorenim sustavima.

Zaključak

Salinitet uzgojnog medija od 25 psu pokazao se pogodnijim za uzgoj mikroalge *I. galabana* od saliniteta 33 psu. Populacija *I. galbana* može uspješno rasti na otpadnoj vodi iz recirkulacijskog akvakulturnog sustava saliniteta 25 psu, proizvodeći dovoljno biomase za eventualnu komercijalizaciju, što ujedno potvrđuje njenu podobnost za biološko pročišćavanje otpadne vode.

Literatura

Alkhamis Y, and Qin J.G. (2013). Cultivation of *Isochrysis galbana* in Phototrophic, Heterotrophic, and Mixotrophic Conditions. BioMed Research International. 2013: 983465.

Brown M.R., Garland C.D., Jeffrey S.W., Jameson I.D., Leroi M. (1993). The gross and amino acid compositions of batch and semi-continuous cultures of I*sochrysis* sp. (clone T. ISO), *Pavlova lutheri* and *Nannochloropsis oculata*. Journal of Applied Phycology. l5: 285–296.

Cañavate J.P., Hachero-Cruzado I., Pérez-Gavilán C., Fernández-Díaz C. (2020). Lipid dynamics and nutritional value of the eustarine strain Isochrysis galbani VLP from hypo to hyper salinity. Journal of Applied Phycology. 32: 3749-3766.

Chen J., Bai J., Li H, Chang C.., Fang S. (2015). Prospects for Bioethanol Production from Macroalgae. Trends Renewable Energy. 1: 2376-2144.

Cohen Z. (1986). Products from microalgae. *ln*: Handbook of miaoalgal mass cultures (Richmond, A., ed), p.421-454. Boca Raton, USA: CRC Press.

Crab R., Avnimelech Y., Defoirdt T., Bossier P., Verstraete W. (2007). Nitrogen Removal Techniques in Aquaculture for a Sustainable 342 Production. Aquaculture. 270 (1–4): 1–14.

Ebeling J.M., Sibrell P.L., Ogden, S.R., Summerfelt S.T. (2003). Evaluation of Chemical Coagulation–Flocculation Aids for the Removal of Suspended Solids and Phosphorus from Intensive Recirculating Aquaculture Effluent Discharge. Aquaculture Engineering. 29(1-2): 23–42.

Fon-Sing S., Borowitzka M.A. (2016). Isolation and screening of euryhaline *Tetraselmis* spp. suitable for large-scale outdoor culture in hypersaline media for biofuels. Journal of Applied Phycology. 28: 1–14.

Gavrilović A., Jug-Dujaković J., Ljubičić A., Iveša N. (2021). Dizajn i menadžment mrijestilišta školjkaša. Pula, Hrvatska: Sveučilište Jurja Dibrile u Puli.

Gong M., Bassi A. (2016). Carotenoids from microalgae: a review of recent development. Biotechnol. Adv. 34: 1396–1412.

Hawrot-Paw M., Koniuszy A., Gałczynska M., Zajac G., Szyszlak-Bargłowicz J. (2020) Production of Microalgal Biomass Using Aquaculture Wastewater as Growth Medium. Water. 12 (1):106.

Hotos G., and Avramidou D. (1995). Growth study of the one-celled halophilous alga *Asteromonas gracilis* (Chlorophyta) in mass culture condition using different salinities, photoperiod and without addition of vitamins (in Greek with English summary). Geotechnical Scientific Issues of the Geotechnic Chamber of Greece. 6(2): 37-45.

Lananan F., Abdul Hamid S.H., Din W.N.S., Ali N., Khatoon H., Jusoh A., Endut A. (2014). Symbiotic Bioremediation of Aquaculture Wastewater in Reducing Ammonia and Phosphorus Utilizing Effective Microorganism (EM-1) and Microalgae (*Chlorella* sp.). International Biodeterioration& Biodegradation. 95: 127–134.

Moravvej Z., Makarem M.A., Rahimpour M.R. (2019). The fourth generation of biofuel. *In*: Second and Third Generation of Feedstocks, Basile, A., and Dalena F. (eds), 557-597. Elsevier Inc.

Renaud S.M., Parry D.L. (1994). Microalgae for use in tropical aquaculture. II: Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. Journal of Applied Phycology. 6: 347–356.

Sathasivam R., Radhakrishnan R., Hashem A., Abd_Allah E.F. (2019). Microalgae metabolites: a rich source for food and medicine. Saudi Journal Biology of Science. 26: 709–722.

Sun Z., Chen Y., Mao X., Liu J. (2017). Physiological and biochemalchanges reveal differential patterns of docosahexaenoic acid partitioning in two marine algal strains of *Isochrysis.* Marine Drugs. 15(11): 357.

Sun Z., Wei H., Zhou Z.G., Ashokkumar M., Liu J. (2018). Screening os *Isochrysis* strains and utilization of a two stage outdoor cultivation strategy for algal biomass and lipid production. Apllied biochemestry and biotechnology. 185(4): 1100-1117.

Zuorro A. (2015). Optimization of Polyphenol Recovery from Espresso Coffee Residues Using Factorial Design and Response Surface. Methodology. Separation and Purification Technology. 152: 64-69.

Possibilities of cultivation of microalga *I. galbana* in wastewater from aquaculture cultivation systems

Abstract

More and more attention is being paid to the possibility of using wastewater from aquaculture farming systems. An attractive option is the cultivation of microalgae, which, with traditional use in hatcheries, are becoming increasingly popular raw materials in the pharmaceutical, cosmetic, food and biofuel industries. In this paper, the suitability of 25 psu salinity wastewater from the aquaculture production system for the cultivation of the microalga *I. galbana* was investigated. The data were then compared with the growth of breeding populations of this species in sterilized seawater with the addition of commercial breeding medium F / 2 at two different salinities (25psu and 33psu). The results show that the composition and amount of nutrients in wastewater are sufficient for successful cultivation of the microalga *I. galbana*. and that reduced salinity has a beneficial effect on the growth of its population in wastewater and commercial culture medium.

Key words: aquaculture waste water, salinity, biofuel, phytoplankton

ORIGINAL SCIENTIFIC PAPER

Feeding habits of commercially important gastropod species *Hexaplex trunculus* (Linnaeus, 1758) under ocean acidification conditions

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Abstract
This paper presents a long-term experiment aiming to assess the feeding habits of a predatory marine gastropod species, *Hexaplex trunculus* (Linneaeus, 1758), under a range of pH conditions relevant in the context of ocean acidification (8.1 to 7.4). There was no significant relationship among the feeding rate (mussel week^{-1}) and pH after a 40-week exposure ($F_{1,8}$=2.31, R^2=0.25, p=0.25). After 60 weeks of exposure, no difference was observed among pH treatments in number of snails successfully reaching their food (success 66.7%), but the time to reach the food significantly differed ($F_{2,21}$=3.98, p=0.034) and showed a positive trend toward shorter duration (min) at lower pH. The data suggest that, when fed on Mediterranean mussels, ocean acidification will not negatively impact feeding habits of this species.

Key words: Mali Ston Bay, ocean acidification, marine gastropod, feeding habits, long-term experiment,

Introduction

Since the beginning of the Industrial Revolution, excessive fossil fuel burning and intensive agriculture have increased atmospheric CO_2 levels by 40% as compared to pre-industrial times (Feely et al., 2009). Approximately 30% of the CO_2 released into the atmosphere is absorbed by the world's oceans, leading to a perturbation of seawater carbonate chemistry (Caldeira and Wickett, 2005), including a reduction in seawater pH, carbonate ion concentration, and the saturation state of calcite and aragonite (Feely et al., 2009). As a result, the average pH of surface seawater has decreased from 8.2 to 8.1 over the past 200 years and is estimated to continue to decrease by up to 0.4 pH units by 2100 (IPCC, 2019). This phenomenon is known as ocean acidification and is already impacting a range of marine organisms. A growing body of research has shown that the biological response to ocean acidification is highly variable, even among closely related species (Doney et al., 2020). The physiological response may even vary between different populations of the same species and recent work identified adaptation to the high local variability with pH as a key factor explaining these differences (Vargas et al., 2017).

Banded-dye murex, *Hexaplex trunculus* (Linnaeus, 1758) is predatory gastropod species belonging to the family Muricidae. In addition to its commercial value for small-scale fisheries in the Mediterranean Sea, it also has an important role in controlling benthic communities. That especially comes to attention in shellfish farming areas where it may be considered a pest (Peharda and Morton, 2006) *H. trunculus* is a common species in Mali Ston Bay, a protected area known for shellfish farming since the 16th century (Benović,

1997). This paper presents a long-term experiment aiming to assess the feeding habits of the *H. trunculus* population from Mali Ston Bay when fed on Mediterranean mussels under a range of pH conditions relevant in the context of ocean acidification.

Material and methods

Adult gastropods (shell length, SL=49.76±0.86 mm) were collected in Mali Ston Bay (42°52'19.1 "N 17°42'02.3 "E) using plastic trays filled with the Mediterranean mussel *Mytilus galloprovincialis* Lamarck, 1819. Individuals were immediately transferred to the laboratory and kept in a flow-through system for two weeks prior to the start of the experiment to allow them to acclimate. The pH treatments related to future ocean acidification were designed to represent a range of nine pH conditions, including the present-day variability in Bistrina Bay (8.2-7.9, this study) and 0.5 pH units lower. Randomly selected 40 individuals were placed in each of the nine treatment tanks in a flow-through system with filtered, UV-sterilized, and aerated ambient seawater. The pH in each tank was manipulated by bubbling pure CO_2 gas and using pH controllers (Milwaukee MC122) connected to solenoid valves to maintain the desired pH. In one tank, pH was regularly monitored and measured but not manipulated. Temperature (T; ºC), salinity (S; ppt), dissolved oxygen concentration (DO, mgL^{-1}), and pH (on the National Bureau of Standards scale – pH_{NBS}) were measured using multiparametric probes (YSI Pro 30, Oxygen Handy Polaris, and SI Analytics Lab Meter 845, respectively). Total alkalinity (TA, µmol kg^{-1}) was determined every two weeks using the potentiometric two-point open-cell titration method (Dickson et al., 2007). Other seawater carbonate chemistry parameters (Riebsell et al., 2011) were calculated based on known TA and pH_{NBS} for a given salinity using CO2SYS software, with dissociation constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Feces and debris were siphoned every other day, and once a week a complete exchange of the seawater and detailed cleaning was done.

Specimens were fed ad libitum for 40 weeks (start: August 24; end: June 2) with commercial size Mediterranean mussels (SL =67.10±0.83 mm), *M. galloprovincialis*, obtained from a local shellfish supplier. Mussels were replaced once a week. Empty shells were counted and divided by the number of snails in each treatment. The feeding rate (mussel $week^{-1}$) was calculated as the average number per mussels consumed per week and per snail. The effect of pH was estimated using a linear relationship between pH and feeding rate.

For the second part of the experiment, three representative pH treatments were selected to cover the range of the experimental design - pH 8.09, pH 7.81, pH 7.43. The percentage of individuals (%) reaching their food and time needed (min) was estimated for gastropods exposed for 60 weeks to each pH condition. All individuals were starved for two weeks prior to the experiment. Plastic trays (52x35x20 cm) were filled with seawater from each respective pH treatment. Four mussels were manually opened and placed on one side of the tray. Four randomly selected individuals were chosen and placed on the opposite side with the anterior facing mussels. The time to reach the food (min) was measured with a stopwatch, for a maximum of 60 minutes. The number of individuals that successfully reached their food was noted. Each treatment was repeated 3 times. All data are presented as mean±SD. Statistical analysis was performed using the Minitab.v17.1.0 package. Data were tested for normality using the Anderson-Darling test (AD =0.514, p=0.174) and ANOVA were used to test for pH effect.

Results and discussion

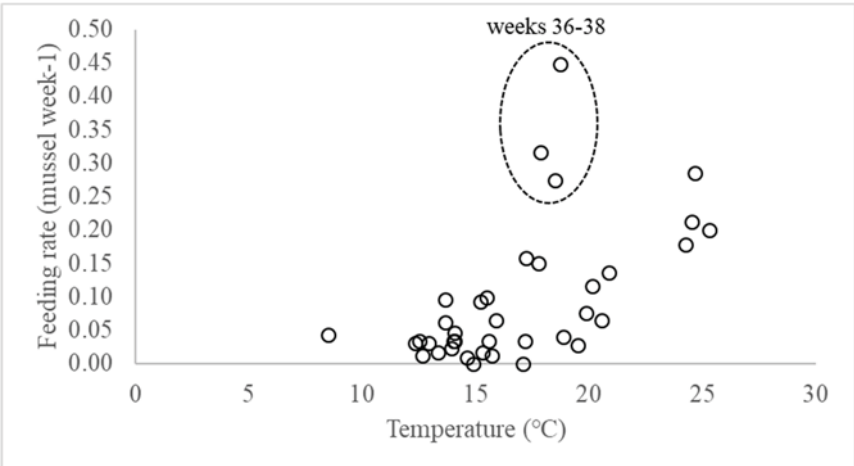
During the experiment, temperature and salinity varied with seasonal changes, between 8.4 and 26.6 °C for temperature and 22.6 and 35.3 ppt for salinity. The dissolved oxygen concentration never fell below 6.28 mg L^{-1} O_2. The pH of seawater in the unmanipulated pH

treatment varied between 7.91 and 8.23 during the experiment, corresponding to the nearshore pH variability in Mali Ston Bay. The measured and calculated carbonate chemistry parameters are listed in Table 1.

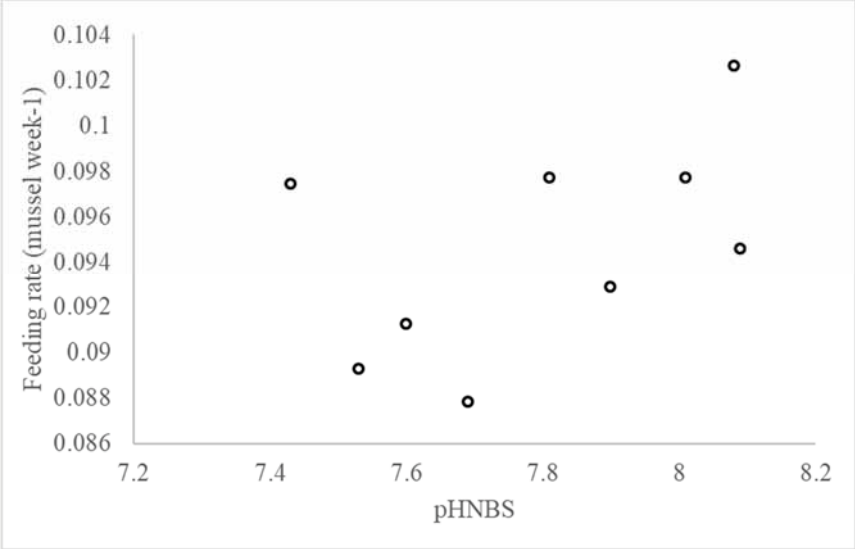
Table 1. Seawater carbonate chemistry parameters during experiment presented as mean ±SD. Seawater pH on NBS scale (pH_{NBS}), salinity (S; ppt), temperature (T; ºC) and total alkalinity (TA; mmol kg^{-1}) were used to calculate CO_2 partial pressure (pCO_2; µatm), calcite and aragonite saturation states (Ω Ca and Ω Ar, respectively)

Measured				Calculated		
pH_{NBS}	S (ppt)	T (ºC)	TA (mmol kg^{-1})	pCO_2 (µatm)	Ω Ca	Ω Ar
8.09±0.07	31.3±2.8	17.4±4.6	2976±216	692±181	3.9±0.8	2.5±0.6
8.08±0.06	31.3±2.8	17.4±4.6	2950±176	698±194	3.9±0.9	2.5±0.6
8.01±0.05	31.4±2.8	17.4±4.6	2940±190	809±77	3.3±0.4	2.1±0.3
7.90±0.07	31.3±2.8	17.4±4.5	2955±201	1064±99	2.7±0.4	1.7±0.3
7.81±0.05	31.3±2.9	17.5±4.6	2935±207	1335±125	2.2±0.3	1.4±0.2
7.69±0.05	31.3±2.9	17.5±4.6	2917±208	1759±170	1.7±0.2	1.1±0.2
7.60±0.06	31.3±2.8	17.4±4.5	2946±179	2187±182	1.4±0.9	0.9±0.1
7.53±0.04	31.3±2.9	17.4±4.6	2891±207	2601±249	1.2±0.2	0.7±01
7.43±0.05	31.4±2.8	17.4±4.6	2851±184	3221±258	0.9±06	0.6±0.1

The amount of food consumed varied throughout the experiment, mainly as a function of temperature, with lower consumption rates during the colder winter period. A significant increase in consumption in all treatments was observed during weeks 36-38 of the experiment (Graph 1), presumably due to higher energy requirements prior to spawning, when females are known to interrupt feeding (Vasconcelos et al., 2004). There was no significant relationship between pH and feeding rate (Graph 2; $F_{1,8}$=2.31, R^2=0.25, p=0.17), suggesting that future ocean acidification may not affect the consumption rate of this species, assuming an abundant food supply is implied.



Graph 1. Relationship between the temperature (°C) in the seawater and the average weekly feeding rate (mussel $week^{-1}$) across pH treatments. Increase in consumption (weeks 36-38) is highlighted.



Graph 2. Relationship between feeding rate (mussel week^{-1}) and pH treatments (8.09-7.43)

After 60 weeks, there was a significant difference in the time to reach the food among treatments ($F_{2,21}$=3.98, p=0.034). A post hoc Tukey's pairwise analysis showed that the snails at pH 7.43 took significantly less time to reach their food than at pH 8.09 (T=-2.78, p=0.029), although the number of individuals that successfully reached their food (success, %) had not changed, with a success rate of 66.7% in all treatments (Table 2). This could indicate that individuals from the low pH treatments had less energy reserves and were more affected by starvation than under high pH treatment, even though there was no difference in the amount of food consumed over the course of the experiment.

Table 2. Average values (±SE) of time to reach the food (duration, min) and success of individuals that reached the food (success, %) for a given number of individuals (N). pH_{NBS} and temperature in respective treatments (T, °C) at the time of experiment

Treatment	N	pH_{NBS}	T (C)	Duration (min)	Success %
pH 8.09	12	8.01	26.7	32.88 ± 5.6	66.7
pH 7.81	12	7.80	26.8	27.75 ± 2.6	66.7
pH 7.43	12	7.40	26.7	18.13 ± 2.1	66.7

Chatzinikolaou et al. (2019) observed negative effects of low pH (pH 7.6) on successful foraging of *H. trunculus* (the coast of Gournes, North Crete) in a long-term study (2.5 years). Their data showed that higher temperature can mitigate the effects of low pH. Individuals acclimated at 23°C had 52.9% success in reaching food at low pH (7.6), compared to only 8.3% at 20 °C. Temperature was controlled and kept constant at 20°C, while in this study temperature followed natural variations.

Conclusions

Our results suggest that lower pH relevant in the context of future ocean acidification is not expected to significantly affect the feeding behaviour of *H. trunculus* population from Mali Ston Bay when fed on Mediterranean mussels. Presented data support the hypothesis that the response to ocean acidification is not only species-specific, but also that different populations of the same species may respond differently to the same absolute pH values. While local adaptation has been shown to be a contributing factor, resolving these population-specific differences is a priority for future research.

References

Benović A. (1997). The history, present condition, and future of the molluscan fisheries of Croatia. In *The History, Present Condition,and Future of the Molluscan Fisheries of North and Central America and Europe*, Mackenzie C. L., Burrell V. G., Rosenfield A., Hobart W. L. (ed.), 240 p. NOAA Technical Report 129: U.S. Department of Commerce. Available from: https://spo.nmfs.noaa.gov/sites/default/files/tr127opt.pdf

Caldeira K., and Wickett M. E. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions of three intertidal gastropods in Australia. Marine Biology. 83: 163-169.

Chatzinikolaou E., Sterioti A., Grigoriou P. (2019). Impact of ocean acidification and warming on the feeding behaviour of two gastropod species. Mediterranean Marine Science. 20(4): 669-679.

Dickson A. G., and Millero F. K. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep-Sea Research Part A. Oceanographic Research Papers. 34(10): 1733-1743.

Dickson A. G., Sabine C. L., Christian J. R. (2007). Guide to Best Practices for Ocean CO_2 measurements. PICES Special Publication3. 191p. Available from: https://repository.oceanbestpractices.org/handle/11329/249

Doney S. C., Busch D. S., Cooley S. R., Kroeker K. J. (2020). The Impacts of Ocean Acidification on Marine Ecosystems and Reliant Human Communities. Annual Review of Environment and Resources. 45: 83-112.

Feely R. A., Doney S. C., Cooley S. R. (2009). Ocean Acidification. Present conditions and future changes in high-CO_2 world. Oceanography. 22 (4): 36-47.

IPCC. (2019). IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Pörtner H. O., Roberts D. C., Masson-Delmottee V., Zhai P., Poloczanska E., Mintenbeck K., Tignor M., Alegria A., Nicolai M., Okem A., Petzold, J. Rama B., Weyer N. M. (ed.), In press. Available from: https://www.ipcc.ch/srocc/

Mehrbach C., Culberson C., Hawley J. E., Pytkowicx R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography. 18(6): 897-907.

Peharda M., and Morton B. (2006). Experimental prey species preferences of *Hexaplex trunculus* (Gastropoda: Muricidae) and predator-prey interactions with the Black mussel *Mytilus galloprovincialis* (Bivalvia: Mytilidae). Marine Biology. 148 (5): 1011-1019. A

Riebsell U., Fabry V. J., Hansson L., Gattuso J. P. (2011). Guide to best practices for ocean acidification research and data reporting. Oceanography. 22(4): 260.

Vargas C. A., Lagos N. A., Lardies M. A., Duarte C., Manriquez P. H., Aguilera V., Broitman B. R., Widdicombe S., Dupont S. (2017). Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nature Ecology & Evolution. 1(4): 0084.

Vasconcelos P., Joaquim S., Gaspar M. B., Martias D. (2004). Spawning of *Hexaplex* (Truncularіopsis) *trunculus* (Gastropoda: Muricidae) in the laboratory: description of spawning behaviour, egg masses, embryionic development, hatchling and juvenile growth rates. Invertebrate Reproduction and Development. 46(2-3): 125-138.

IZVORNI ZNANSTVENI RAD

Skupljačka aktivnost sive pčele (*Apis mellifera carnica* Pollmann 1879) na području Zabiokovlja

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Sažetak
Cilj je istraživanja bio utvrditi skupljačku aktivnosti radilica sive pčele (*Apis mellifera carnica* Pollmann 1879) na području Zabiokovlja tijekom jedne sezone na tri pčelinje zajednice, analizom sadržaja mednog mjehura i peludnog tereta. U 8:00 je bila utvrđena značajno veća masa sadržaja mednog mjehura (18,58 mg) u odnosu na uzorke prikupljene u 13:00 (11,45 mg) i one prikupljene u 18:00 (11,93 mg) sati. Masa peludnog tereta u 18:00 je bila značajno niža (7,83 mg) u odnosu na uzorke prikupljene u 8:00 (10,88 mg) i 13:00 (9,82 mg). U najvećem je udjelu mednih mjehura (62,21%) bila utvrđena medna rosa, što ukazuje na prisutnost cvrčka (*Acericerus heydenii*) na istraživanom području.

Ključne riječi: skupljačka aktivnost, *Apis mellifera carnica*, medni mjehur, peludni teret, Zabiokovlje

Uvod

Područje je Zabiokovlja zbog specifičnosti mediteranske i submediteranske klime prepoznato po medonosnoj flori koja omogućava proizvodnju raritetnih vrsta meda. Među koje se, od uniflornih vrsta, ističu med od kadulje (*Salvia officinalis* L.), zatim med od modrog lasinja (*Moltkia petraea* (Tratt) Grisseb.) te med od bijelog vriska (*Satureja montana* L.). Osim uniflornih vrsta meda, navedeno područje također pruža zanimljiv medonosni potencijal i za proizvodnju multiflornih vrsta meda. Također, zanimljivost Zabiokovlja predstavljaju i značajne sastojine maklena (*Acer monspessulanum* L.). Naime, u posljednjih nekoliko godina na maklenu je primijećena pojava značajne količine medne rose. Isprva se mislilo da mednu rosu izlučuju, kao što je i uobičajeno, lisne ili štitaste uši, međutim pomnim je praćenjem utvrđeno da se radi o vrsti cvrčka (*Acericerus heydenii* Kirschbaum, 1868) (Bubalo i sur. 2021.). Navedena vrsta cvrčka pripada koljenu člankonožaca (*Arthropoda*), razredu kukaca (*Insecta*), redu polukrilaca (*Hemiptera*), porodici cvrčaka (*Cicadellidae*) i rodu *Acericerus*. Ima jednu generaciju godišnje. Ženka polaže jaja u biljno tkivo grančica biljaka domaćina pomoću leglice oblika male pile. Razvoj se nimfe odvija u pet stadija te tijekom petog stadija ulazi u hibernaciju, koji kreće početkom kolovozu i traje do početka rujna. Prezimljuje kao odrasli oblik (Stöckmann i sur. 2010.). Kako je navedena vrsta cvrčka nedavno determinirana na ovom području, a po količinama medne rose koju izlučuju različiti stadiji nimfi, predstavlja zanimljiv pašni potencijal. Jedan od prvih koraka u istraživanju pašnog potencijala je ponašanje pčelinje zajednice, odnosno, njezina skupljačka aktivnost. Naime, skupljačka aktivnost bila je u posljednje vrijeme predmet nekoliko istraživanja. Tako je obrađena paša na ljekovitoj kadulji (Valentić, 2009.), zatim na metvici (Hegić, 2016.) te na unšijskoj mandarini (Prđun, 2017.). Međutim, kako u znanstveno dostupnoj literaturi nema podatka o skupljačkoj aktivnosti u području Zabiokovlja, a i navedeno područje zbog svojih specifičnosti pašnog potencijala, jer se nalazi na prijelazu iz submediteranske u

planinsku klimu, nije do sada istraženo. Stoga je cilj rad bio utvrditi skupljačku aktivnosti radilica sive pčele (*Apis mellifera carnica* Pollman 1879) na području Zabiokovlja.

Materijali i metode

Istraživanje je bilo provedeno na lokaciji Duge njive u području Zabiokovlja. Uzorkovanje je pčela bilo provedeno na tri pčelinje zajednice sive pčele (*A. mellifera* carnica Pollmann 1897) smještenih u Langstroth-Root (LR) košnicama. Uzorci pčela skupljačica bili su prikupljani tri puta dnevno (8:00, 13:00 i 18:00) u razdoblju od 10 dana tijekom lipnja 2017. godine. Po uzorku se prikupilo između 150 i 200 skupljačica. Analiza sadržaja mednog mjehura bila je provedena po metodi Soehngen i Jay (1974). Na skupljačicama, na kojima je bio utvrđen peludni teret isti je bio skidan s peludnih košarica i zatim odlagan na predmetnice na kojima je bilo izvršeno njihovo vaganje. Statistička obrada podataka provedena je programom SAS 9.4 (SAS, 2012). Za mjerene parametre (masa mednog mjehura, masa peludnog tereta) opisna statistika utvrđena je korištenjem procedura MEANS, dok je za broj skupljačica korištena procedura FREQ. Statistička značajnost pojedinih utjecaja na mjerene parametre utvrđena je GLM procedurom prema modelu $Y_{ij}=\mu+\tau_i+\varepsilon_{ij}$, gdje je Y_{ij} = svojstvo, μ = srednja vrijednost, τ_i= fiksni utjecaj te ε_{ij}= neprotumačeni ostatak. Razlike između eksperimentalnih skupina utvrđene su primjenom Duncan post hoc testa. Za provjeru razlika očekivane i utvrđene frekvencije skupljačica primijenjen je χ^2 kvadrat test.

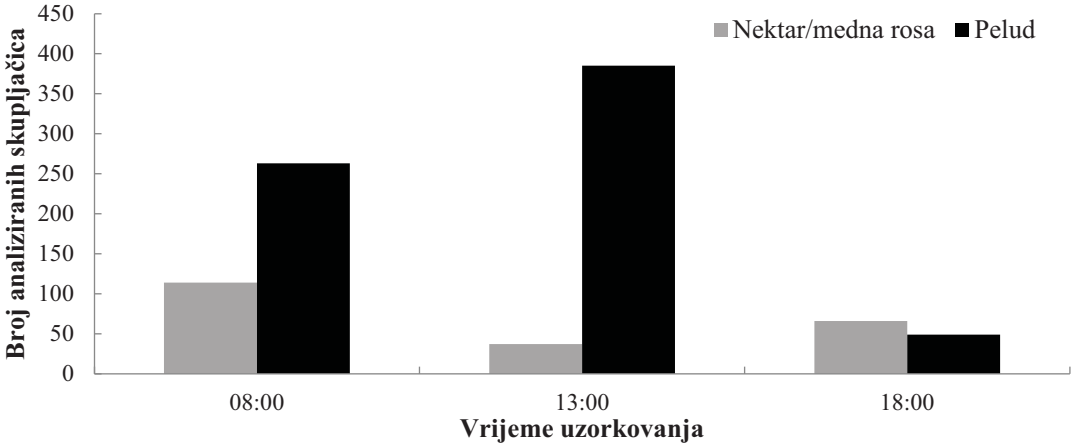
Rezultati i rasprava

Na osnovu provedene analize sadržaja mednih mjehura, od ukupno 217 analiziranih uzoraka u najvećem je udjelu (62,21%) utvrđena medna rosa, zatim nektar (23,35%) te mješavina nektara i medne rose (4,15%), dok je 8,29% uzoraka bilo slabo ili neispravno (Tablica 1). Usporedivši broj pčela prema sadržaju mednog mjehura, utvrđene su statistički opravdane razlike ($\chi2$ = 279,89; SS=4, p <0,0001) u broju skupljačica koje su donijele teret. Stoga se smože pretpostaviti da je utvrđena medna rosa posljedica izlučivanja cvrčka (*A. heydenii*), jer je isti utvrđen na istraživanom području (Bubalo i sur., 2021.).

Tablica 1. Analiza sadržaja mednog mjehura

Sadržaj mednog mjehura	N	%
Medna rosa	135	62,21
Nektar	55	25,35
Mješavina nektara i medne rose	9	4,15
Neispravan/slab uzorak	18	8,29
Ukupno	**217**	**100,00**

Na osnovu podataka skupljačke aktivnosti prikazanih u grafikonu 1 vidljivo je da je bila utvrđena statistički značajna razlika s obzirom na broj skupljačica koje su skupljale nektar ($\chi2$= 41,8199 p<0,0001) i pelud ($\chi2$= 249.0579 p<0,0001). Promatrajući broj skupljačica s punim mednim mjehurom (114) utvrđeno je da je on bio najveći u 8:00, dok je broj skupljačica u 13:00 (37) i 18:00 (66) bio značajno manji. S obzirom na broj skupljačica koje su skupljale pelud utvrđeno je da je najveći broj njih bio ustanovljen u 13:00 (385), zatim u 8:00 (263), dok je u 18:00 (49) bio značajno manji.



Grafikon 1. Broj analiziranih skupljačica prema vrsti tereta i vremenu uzorkovanja

Analizirajući skupljačice utvrđeno je da su se tijekom pokusnog razdoblja prosječne mase sadržaja mednog mjehura kretale od 6,33 (26. lipnja) do 22,26 mg (16. lipnja), a raspon se kretao od minimalnih 0,60 mg do maksimalnih 53,40 mg, koji je bio utvrđen 16. lipnja (Tablica 2). Dobivene su se vrijednosti podudarale s vrijednostima utvrđenim u istraživanjima Valentić (2009.) na paši kadulje (1,40 - 54,50 mg), Hegić (2016.) na paši metvice (0,20 - 50,60 mg) te na paši unšijske mandarine, gdje se raspon kretao od 0,70 do 58,60 mg (Prđun, 2017.). Međutim, u istraživanju Soehngen i Jay (1974.) utvrđene su znakovito niže vrijednosti istraživanog parametra i one su se kretale od 15 do 23 mg. Promatrajući sljedeći parametar, masu peludnog tereta, utvrđeno je da se prosječna masa kretala od 4,34 mg (15. lipnja) do 14,70 mg (16. lipnja), a raspon se kretao od minimalnih 0,50 mg (15. i 16. lipnja) do maksimalnih 65,90 mg, koji je bio utvrđen 16. lipnja (Tablica 2).

Tablica 2. Deskriptivna statistika mase sadržaja mednog mjehura (mg) skupljačica i mase peludnog tereta (mg) po danima

	Masa sadržaja mednog mjehura (mg)				**Masa peludnog tereta (mg)**			
Datum	**N**	$\bar{x}$	**Sd**	**Raspon**	**N**	$\bar{x}$	**Sd**	**Raspon**
15. lipnja	26	18,97 a b	11,75	0,60 – 45,40	5	4,34 e	5,90	0,50 – 14,80
16. lipnja	57	22,26 a	11,38	2,10 – 53,40	59	14,70 a	11,66	0,50 – 65,90
17. lipnja	24	11,32 b c	9,31	0,40 – 38,20	93	10,96 b c	5,08	1,80 – 22,40
18. lipnja	13	8,59 c	4,37	2,90 – 16,70	41	10,13 b c d	5,18	0,70 – 26,40
20. lipnja	18	11,82 b c	12,35	0,90 – 53,10	27	8,55 c d	6,12	0,50 – 27,60
21. lipnja	15	11,17 b c	7,48	3,60 – 33,80	40	13,42 a b	11,32	1,20 – 56,70
22. lipnja	28	10,75 b c	6,75	1,70 – 30,90	79	8,89 c d	4,87	1,10 – 26,40
23. lipnja	32	14,90 a b c	12,03	1,60 – 46,50	46	6,91 e d	4,74	1,30 – 21,20
26. lipnja	4	6,33 c	4,07	1,10 – 9,70	129	9,43 c d	5,44	1,30 – 33,60

Srednje vrijednosti označene različitim slovima unutar istog parametra statistički su značajno različite (p< 0,05)

Uspoređujući prosječne mase mednog mjehura analiziranih skupljačica iz tablice 2 je razvidno da su bile utvrđene statistički značajne razlike po danima uzorkovanja (F=6,43; p<0,0001). Najveća je prosječna masa sadržaja mednog mjehura (22,26 mg) bila ustanovljena u uzorcima skupljačica od 16. lipnja i ona se statistički značajno razlikovala od uzoraka skupljačica od 17. (11,32 mg), 18. (8,59 mg), 20. (11,82 mg), 21. (11,17 mg) i 22. (10,75 mg) lipnja, dok u odnosu na skupljačice od 15. (18,97 mg) i 23. (14,90 mg) lipnja

nije bilo statistički značajne razlike. Također, usporedbom prosječnih masa peludnog tereta bile su ustanovljene statistički značajne razlike po danima uzorkovanja (F=6,88; p<0,0001). Najveća prosječna masa peludnog tereta (14,70 mg) je bila ustanovljena u uzorcima skupljačica od 16. lipnja i ona se statistički značajno razlikovala od uzoraka skupljačica od 15. (4,34 mg), 17. (10,96 mg), 18. (10,13 mg), 20. (8,55 mg), 22. (8,89 mg), 23. (6,91 mg), 25. (9,43 mg) i 26. (9,55 mg) lipnja, dok u odnosu na skupljačice od 21. lipnja (13,42 mg) nije bilo statistički značajne razlike. Također, statistički značajna razlika ispitivanog parametra je bila utvrđena i između uzoraka skupljačica od 21. lipnja u odnosu na one od 22., 23., 25. i 26. lipnja, dok statistički značajnih razlika između skupljačica od 22., 23., 25. i 26. lipnja nije bilo. Statistički značajna razlika bila je utvrđena i između uzoraka skupljačica od 17. i 23. lipnja (Tablica 2).

Prosječne mase mednog mjehura analiziranih skupljačica su se statistički značajno razlikovale (F=10,82; p<0,0001) i po satima uzorkovanja. U uzorcima pčela prikupljenih u 8:00 bila je utvrđena statistički značajno veća masa sadržaja mednog mjehura (18,58 mg) u odnosu na uzorke prikupljene u 18:00 (11,93 mg) i one prikupljene u 13:00, kod kojih je i utvrđena najmanja masa od 11,45 mg. Međutim, uspoređujući vrijednosti ispitivanog parametra između uzorka skupljačica prikupljenih u 13:00 i 18:00 nije bila utvrđena statistički značajna razlika (Tablica 3). Vjerojatno su manje mase nektara tijekom dana posljedica vremenskih uvjeta, prije svega rasta temperatura zraka, a možda i strujanja zraka (vjetra), utjecale na sušenje nektara i medne rose, dok na skupljanje peludi navedeni vremenski uvjeti nisu imali toliki upliv. Također, navedene su činjenice vjerojatno utjecale i na razlike istraživanih parametra po danima praćenja.

Tablica 3. Deskriptivna statistika mase sadržaja (mg) mednog mjehura i mase peludnog tereta (mg) skupljačica tijekom dana

	Masa sadržaja (mg) mednog mjehura				**Masa peludnog tereta (mg)**			
Sati	**N**	$\bar{x}$	**Sd**	**Raspon**	**N**	$\bar{x}$	**Sd**	**Raspon**
8:00	114	18,58 a	12,26	0,60 – 53,40	263	10,88 a	5,47	0,70 – 33,60
13:00	37	11,45 b	8,01	1,70 – 38,90	385	9,82 a	7,47	0,50 – 65,90
18:00	66	11,93 b	9,36	0,40 – 41,00	49	7,83 b	7,66	0,50 – 39,20

Srednje vrijednosti označene različitim slovima unutar istog parametra statistički su značajno različite (p< 0,05)

Promatrajući prosječne mase peludnog tereta analiziranih skupljačica utvrđeno je da postoje statistički značajno razlikovale (F=4,78; p=0,0087) tijekom dana, tj. vremena uzorkovanja. U uzorcima pčela skupljenih u 18:00 utvrđena je statistički značajno niža prosječna masa peludnog tereta (7,83 mg) u odnosu na uzorke prikupljene u 8:00 (10,88 mg) i 13:00 (9,82 mg). Međutim, uspoređujući vrijednosti ispitivanog parametra između uzorka skupljačica prikupljenih u 8:00 i 13:00 nije bila utvrđena statistički značajna razlika (Tablica 3). Slične su vrijednosti utvrdili Garcia-Garcia i sur. (2004.), gdje je prosječna masa peludnog tereta iznosila 7,89 mg te 10,30 mg u istraživanju Keller i sur. (2005.), kao i u istraživanjima Vaissiere i Vinson (1994.), gdje su se prosječne mase peludnog tereta kretale od 0,42 do 8,23 mg. Također, Joshi i Joshi (2010.) iznose podatke da su prosječne masa peludnog tereta prikupljenog u 9:00 iznosila 8,33 mg u pčelinjim zajednicama istočne pčele (*A. cerana*) te 10,11 mg u zajednicama zapadne pčele (*A. mellifera*), dok su u 12:00 izmjerene mase iznosile 9,16 mg (*A. cerana*) i 11,19 mg (*A. mellifera*). Međutim, u istraživanju Lindtner (1981) utvrđene su više vrijednosti istraživanog parametra te se masa peludnog tereta kretala od 5 do 15 mg, kao i u istraživanju Winston (1987.) od 10 do 30 mg.

Zaključci

Radilice su na istraživanoj lokaciji u jutarnjim satima skupljale veće mase nektara nego u kasnijim uzorkovanjima tijekom dana. Najveći broj skupljačica s peludnim teretom utvrđen je u mjerenjima sredinom dana, dok se masa peludnog tereta smanjivala od jutra prema večeri. Na osnovu analize sadržaja mednog mjehura u najvećem je udjelu bila utvrđena medna rosa, čime se potvrdila aktivnost cvrčka (*A. heydenii*) na istraživanom području.

Literatura

Bubalo D., Houra M., Prđun S. (2021). Pojavnost medne rose na maklenu. Hrvatska pčela. 140 (5): 166-168.

Garcia-Garcia M. C., Ortiz L. P., Diez Dapena J. M. (2004). Variations in the weights of pollen loads collected by Apis mellifera L. Grana. 43: 183–192.

Hegić G. (2016). Peludni spektar i isparljivi spojevi u sadržaju mednog mjehura i medu od metvice (*Mentha* spp.). Doktorska disertacija. Sveučilište u Zagrebu Agronomski fakultet

Joshi N. C., Joshi P. C. (2010). Foraging behaviour of Apis spp., on Apple Flowers in a subtropical environment. New York Science Journal. 3: 71-76.

Keller I., Fluri P., Imdorf A. (2005). Pollen nutrition and colony development in honey bees: Part I. Bee World. 86: 3-10.

Lindtner P. (1981). Indentification of honey bee pollen loads in Hagley yard Wilmington, Delaware. Master thesis. Faculty oft he University of Delaware

Prđun S. (2017). Skupljačka aktivnost pčelinje zajednice na paši i sastav nektara i meda unšijske mandarine (*Citrus unshiu* Marc.). Doktorska disertacija. Sveučilište u Zagrebu Agronomski fakultet

SAS Inst. Inc. 2012. The SAS System for Windows. Version 9.4. Cary. NC. SAS Institute

Soehngen U., Jay S. C. (1974). Studies on the honey-sac contents and pollen loads of honey bees. 2. Honey-sac contents of foraging bees. Journal of Apicultural research. 13: 199-206.

Stöckmann M., Biedermann R., Niedringhaus R. (2010). Key for the identification of the nymphs of the leafhopper subfamily Idiocerinae in Germany. Cicadina. 11: 59-72.

Vaissiere B. E., Vinson S. B. (1994). Pollen morphology and its effect on pollen collection by honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), with special reference to upland cotton, *Gossypium hirsutum* L. (Malvaceae). Grana. 33: 128-138.

Valentić M. (2009). Skupljačka aktivnost pčelinje zajednice na paši ljekovite kadulje (*Salvia officinalis* L.). Diplomski rad. Sveučilište u Zagrebu Agronomski fakultet

Winston M. (1987). The biology of the honeybee. Harward. First Harward University Press.

Foraging activity of the Carniolan bee (*Apis mellifera carnica* Pollman 1879) in the Zabiokovlje area

The aim of this study was to determine the foraging activity of the Carniolan bee (*Apis mellifera carnica* Pollman 1879) in the Zabiokovlje area during one season on three bee colonies, by analysing the content of honey sac and pollen loads. At 8:00 a significantly higher mass of honey sac content (18.58 mg) was found compared to samples collected at 13:00 (11.45 mg) and those collected at 18:00 (11.93 mg) hours. The weight of pollen loads at 18:00 was significantly lower (7.83 mg) compared to samples collected at 8:00 (10.88 mg) and 13:00 (9.82 mg). Honeydew was found in the largest share of analysed honey sacs (62.21%), which indicates the presence of cicada (*Acericerus heydenii*) in the study area.

Keywords: foraging activity, *Apis mellifera carnica*, honey sac, pollen load, Zabiokovlje area

IZVORNI ZNANSTVENI RAD

Preliminarna istraživanja sezonskih promjena indeksa kondicije i omjera spolova plavog raka (*Callinectes sapidus*, Rathbun 1896) u estuariju Neretve i Neretvanskom kanalu

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Sažetak
Cilj ovog istraživanja bio je utvrditi sezonske promjene kondicije i spolnu zastupljenost plavog raka (*Callinectes sapidus*, Rathbun 1896), alohtone invazivne vrste, koja je sve učestalija u lovinama ribara na području estuarija Neretve i Neretvanskog kanala. Uzorci su u suradnji s ribarima prikupljani iz lovina jednostrukih i trostrukih mreža stajaćica tijekom sva četiri godišnja doba u 2021. Ukupni broj jedinki i spolna zastupljenost varirali su u ovisnosti o sezoni. Tijekom istraživanog razdoblja zastupljeniji su bili mužjaci (63 %), a najveći broj jedinki utvrđen je u toplijem dijelu godine. Promjene indeksa kondicije i nalaz spolno zrelih ženki upućuju na uspostavu stabilne populacije. S obzirom da se radi o potencijalno tržišno značajnoj vrsti predlažu se mjere poticanja njezine komercijalizacije.

Ključne riječi: plavi rak**,** ušće Neretve, Neretvanski kanal, indeks kondicije, omjer spolova

Uvod

Plavi rak (*Callinectes sapidus*, Rathbun 1896.) prirodno nastanjuje područja uz zapadnu obalu Atlantika gdje predstavlja gospodarski značajan resurs (Miller i sur., 2006.). Početkom dvadesetog stoljeća prvi je put utvrđen u europskim vodama (Bouvier, 1901.) otkada se postupno širio te danas nastanjuje brojna područja Sredozemnog mora (Galil, 2011.). Streftaris i Zanetos (2006.) te FAO (2017.) ubrajaju ga među 100 najinvazivnijih stranih vrsta u Sredozemnom moru. Postoji mišljenje da je ova vrsta namjerno unesena u nova područja zbog potencijalno visoke tržišne vrijednosti (Nehring, 2011.). Na području estuarija rijeke Neretve u prošlom desetljeću zabilježeno je više jedinki ove vrste (Dulčić i Dragičević, 2010.). Novija su istraživanja pokazala povećanje njegove brojnosti, a utvrđen je kompeticijski i predatorski odnos s nekim lokalnim vrstama riba i autohtonim rakom *Carcinus maenas* (Mancinelli i sur., 2016.). Međutim, indeks kondicije, koji predstavlja pokazatelj općeg i zdravstvenog stanja morskih organizama te ujedno ukazuje na pogodnost određenog staništa za uspostavu populacije određene vrste (Bagenal i Tesch, 1978.), na ovim prostorima do sada nije istraživan. Radi toga je cilj ovog rada utvrditi vrijednosti indeksa kondicije plavog raka u različitim sezonama u biološki i ribolovno vrlo značajnom području u Hrvatskoj – estuariju rijeke Neretve i Neretvanskom kanalu.

Materijali i metode

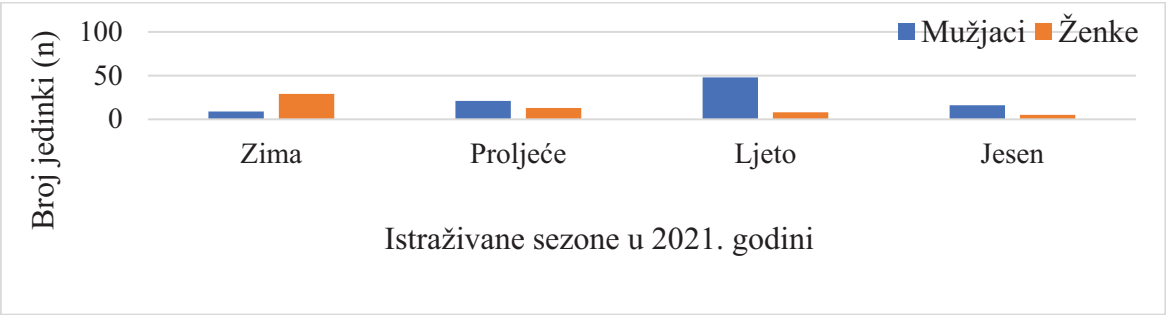
Jedinke plavog raka prikupljane su tijekom sva četiri godišnja doba u 2021. u području estuarija rijeke Neretve i Neretvanskog kanala uz primjenu jednostrukih i trostrukih mreža

stajaćica. Sakupljanje se odvijalo u sklopu uobičajenih ribolovnih operacija lokalnih ribara. Nakon izdvajanja iz mreža i brojanja, jedinkama su pomoću trake za izolaciju fiksirani distalni dijelovi kliješta kako ne bi došlo do ozljeda, oštećenja i gubitka tjelesnih nastavaka. Potom su rakovi očišćeni od obraštaja (ramenonošci, rakovi vitičari, alge i mulj) i sortirani prema spolu (Slika 1A. i 1B.) te privremeno skladišteni u prijenosnim hladnjacima do sljedećeg dana kada su vagani i izmjereni digitalnim kaliperom preciznosti 0.01 mm. Jedinke bez jednog ili obiju kliješta i/ili bez drugih tjelesnih nastavaka (*pereiopodi*), kao i u slučaju izostanka njihovih dijelova, izuzete su iz dalje analize. Za izračun indeksa kondicije korišten je Fultonov indeks ($FI = W/L^3*100$) gdje su W – masa u gramima, a L – širina glavopršnjaka u centimetrima (Hung i Deng, 2002.). Podatci su bilježeni i obrađeni u programu *Excel, Microsoft Office 2010.*

Slika 1A i 1B. Ženka i mužjak plavog raka iz estuarija Neretve očišćeni od obraštaja prije daljne analize

Rezultati i rasprava

Provedenim istraživanjem prikupljeno je ukupno 149 jedinki plavog raka, pri čemu su tijekom istraživanog razdoblja zastupljeniji bili mužjaci (63 %). Najviše jedinki ove vrste utvrđeno je u toplijem dijelu godine (Slika 2.). Ženke su zimi bile brojnije dok su u ostalim sezonama dominirali mužjaci. Razlike u sezonskoj prisutnosti ženki na istraživanom području u suglasju su s literaturnim podacima o reprodukciji ove vrste pri čemu je utvrđeno da ljeti, u vrijeme polaganja jaja, ženke napuštaju estuarij i odlaze na područja višeg saliniteta (Shapiro, 2011.). Berglund (1981.) naglašava da se varijacije u omjerima spolova kod rakova mogu objasniti njihovom različitom smrtnošću i specifičnim migracijskim obrascima u lagunama, odnosno specifičnim ekološkim uvjetima. Međutim, na nekim lokalitetima u Sredozemnom moru gdje je ova vrsta izuzetno brojna (npr. Beymelek laguna u Turskoj), u populaciji dominiraju ženke, što može ukazati na stabilnost populacija (Sumer i sur., 2013.).



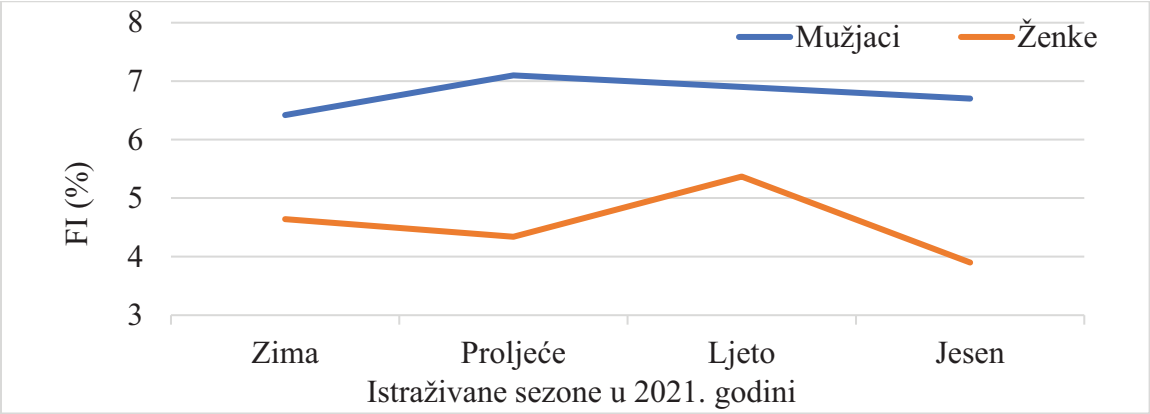
Slika 2. Brojnost mužjaka i ženki plavog raka na području estuarija Neretve u istraživanim sezonama tijekom 2021

Raspon mase mužjaka tijekom istraživanog razdoblja kretao se od 28,44 g do 473 g, dok je kod ženki bio od 55,21 g do 269 g (Tablica 1.). Širina glavopršnjaka bila je od 7,3 do 19,3 cm kod mužjaka, a 10 do 18,6 cm kod ženki. Od svih prikupljenih jedinki, sve ženke bile su spolno zrele. Utvrđen je samo jedan spolno nezreo mužjak, čija je masa iznosila 28,44 g.

Tablica 1. Srednje vrijednosti mase (W) i širine glavopršnjaka (L) mužjaka i ženki plavog raka uzorkovanih u području estuarija Neretve i Neretvanskog kanala tijekom 2021.; raspon mjerenih vrijednosti (min - minimum, maks - maksimum), n – broj jedinki

Spol	n	W(g) ±SD	W(g) (min-maks)	L(cm) ±SD	L(cm) (min-maks)
Mužjaci	94	250,81±81,97	28,44-473	15,29±1.97	7,3-19,3
Ženke	55	157,79±39,19	55,21-269	15,05±1.52	10-18,6

Na istraživanom području srednje vrijednosti indeksa kondicije plavog raka pokazuju sezonske razlike (Slika 3.). U istraživanom razdoblju veće vrijednosti imali su mužjaci. Najviše vrijednosti su kod oba spola utvrđene u toplijem dijelu godine (7,1 kod mužjaka na proljeće i 5,37 kod ženki u ljeto), dok su najmanje kod mužjaka zabilježene zimi (6,42). Kod ženki su najmanje vrijednosti (3,9) utvrđene nakon mrijesta, u jesen. Navedeno je u suglasju i s literaturnim podacima istraživača koji su proučavali indeks kondicije u lagunama Turske, gdje je ova vrsta uspostavila stabilnu populaciju (Atar i Secer, 2003.).



Slika 3. Indeks kondicije mužjaka i ženki plavog raka tijekom 2021. godine u estuariju Neretve

Zaključak

Kondicijsko stanje i prisutnost spolno zrelih jedinki plavog raka na području estuarija Neretve i Neretvanskog kanala ukazuju da je na ovom području uspostavljena stabilna populacija koja je pronašla svoju hranidbenu nišu. To s jedne strane može biti prijetnja lokalnim autohtonim vrstama, ali ujedno može predstavljati novi gospodarski resurs. S obzirom na sve učestaliju prisutnost u ribarskim lovinama te njegov neosporan tržišni i gastronomski potencijal potrebno je marketinškim aktivnostima povećati interes javnosti za ovu vrstu kako bi se potaknula njegova komercijalna eksploatacija i uspješan plasman na tržište. Uz navedeno, potrebno je stvoriti preduvjete za prikupljanje realnih podataka o količini ulova ove vrste. Sukladno Pravilniku o obliku, sadržaju i načinu vođenja i dostave podataka o ulovu u gospodarskom ribolovu na moru (NN br. 38/18, 48/18, 64/18, 35/20) plavi rak evidentira se pod FAO šifrom CRU (ostali rakovi) te bi bilo uputno popis komercijalnih vrsta nadopuniti unošenjem u Pravilnik FAO šifre za plavog raka - CRB. Na ovaj način dobili bi se potpuniji podatci potrebni za realnu procjenu komercijalnog potencijala i učinkovito definiranje i provođenje mjera upravljanja.

Literatura

Atar, H. H., Seçer, S. (2003). Width/length-weight relationships of the blue crab (Callinectes sapidus Rathbun 1896) population living in Beymelek Lagoon Lake. Turkish Journal of Veterinary and Animal Sciences. 27(2): 443-447.

Bagenal, T. B., Tesch, F. W. (1978). Age and growth. U: *Methods for assessment of fish production in fresh waters*, 3rd edition, Bagenal, T., (ed.), 101–136. Blackwell Scientific Publications, Oxford.

Berglund, A. (1981). Sex dimorphism and skewed sex rations in the prawn species Palaemon adspersus and P. squilla. Oikos. 36: 158-162.

Bouvier, E. L. (1901). Sur un *Callinectes sapidus* M. Rathbun trouvé à Rocheford. Bulletin du Museum d'Histoire Naturelle, 7: 16-17.

Dulčić, J., Dragičević, B. (2010). New record of the blue crab, *Callinectes sapidus* Rathbun, 1896, (Decapoda: Brachyura) in the Adriatic Sea. Annales: Series Historia Naturalis. 20 (1): 23.

Food and Agriculture organization (2017). Species Facts Sheets: Callinectes sapidus (Rathburn, 1896). Food and agriculture organization of the United Nations, FAO Fisheries and Aquaculture Department.

Galil, B. S. (2011). The alien Crustaceans in the Mediterranean Sea: U: *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts*, Galil, B. S., Clark, P. F., Carlton, J. T., (eds.), 377- 401. Springer-Verlag, Berlin-Heidelberg.

Hung, S. S. O., Deng, D. F. (2002). Sturgeon, *Acipenser* spp. U: *Nurient requirements and feeding of finfish for aquaculture*, Webster, C. D., Lim, C. (eds.), 344–357. CABI Publishing, Walligford.

Mancinelli, G., Glamuzina, B., Petrić, M., Carrozzo, L., Glamuzina, L., Zotti, M., Raho, D., Vizzini, S. (2016). The trophic position of the Atlantic blue crab Callinectes sapidus Rathbun 1896 in the food web of the Parila Lagoon (South Eastern Adriatic, Croatia): a first assessment using stabile isotopes. Mediterranean Marine Science. 17(3): 634-643.

Miller, T. J., Martell, S. J. D., Davis, G., Fegley, L., Sharov, A., Bonzek, C., Hewit, D., Hoenig, J., Lipcus, R. N. (2005). Stock assessment of blue crab in Chesapeake Bay. Final report. Technical report series No. TS-487-05 of the University of Maryland for environmental science.

Nehring, S. (2011). Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. U: *In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts*, Galil, B. S., Clark, P. F., Carlton, J. T. (eds.), 607-624. Springer, Dordrecht.

Pravilnik o obliku, sadržaju i načinu vođenja i dostave podataka o ulovu u gospodarskom ribolovu na moru (Narodne novine, br. 38/18, 48/18, 64/18, 35/20).

Shapiro, L. (2011). "*Callinectes sapidus* M. J. Rathbun**,** 189" (On-line). EOL species rapid response. Pristupljeno: 22. lipnja 2011 na http://eolspecies.lifedesks.org/pages/18927.

Streftaris, N., Zanetos, A. (2006). Alien marine species in the Mediterranean – the 100 "worst invasives" and their impact. Mediterranean Marine Science. 7(1): 87-118.

Sumer, C., Teksam, I., Karatas, H., Beyhan, T., Mandares Aydin, C. (2013). Growth and reproduction biology of the blue crab, *Callinectes sapidus* Rathbun, 1896, in the Beymelek Lagoon (southwestern coast of Turkey). Turkish journal of fisheries and aquatic science. 13: 675-684.

Preliminary research of the seasonal changes in the condition index and sex ratio of blue crab (*Callinectes sapidus*, Rathbun 1896) in the Neretva estuary and the Neretva Channel

Abstract
The aim of this study was to determine the seasonal changes in the condition index and sex ratio of blue crab (*Callinectes sapidus*, Rathbun 1896.) in fishing catch. Blue crab is non-indigenous invasive species, which is becoming increasingly common in the Neretva estuary and the Neretva channel. Samples were collected in collaboration with fishermen using single and triple trammel nets during all four seasons in 2021. The total number of individuals and sex ratio varied depending on the season. During the research period, males were more represented (63%), and the largest number of individuals was found in the warmer part of the year. Changes in the condition index and the finding of sexually mature females suggest the establishment of a stable population. Since this is a potentially market-significant species, measures to encourage its commercialization are proposed.

Key words: blue crab, Neretva estuary, Neretva channel, condition index, sex ratio

IZVORNI ZNANSTVENI RAD

Prisutnost spora nozeme (*Nosema spp.)* u zajednicama medonosne pčele (Apis mellifera) na kraju razdoblja zimovanja

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Sažetak
Cilj istraživanja bio je utvrditi prisutnost spora *Nosema spp.* nakon razdoblja zimovanja. Analizirana je prisutnost spora nozeme na 563 uzorka pčela s 19 pčelinjaka iz dvije regije u Hrvatskoj. Iz regije Osijek i okolice obrađeno je 299 uzoraka s 10 pčelinjaka, a iz regije Sisak i okolice 264 uzorka s 9 pčelinjaka. Sa svakog pčelinjaka uzorci su skupljeni iz 30 pčelinjih zajednica. Prosječno je utvrđeno 3,2 milijuna spora po pčeli. U regiji Osijek prosječan broj spora iznosio je oko 900.000, dok je u regiji Sisak prosječan broj spora bio znatno veći i iznosio 5.8 milijuna spora. Na području Osijeka i okolice 41,33% uzoraka bilo je pozitivno na nozemozu (utvrđena barem jedna spora), dok je na području Siska i okolice utvrđena puno veća invadiranost gdje je 70,48 % uzoraka bilo pozitivno na prisutnost spora nozeme.

Ključne riječi: medonosna pčela, zimovanje, nozemoza, spore

Uvod

Nozemoza je bolest čiji su uzročnici mikrosporidije iz roda *Nosema spp.* koje se razvijaju u probavnom sustavu pčele. Nakon što spore uđu u probavni sustav pčele, razmnožavaju se brzo i prodiru u stanice probavnog sustava (FAO, 2020.). Ulaze u epitelne stanice srednjeg crijeva i tamo se razmnožavaju. Napadnute stanice propadaju, a spore ulaze u crijeva odakle odlaze u nove crijevne stanice. Nozemozu uzrokuju dvije vrste gljivica, a to su *Nosema apis* i *Nosema ceranae.* Simptomi bolesti razlikuju se s obzirom na vrstu gljivica kojima je zajednica zaražena. Simptomi bolesti uzrokovane *Nosemom apis* su crijevni poremećaj odnosno proljev koji se može uočiti na podnici, letu i saću, kod pčela skupljačica smanjuje se letačka aktivnost, a pčele slabije proizvode matičnu mliječ. Ako je matica bolesna, smanjuje polaganje jaja, pčele koje nisu u stanju letjeti hodaju s raširenim krilima i paralizirane su, mogu se naći mrtve pčele natečenog zatka i nogu uvučenih ispod prsa. Nozemoza uzrokovana *Nosemom ceranae* ima nešto drugačije simptome. Smatralo se kako bolest napada samo azijsku medonosnu pčelu (*Apis cerana)* no pronađena je na europskoj medonosnoj pčeli (*Apis mellifera)* 2005. godine u Španjolskoj (Higes i sur., 2006.). Za bolest je tipična odsutnost proljeva i te pčele ugibaju dalje od košnice što uzrokuje smanjen broj pčela u košnici koji se teže primjećuje. Nozemoza se može kontrolirati usvajanjem dobre pčelarske prakse i biotehničkim mjerama. Dobra priprema zajednica za zimovanje ključna je u suzbijanju bolesti. Zdrave pčele za vrijeme zimskog razdoblja zadržavaju neprobavljenu hranu u rektumu. U slučaju zaraze nozemozom, za vrijeme zimovanja pčele će prazniti neprobavljenu hranu iz crijeva u košnicu. Takav izmet je tekuć, ljepljiv i sladak što privlači druge pčele. Na taj način zaraza nozemozom se brzo širi jer pčele nisu u mogućnosti ići na pročisne letove i dolazi do zaraze zdravih pčela. Pčele zaražene nozemozom imaju poremećenu probavnu funkciju i pojačanu osjetljivost što omogućava razvoj drugih bolesti (Higes i sur., 2010). Bolest je izraženija kod pčela radilica jer su one uključene u čišćenje saća. Wang i Moeller (1970.) su utvrdili kako bolesne pčele ne prilaze

i ne hrane maticu i nisu u mogućnosti proizvoditi matičnu mliječ. Također, kod zaraženih pčela može doću do atrofije hipofaringealnih žlijezda, koje luče glavne proteine matične mliječi (Vidau i sur., 2014.).
Cilj ovog istraživanja bio je utvrditi broj spora *Nosema spp.* u pčelinjim zajednicama nakon razdoblja zimovanja na 19 pčelinjaka na području istočne i središnje Hrvatske.

Materijal i metode
Istraživanje je provedeno tijekom ožujka 2021. godine kada su prikupljeni uzorci pčela radilica s 19 pčelinjaka. S područja Osijeka i okolice bilo je uključeno 10 pčelinjaka, a s područja Siska i okolice 9 pčelinjaka. Prilikom prvog proljetnog pregleda zajednica sa svakog pčelinjaka skupljeni su uzorci pčela radilica iz 30 nasumično odabranih pčelinjih zajednica. Uzorci pčela su nakon skupljanja čuvani na temperaturi od -18 °C te su ubrzo nakon skupljanja analizirani na prisutnost spora nozeme u laboratoriju Fakulteta agrobiotehničkih znanosti u Osijeku prema standardnim metodama (Human i sur., 2013.; Fries i sur., 2013). Za pripremu uzorka za analizu pincetom je odvojen zadak s 20 pčela radilica. Odvojenim zatcima u tarioniku je pomoću pipete dodano 0,5 ml destilirane vode po zatku odnosno (10 ml vode sveukupno). Uzorak je zdrobljen pomoću tučka kako bi iz crijeva izašao sadržaj te je promješan. Od ovako pripremljenog preparata čistom pipetom stavljena je kap preparata na hemocitometar i pokriven pokrovnim stakalcem. U uzorku se pod svjetlosnim mikroskopom na povećanju od 400x utvrdio broj spora na 5 polja hemocitometra te je izračunat broj spora po pčeli množenjem utvrđenog broja spora s 50.000.

Rezultati i rasprava
U ovom istraživanju na ukupnom broju od 563 uzorka (299 iz regije Osijek i 264 iz regije Sisak) kod pčela radilica utvrđen je prosječan broj spora od 3,2 milijuna (tablica 1). U regiji Osijek prosječan broj spora je oko 912.207 ± 2.911.490 (srednja vrijednost ± SD) dok je u regiji Sisak prosječan broj spora 5.815.720 ± 11.771.513. Mann-Whitney U test pokazao je kako je između regija utvrđena statistička značajna razlika (U = 21.798,500, p < 0,001).

Tablica 1. Prosječni broj spora u uzorku utvrđen brojanjem pod hemocitometrom (utvrđeni broj spora) te izračunati broj spora po pčeli (broj spora po pčeli) za regiju Osijek i regiju Sisak

		N	Srednja vrijednost	Standardna devijacija	Minimum	Maksimum
Regija Osijek	Utvrđeni broj spora	299	18,24	58,26	0	551
	Broj spora po pčeli	299	912.207	2.911.458	0	27.550.000
Regija Sisak	Utvrđeni broj spora	264	116,31	235,43	0	2.100
	Broj spora po pčeli	264	5.815.719	11.771.513	0	105.000.000
Ukupno	Utvrđeni broj spora	563	64,23	173,597	0	2.100
	Broj spora po pčeli	563	3.211.545	8.679.830	0	105.000.000

Na području Osijeka i okolice od svih analiziranih uzoraka, 41,33 % bilo je pozitivno na prisutnost spora nozeme (utvrđena barem 1 spora) dok je na području Siska i okolice kod 70,48% uzoraka utvrđena prisutnost spora nozeme. Međutim, ukoliko se kao granica uzme 1 milijun spora nozeme, tada je u regiji Osijek 16,1% uzoraka bilo pozitivno (48 uzoraka od 299 s više od milijun spora po pčeli) dok je u regiji Sisak 53,8 % uzoraka bilo pozitivno (142 od 264 uzorka s više od milijun spora po pčeli).
U regiji Osijek utvrđeni prosječni broj spora je oko 900.000 dok je u regiji Sisak broj spora bio statistički značajno veći (p<0,001) i iznosio prosječnih 5,8 milijuna.

Slično istraživanje proveli su Retschnig i sur. (2017.) u Švicarskoj koji su tijekom dvije godine uzimali uzorke pčela u zimu i rano proljeće. Od 900 analiziranih uzoraka, 420 je bilo zaraženo *Nosema spp.* Za uzorke su uzimali 60 nasumično odabranih pčela radilica iz zajednice. Kroz cijelo istraživanje broj spora je bio od 200 tisuća do 4,2 milijuna spora po pčeli. U zimu 2011. godine prosječan broj spora je bio 850 tisuća, a u zimu 2012. godine 4,2 milijuna. Spore nozeme su detektirane u 57,6 % uzoraka od čega je 89,47 % zaraženo *Nosema ceranae*, a samo 4,21% je *Nosema apis.* U 6,32 % uzoraka su pronađene obje vrste. Autori spomenutog istraživanja navode kako je na veliku razliku u broju spora između uzoraka iz 2011. i 2012. godine moglo utjecati vrijeme uzimanja uzoraka kao i broj letnih dana tijekom zime.

Özkırım i sur. (2019.) proveli su istraživanje u Turskoj u 72 različite regije u razdoblju između 2009. i 2016. godine. U svako godišnje doba prikupljena su 4 uzorka s 4 pčelinjaka iz svake regije. U tom razdoblju prikupljen je 1041 uzorak. Najveći prosječan broj spora nađen je 2009. godine (23,5 milijuna spora) i 2015. godine (21 milijun spora). Autori zaraženost povezuju s temperaturom i klimatskim čimbenicima. U godinama u kojima su zimske temperature bile najniže povećao se broj spora *N. apis*, ali se broj spora *N. ceranae* smanjio. Navode kako se uzročnici zaraze „zamjenjuju“ ovisno o vanjskim čimbenicima te da je zaraženost s *N. ceranae* najčešća. Također navode kako zaraza ovisi o prosječnim zimskim temperaturama, ali ne i o lokaciji pčelinjaka. U našem istraživanju utvrđena je značajna razlika u prisutnosti spora nozeme između regija te je daljnjim istraživanjima potrebno utvrditi koji je uzrok ove razlike. Istraživanje na 38 pčelinjaka koje su proveli Papini i sur (2016.) u 6 regija u centralnoj Italiji pokazala su prisutnost spora nozeme na 24 od 38 pčelinjaka. Svi pčelinjaci pozitivni na nozemozu bili su zaraženi sporama *N. ceranae*. Istraživanja u Hrvatskoj također su utvrdila prisutnost samo spora *N. ceranae* što pokazuje kako je iz našeg područja *N. apis* istisnuta te više nije sezonalnog karaktera (Tlak Gager i sur., 2010a).

Istraživanje Tlak Gajger i suradnika (2010.) nakon razdoblja zimovanja u tri klimatske regije u Hrvatskoj pokazala su visoki udio zaraženih zajednica sporama nozeme kod zajednica koje su bile slabe ili su stradale tijekom zime. Prosječan broj zaraženih zajednica nozemozom u regiji Osijek (16,1 %) i u regiji Sisak (53,8 %) kod zdravih, vitalnih zajednica pokazuju kako je ova bolest prisutna u značajnom broju pčelinjih zajednica na istraživanom području.

Zaključak

Na temelju rezultata dobivenih u provedenom istraživanju, kao i rezultata istraživanja drugih autora možemo zaključiti kako su spore nozeme prisutne u većini uzoraka pčela nakon razdoblja zimovanja. U ovom istraživanju, značajno manji broj spora utvrđen je u regiji Osijek. Druga istraživanja pokazala su kako je značajnija invadiranost sporama *Nosema ceranae* u odnosu na *Nosema apis* te kako je ona sada dominantna u Europi. Istraživanja drugih autora koji su pratili prisutnost spora nozeme tijekom cijele godine pokazala su kako je prisutnost spora kod radilica najveća nakon razdoblja zimovanja jer pčele nisu bile u mogućnosti ići na pročisne letove i spore su se širile unutar pčelinje zajednice. Zaraza se najčešće širi među pčelama radilicama, a puno rjeđe se javlja kod matice i trutova.

Napomena

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Literatura

FAO. (2020). Dobra pčelarska praksa: Glavne bolesti medonosne pčele (*Apis mellifera*): Kako ih prepoznati, spriječiti i liječiti: Praktični priručnik. Geromar d.o.o., Sveta Nedelja, 65-70.

Fries I, Chauzat M.P., Chen Y.P., Doublet V., Genersch E., Gisder S., Higes M., McMahon D.P., Martín-Hernández R., Natsopoulou M., Paxton R.J., Tanner G., Webster T.C., Williams G.R. (2013). Standard methods for Nosema research. Journal of Apicultural Research. 52(1):1-28.

Higes M., Martín R., Meana A. (2006). *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J Invertebr Pathol. 92(2): 93-5.

Higes M., Martín-Hernández R., Meana A. (2010): *Nosema ceranae* in Europe: An emergent type C nosemosis. Apidologie. 41: 375–392.

Human H., Brodschneider R., Dietemann V., Dively G., Ellis J. D., Forsgren E., Zheng H.-Q. (2013). Miscellaneous standard methods for Apis mellifera research. Journal of Apicultural Research. 52(4): 1–56.

Özkırım A., Schiesser A., Keskin N. (2019.): Dynamics of *Nosema apis* and *Nosema ceranae* Co-Infection Seasonally in Honey Bee (*Apis mellifera* L.) Colonies. Journal of Apicultural Science. 63(1): 41-48.

Papini R., Mancianti F., Canovai R., Cosci F., Rocchigiani G., Benelli G., Canale A. (2016). Prevalence of the microsporidian *Nosema ceranae* in honeybee (*Apis mellifera*) apiaries in Central Italy. Saudi Journal of Biological Sciences. 24(5): 970-982

Retschnig G., Williams G.R., Schneeberger A., Neumann P. (2017). Cold Ambient Temperature Promotes Nosema spp. Intensity in Honey Bees (*Apis mellifera*). Insects. 8(1): 20

Tlak Gajger I., Vugrek O., Grilec D., Petrinec Z., (2010a). Prevalence and distribution of Nosema ceranae in Croatian honeybee colonies. Veterinarni Medicina. 55(9): 457-462.

Tlak Gajger I., Vugrek O., Petrinec Z., Grilec D., Tomljanović Z. (2010b). Detection of *Nosema ceranae* in honey bees from Croatia. Journal of Apiculural Research. 49(4): 340-341

Vidau C., Panek J., Texier C., Biron D.G., Belzunces L.P., Le Gall M., Broussard C., Delbac, F., El Alaoui H. (2014). Differential Proteomic Analysis of Midguts from Nosema ceranae-Infected Honeybees Reveals Manipulation of Key Host Functions. J. Invertebr. Pathol. 121: 89–96.

Wang Der-I., Moeller F. E. (1970). The division of labor and queen attendance behaviour of nosema-infected worker honey bee. J. Econ. Ent. 63: 1539-1541.

Presence of Nosema spores (*Nosema spp.*) in honey bee colonies (*Apis mellifera*) at the end of the wintering period

Abstract

The aim of the study was to determine the presence of *Nosema spp.* spores after the wintering period. The presence of nosema spores was analyzed on 563 samples from 19 apiaries from two regions in Croatia. 299 samples were processed from the Osijek region and its surroundings, and 264 samples from the Sisak region and its surroundings. Samples from each apiary were collected from 30 honey bee colonies. An average number of 3.2 million of spores has been determined. In the Osijek region, the average number of nosema spores was around 900.000, while in the Sisak region the average number of spores was 5.8 million. In the area of Osijek and its surroundings, 41.33% of the samples were positive for nosemosis (at least one spore was found), while in the area of Sisak and its surroundings, a much higher infection was found where 70.48% of samples were positive for nosemosis.

Key words: honey bee, wintering, nosemosis, spores

ORIGINAL SCIENTIFIC PAPER

Yields of grass species for large game in the Eu-Mediterranean zone of the North Adriatic

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Abstract
Previous experiences with wild (and mainly allochthonous) ruminants in the Mediterranean area point out on the detrimental impact on indigenous vegetation, mainly indigenous species of grasses and herb species. The analysis of the yields of fresh mass of four species of grasses showed that it is possible to achieve green fodder yields in the Mediterranean (arid) conditions, which by far exceeds the biomass of natural vegetation. A statistically significant difference in the yields of fresh mass in the first and second year of the experiment was found in three of the four species of grasses. The establishment of such grassland draws could reduce the pressure on indigenous vegetation and enable sustainable breeding of wild ruminants in areas where livestock-breeding has been abandoned.

Key words: wild ruminants, grasses, herb species, defoliation, yield, Mediterranean

Introduction

The Mediterranean is one of the most complex parts of the Earth in terms of geology, geography, morphology and natural history (Blondel and Aronson, 1999). Its landscape was influenced by three fundamental factors making it different from other European landscapes. These factors are climate, fires and intense human impact. Grazing has been present in the Mediterranean for 5,000 years (Le Houerou, 1981), and represent one of the most pronounced human influence that has affected the current state of forest vegetation. Since the end of the 20th century, abrupt and often underestimated landscape changes have become visible in this area, resulting from the pronounced depopulation of rural areas (Debussche et al., 1999). In addition to the decline in human population, the decline in the number of small ruminants has been even more rapid, accompanied with the lack of traditional management of forest ecosystems (felling of trees, coal production and grazing in forests). All this has led to the expansion of forest areas. As an alternative to livestock and their positive role in the reclamation of habitats in the area, primarily by reducing the risk of fire, the question of the justification of breeding wild ruminants is raised. Those are mainly species that have so far been traditionally bred in the certain localities of the Mediterranean area, such as fallow deer (*Dama dama*) and the European mouflon (*Ovis gmelini musimon*). Due to the specific environment (aridity and the skeletal nature of the soil) in the Mediterranean hunting grounds, the variety of grass species is generally quite limited, and very little research has been conducted with the intention of raising the trophic capacity for wild ruminants. Therefore, the aim of this paper is to investigate the amount of yields of certain species of grasses that could be sown in particular forest areas of the Mediterranean basin.

Material and methods

The research was conducted in a fenced part (877 ha) of the state hunting ground number VIII/6 "Kalifront" on the island of Rab. According to Köpen's classification, the area belongs to the Cfsax´´ climate type (Seletković and Katušin, 1992), which is a moderately warm

rainy climate with hot summers, and the driest part of the year falls in the summer season. From the vegetation point of view, the hunting ground belongs to the Eu-mediterranean vegetation zone (Trinajstić, 1986), which is dominated by the forest community of holm oak and black ash (*Fraxino orni-Quercetum ilicis* H-ić / 1956/1958). The experiment was carried out on a cleared area of 2 ha surrounded by forest. The soil belongs to an acid brown soil on clastic sediments. Basic tillage (ploughing) was carried out in late autumn (November 1999), and additional processing (discing and tilling) was carried out in spring (March 2000). Before discing and tilling, mineral fertilizer of NPK formulation 7:20:30 in the amount of 300 kg ha^{-1} was applied to the soil. Sowing of grasses was done in separate strips 1 meter wide and 30 meters long with five species of grasses: Italian ryegrass (*Lolium multiflorum* Lam. cv. Amba), English ryegrass (*Lolium perenne* L. cv. Calibra), cocksfoot (*Dactylis glomerata* L. cv. Amba), red fescue (*Festuca rubra* L. cv. Echo) and meadow-grass (*Poa pratensis* L. cv. Balin). Between each repetition, a 1 by 30 metre strip was left for five repetitions in a random block plan. After sowing, the plots were fenced, to prevent the access of big game to the growing grass. Defoliation was carried out on May 20, 2000. (defoliation 1) and on June 6, 2001 (defoliation 2), using a shearing scythe. The meadow grass did not grow at all, so no measurements were taken for it. On each sown strip of fenced area of 1 m^2 and in each repetition, fresh mass weight was calculated and 1 kg of fresh mass was classified according to floral components: sown species of grasses, herb species and weeds. During defoliation, the species of grasses were in different phenophases. The red fescue was in the late phenophase of forking the shoots, the cocksfoot in the full wilting phenophase, the English ryegrass in the late ear formation phenophase, and the Italian ryegrass in the seed maturation phenophase. The area where the research was carried out is inhabited by two species of big game – the European mouflon (*Ovis gmelini musimon*) and the axis deer (*Axis axis*). During the experiment, over 20 mouflons and up to 5 axis deers graze on the sown area (coverts) (Krapinec, 2000), but the game could not penetrate the fence and reach the biomass in the fenced part of the covert, making the crops protected. Statistical analysis was performed by the analysis of variance (ANOVA), i.e. with Sheffé's post hoc test and the Kruskal-Wallis test. A comparison of the fresh mass yields of the sown species of grasses and the yields of herb species and weeds was made by t-test for controlled samples. The data obtained were analysed using the Statsoft 14.0.0.15 program (TIBCO Software Inc., 2020).

Results and discussion

In the first defoliation, the total fresh mass yields was highest on the experimental plots with Italian ryegrass as the sown species of grasses (Table 1). However, after the fresh mass yields of sown species of grasses is separated from the total yield then the yield of Italian ryegrass is no longer statistically significantly higher compared to the other species of grasses. Namely, although the Italian ryegrass in the first defoliation showed apparently the highest fresh mass yield (26.75 t ha^{-1}), even double the yield of the cocksfoot (12.77 t ha^{-1}), statistically it is only significantly higher than the yield of red fescue (4.77 t ha^{-1}; $\chi 2 = 18.362$; $p < 0.001$). The fresh mass yield of herb species and weeds also did not differ between the areas under the Italian ryegrass (0.57 t ha^{-1}) the English ryegrass (0.69 t ha^{-1}) and the cocksfoot (1.18 t ha^{-1}), but it was significantly higher in the area of the red fescue (4.57 t ha^{-1}) in relation to the yield of herb species and weed species in the other areas. The share of weeds and herb species on the red fescue plot during the first defoliation was so high that there was no difference between the red fescue yield and the yield of herb species and weeds (51.38 ± 3.74 % of the total yield, respectively (48.62 ± 3.74 % of the total yield; $t = 0.83$; $p = 0.456$). In other species, the share of biomass of sown grass species in the first defoliation ranged from 91.2 ± 3.14 % (cocksfoot) to 98.0 ± 0.85 % of the total fresh mass yield (Italian ryegrass).

Table 1. Yield of fresh mass of floral components in the first defoliation (20.05.2000).

Species of grass	Yield of grasses t ha^{-1} (Kruskal-Wallis test)	Yield of herb species and weeds t ha^{-1} (Sheffé test)	Total yield t ha^{-1} (Sheffé test)
Lolium multiflorum Lam. cv. Bofur	26.75a	0.57a	27.32a
Lolium perenne L. cv. Calibra	16.49ab	0.69a	17.18b
Dactylis glomerata L. cv. Amba	12.77ab	1.18a	13.95b
Festuca rubra L.cv. Echo	4.77b	4.57b	9.34b

Different letters within the columns indicate a statistically significant difference at the level of $p < 0.05$. All comparisons were made with the Sheffé test.

Table 2. Yield of fresh mass of floral components in the second defoliation (6.6.2001).

Species of grass	Yield of grasses t ha^{-1} (Kruskal-Wallis test)	Yield of herb species and weeds t ha^{-1} (Sheffé test)	Total yield t ha^{-1} (Sheffé test)
Lolium multiflorum Lam. cv. Bofur	12.57a	0.78a	13.35a
Lolium perenne L. cv. Calibra	7.78b	1.19a	8.97a
Dactylis glomerata L. cv. Amba	9.14ab	1.46a	10,60a
Festuca rubra L. cv. Echo	9.12ab	4.15b	13.27a

Different letters within the columns indicate a statistically significant difference at the level of $p < 0.05$. All comparisons were made with the Sheffé test.

In the second year of the experiment (second defoliation) there was no difference in the amount of total fresh mass yield when researching grass species (Table 2). Regarding the yield of sown of grass species, the highest fresh mass yield was again in the case of Italian ryegrass (12.57 t ha^{-1}), although it was not significantly higher than the yield of cocksfoot (9.14 t ha^{-1}) and red fescue (9.12 t ha^{-1}), but had a significantly higher yield than the English ryegrass (7.78 t ha^{-1}). In the yields of herb species and weed species, the highest fresh mass yields measured were again significantly highest in the areas where the red fescue was sown (4.15 t ha^{-1}). Although in the first defoliation no difference was found in the share of fresh mass yield of sown and unsown crops in the area with red fescue, in the second defoliation the difference in the share of yield between sown red fescue in relation to the share of biomass of herb species and weeds in the total biomass was significant (69.18 ± 3.19 %, respectively 30.82 ± 3.19 %; $t = 13.444$; $p < 0.001$). The difference in the fresh mass yields of sown grass between the two defoliations depends a lot on the species of grass (Table 3). There was no statistically significant difference in the fresh mass yields of the first and second defoliation for the cocksfoot. The Italian and English ryegrasses had statistically significantly higher fresh mass yields in the first defoliation, i.e. during the first year of grass growth. In the case of red fescue, the situation was reversed and the fresh mass yields were significantly higher during the second defoliation, i.e. during the second year of grass growth. The difference in fresh mass yields in all three species of grass was almost double. The dynamics of the fresh mass yield ratio of the first and second defoliation of the tested species (varieties) of grasses completely coincides with the experiences given by Šoštarić-Pisačić and Kovačević (1968). This means that ryegrasses (especially Italian), as very penetrating species of grasses, already in the first year gave the maximum yield of voluminous fodder, and later the yield of these species decreases. On the other hand, the same authors describe red fescue as a perennial that is neutral to repressed grass species, which grows slowly after germination and achieves higher yields in later years of cultivation.

Quantitatively, Italian ryegrass is a short-lived tall species of grass that achieves maximum fresh mass yields of 40-80 t ha^{-1} during the growing season in the first year of production, while yields of other species range from 40 t ha^{-1} (cocksfoot) to 60 t ha^{-1} (English ryegrass), with the lowest yields being achieved by red fescue (Stjepanović et al., 2008). However, the latter species for the production of voluminous fodder is most often sown in mixtures with legumes or other grasses, and rarely in monoculture (Stjepanović et al., 2008). The reason for this is that it develops a loose and long-lasting sod, which is why the parts of the turf at ground level remain unfilled, and thus suitable for the development of weed species.

Table 3. Yield of fresh mass of floral components in the first and second defoliation

Species of grass	Yield species of grass t ha		*t*	*p*	Proportion of herb species and weeds in the total yield %		*t*	*p*
	Defoliation				Defoliation			
	1st	2nd			1st	2nd		
LM	**26.75**	12.57	7.16	0.002	2.00	**5.82**	-4.57	0.01
LP	**16.49**	7.78	2.89	0.044	3.74	**13.28**	-8.11	0.001
DG	12.77	9.14	1.75	0.15	8.80	**13.52**	-13.02	0.0002
FR	4.77	**9.12**	-6.08	0.004	**48.62**	30.82	6.096	0.004

All comparisons were made with the Sheffé test. The numbers printed in bold indicate significantly higher values within each species of grass. Denotation species of grass: LM= *Lolium multiflorum* Lam. cv. Bofur; LP= *Lolium perenne* L. cv. Calibra; DG= *Dactylis glomerata* L. cv. Amba'; FR= *Festuca rubra* L. cv. Echo.

Despite the large difference in fresh mass yields, grass species with lower yields should not be excluded from cultivation because experiments have shown that the mouflon prefers English ryegrass and red fescue compared to Italian ryegrass (Krapinec et al., 2019). In addition, in spring compared to autumn sowing, greater weeding of grass crops is possible because weeds are fast growing and overshadow the desired plant species (Byrne et al., 2018), especially those with slower germination and growth (Paterson and Sim, 2000), which was confirmed by the high proportion of weeds in the red fescue monoculture. Due to the significantly faster growth compared to red fescue, other grasses used in this study, especially Italian ryegrass, had a significantly lower proportion of weeds in both years of the research study. Wild ruminants in unconstrained nature consume green fodder directly. Therefore, the expression of yield in fresh mass from the point of view of game breeding is more practical than the expression of yield in dry matter. However, data on fresh mass yields are rather scarce in literature. In the Pannonian part of Croatia, the pure culture of Italian ryegrass gives fresh mass yields from 27.2 to 33.3 t of ha^{-1} (Vodopija, 2017). Moreover, it is much more profitable to establish DTSs (Deep Tiller Seeders) in the production of voluminous feeds (Bošnjak et al., 2013). For comparison, the yields of clover grass mixture for alfalfa, English ryegrass and red fescue harvested in the Pannonian area amounted to 27.72 t ha^{-1} fresh mass (Tadić, 2018), while the yield of clover grass mixture with a dominance of cocksfoot and ryegrasses, also in the Pannonian area, was on average 38.84 t ha^{-1} fresh mass (Knežević et al., 2004). However, compared to these yields, biomass yields in the Eu-Mediterranean are far lower and range from 0.23 to 0.61 t ha^{-1} in forest stands, and 0.12 to 0.23 t ha^{-1} for grasses and herb species on sunlit paths and forest clearings. Thus, the yields of cultivated grasses in the Eu-Mediterranean area in the year of sowing are lower than the yields of the same in more humid areas, but, there again, they greatly exceed the amount of natural supply of fodder in the Mediterranean area. Therefore, the not surprising results are scientific evidence of the harmful effects of the European mouflon on the indigenous vegetation of the Mediterranean area (Rodríguez et al., 1988; Garcia-Gonzalez

et al., 1989; Alfayate et al., 1991) since offering a higher quality (more palatable) and richer-yielding fodder could reduce the pressure of this ruminant on indigenous vegetation.

Conclusion

The results of the research on the yields of fresh mass of four grass species showed that it is possible to achieve green fodder yields in the Mediterranean (arid) conditions, which by far exceeds the biomass of natural vegetation. A statistically significant difference in the yields of fresh mass in the first and second year of the experiment was found in three of the four grass species. The establishment of such grassland draws could reduce the pressure of wild ruminants on indigenous vegetation and enable acceptable breeding of wild ruminants in areas where livestock-breeding has been abandoned.

Literature

Alfayate M. C., Rodríguez-Luengo J. L. (1991). Microhistological analysis of the feces of the Corsica mouflon during the flowering period in teide national park (Canary islands). XXth Congress of the Internationl Union of Game Biologists, Gődőlo, Hungary, August 21-26, 536-539.

Blondel J., Aronson J. (1999). Biology and Wildlife of the Mediterranean Region; Oxford University Press; New York; 328 pp.

Bošnjak K., Vranić M., Leto J., Kutnjak H., Perčulija G., Uher D., Teskera M. (2013). The effect of the growth stage at cutting on the productivity of grass/red clover binary mixtures. Glasnik zaštite bilja. 36(4): 39-45. (in Croatian with English summary)

Byrne R., Spink J., Freckleton R., Neve P., Barth S. (2018). A critical review of integrated grass weed management in Ireland. Irish Journal of Agricultural and Food Research, 57(1): 15-28.

Debussche M., Lepart J., and Dervieux A. (1999). Mediterranean landscape changes: evidence from old postcards. Global ecology and Biogeography. 8: 3-15

Garcia-Gonzalez R., Cuartas P. (1989). A comparison of the diets of the wild goat *(Capra pyrenaica)*, domestic goat *(Capra hircus)*, mouflon *(Ovis musimon)* and domestic sheep *(Ovis aries)* in the Cazorla mountain range. Acta Biologica Montana. 9: 123-132.

Knežević M. Leto J., Bošnjak, K, Vranić M., Perčulija, G., Kutnjak H. (2004). Effects of different sowing techniques on productivity and botanical composition of grass/clover mixture. Mljekarstvo. 54 (4): 261-274. (in Croatian with English summary)

Krapinec K. (2000). Feeding structure of the Mouflon (*Ovis ammon* L.) and Axis deer (*Axis axis* Erx.) in the area of the island of Rab. Master's thesis; 107 pp. (in Croatian with English summary)

Krapinec K. (2005). Mouflon (*Ovis ammon musimon* PALLAS, 1811) diet in the Eu-Mediterranean zone of the northern Adriatic Sea. PhD Thesis. Zagreb: Department of Forest Protection and Wildlife Management, Faculty of Forestry, University of Zagreb, (in Croatian with English summary).

Krapinec K., Uher D., Vranić M., Kiš G., Maćešić D. (2019). Preference of four grass species in European mouflon *(Ovis gmelini musimon)* diet in the eu-Mediterranean Zone of North Adriatic. *54th and 14th international symposium on agriculture, Vodice*, Croatia, 17th – 22nd February 2019. - Proceedings. Mioč B., Širić I. (eds.), 384-388. Osijek, Croatia: Poljoprivredni fakultet Sveučilišta Josipa Jurja Strossmayera u Osijeku, (in Croatian with English summary)

Le Houerou H.N. (1981). Impact of man and his animals on Mediterranean vegetation. In: Mediterranean-type scrublands. F. Di Castri et al., (ed.) Elsevier Sci. Pub. Co. Academic Press. Amsterdam. 479-520.

Paterson E., Sim A. (2000). Effect of nitrogen supply and defoliation on loss of organic compounds from roots of *Festuca rubra*, Journal of Experimental Botany. 51(349): 1449–1457.

Pausas J.G. (1999). Mediterranean vegetation dynamics: modelling problem and funcional types. Plant Ecology. 140: 27-39.

Rodríguez J. L., Rodríguez J. C., Ramos M. T. (1988). Autumn diet selectivity of the Corsica mouflon (*Ovis ammon musimon* Schreber, 1782) in Tenerife (Canary Islands). Mammalia. 52(4): 476-481

Seletković Z., Katušin Z. (1992). Klima Hrvatske. Iz: Rauš, Đ. (ur.) Šume u Hrvatskoj, Šumarski fakultet Zagreb i Hrvatske šume p. o. Zagreb, 13-19.

Šoštarić-Pisačić K., Kovačević J. (1968). Travnjačka flora i njena poljoprivredna vrijednost. Nakladni zavod Znanje, Zagreb, 443 pp.

Stjepanović M., Štafa Z., Bukvić G (2008). Trave za proizvodnju krme i sjemena; Zagreb.

Tadić V. (2018). Utjecaj smjese lucerne s travama na pojavu korova i prinos krme. Diplomski rad, Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Zagreb, 41 pp.

TIBCO Software Inc. (2020). Data Science Workbench, version 14. http://tibco.com.

Trinajstić I. (1986). Fitogeografsko raščlanjenje šumske vegetacije istočnojadranskog sredozemnog područja – polazna osnovica. Glasnik za šumske pokuse – posebno izdanje, 2. Dio, Zagreb, 53-65.

Vodopija M. (2017). Produktivnost i kvaliteta talijanskog ljulja kao ozime krmne međukulture. Diplomski rad, Sveučilište u Zagrebu, Agronomski fakultet, Zagreb, 22 pp.

IZVORNI ZNANSTVENI RAD

Morfometrijska svojstva jarebice kamenjarke (*Alectoris graeca* Meisn.) u lovištima Šibensko-kninske županije

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Sažetak
Jarebica kamenjarka grivna (*Alectoris graeca* Meisn.) je jedina autohtona vrsta iz roda *Alectoris* te je na teritoriju Republike Hrvatske nalazimo u priobalnom i obalnom području te na otocima. Za potrebe istraživanja prikupljena su 23 uzorka kroz dvije lovne sezone, 2019./20. i 2020./21., na području Šibensko-kninske županije. Svakoj jedinki određen je spol (14 ženki i 9 mužjaka), a nakon toga određena su morfometrijska svojstva. Statistički značajne razlike između mužjaka i ženki utvrđene su za visinu kljuna, tjelesnu masu, masu trupa i masu srca. Nakon analize i usporedbe vrijednosti morfoloških svojstava grivni iz Šibensko-kninske županije s podatcima iz ostalih istraživanja čini se da se populacije iz Hrvatske razlikuju od populacija iz Francuske, odnosno pokazuju sličnost s populacijama jarebice kamenjarke u Italiji i Turskoj.

Ključne riječi: jarebica kamenjarka, morfometrijska svojstva, spolni dimorfizam

Uvod

Jarebica kamenjarka grivna (*Alectoris graeca* Meisn.) pripadnik je poljskih koka, u lovačkom žargonu poznata je kao kraljica krša. Od nekoliko predstavnika roda *Alectoris* jedino je grivna autohtona kod nas, a obitava na području Sredozemlja, Alpa, Grčke i Albanije te Apeninskog poluotoka i Sicilije (Grubešić i sur., 2011.). Uz jarebicu kamenjarku na području Republike Hrvatske nalazimo još jednog pripadnika roda *Alectoris*, jarebicu čukar (*Alectoris chukar* Gray), koja je alohtona (Janicki i sur., 2007.). Na temelju Zakona o lovstvu (Anonimus, 2018.) jarebica kamenjarka svrstana je u sitnu pernatu divljač, a Pravilnikom o lovostaju (Anonimus, 2019.) lov na kamenjarku zabranjen je u razdoblju od 16. siječnja do 30. rujna.

Uz određene razlike u veličini spolni dimorfizam je slabo izražen. Mužjaci imaju širi crni prsten te snažniju ostrugu na nogama na kojoj se mogu uočiti sitne kvržice kojih kod ženki nema (Pintur, 2010.). Kvržice na ostruzi se kod određenog broja juvenilnih mužjaka ne vide prije dobi od 8 mjeseci pa ni taj pokazatelj nije uvijek pouzdan kod određivanja spola na temelju vanjskog izgleda (Bernard-Laurent i sur., 2003.). Grivnu odlikuje veliki broj boja perja. Tjeme, gornji dio tijela i prsa su sive boje. Grlište i prednji dio vrata su bijeli, obrubljeni crnom prugom koja je u mužjaka nešto šira. Trbuh je žućkasto smeđi, a bočne strane su plavkaste s poprečnim tamnim i svijetlim prugama. Kljun i noge su crvene (Janicki i sur., 2007.).

Prema dostupnoj literaturi, u Republici Hrvatskoj nisu provedena istraživanja vezana uz morfometrijska obilježja jarebice kamenjarke, dok u svijetu postoji više istraživanja u ovom području. Stoga je cilj ovog istraživanja bio utvrditi morfometrijska svojstva (raspon i duljinu krila, duljinu, širinu i visinu kljuna, duljinu i širinu glave, duljinu i širinu tarzusa, duljinu trupa, ukupnu masu, masu glave, masu trupa i randman) jarebice kamenjarke na

području Šibensko-kninske županije i usporediti ih sa istim svojstvima populacija s drugih područja.

Materijal i metode

Za potrebe ovog istraživanja redovnim odstrelom prikupljene su 23 jedinke jarebice kamenjarke. Uzorkovanje je provedeno u Šibensko-kninskoj županiji u lovištima XV/3 „Promina", XV/106 „Lozovac“, XV/115 „Siverić“, XV/116 „Drniš“ te XV/117 „Pakovo selo-Pokrovnik“ tijekom lovnih godina 2019./20. i 2020./21., a odstrel je izvršen sukladno planu gospodarenja za navedena lovišta. Jarebice su lovljene pojedinačnim i skupnim tehnikama lova prema propisima iz Pravilnika o uvjetima i načinu lova, nošenju lovačkog oružja, obrascu i načinu davanja lovačke iskaznice, dopuštenja za lov i evidenciji o obavljenom lovu (Anonimus, 2010.). Uzorci su pohranjeni u PVC vrećice na temperaturu od oko -20 °C. Mjerenja su izvršena koncem svake lovne sezone. Spol je određen prema prisutnosti ostruge kod mužjaka. Masa je određena digitalnom vagom marke „Sencor“, a duljina, širina i visina „Alpha Tools“ digitalnom pomičnom mjerkom s točnošću od 0,1 mm i mjernom vrpcom s točnošću 1 mm. Izmjerena su sljedeća morfološka svojstva: raspon krila, duljina krila, duljina glave, širina glave, duljina kljuna, širina kljuna, visina kljuna, duljina tijela, duljina tarzusa, širina tarzusa, ukupna masa, masa trupa, masa srca, masa jetre i masa želuca. Određen je i terenski randman (odnos mase cijeloga trupa i mase trupa bez unutrašnjih organa).

Rezultati i rasprava

Morfometrijska svojstva istraživanih jedinki jarebice kamenjarke prikazana su odvojeno po spolu. U Tablici 1 dane su prosječne izmjere morfometrijskih svojstava muških jedinki iz svih pet lovišta dok je u Tablici 2 dan prikaz morfometrijskih svojstava ženki. Iz spomenutih tablica se može vidjeti da je od ukupno odstrijeljenih 23 jedinke bilo 9 mužjaka i 14 ženki.

Tablica 1. Morfometrijske izmjere muških jedinki

	N	xmin	xmax	R	x	sta.dev.	sta.gr.
Raspon krila (mm)	9	451	555	104	521,11	33,65	11,21
Duljina krila (mm)	9	186	264	78	228,44	25,63	8,54
Duljina glave (mm)	9	51,51	5,43	4,92	54,05	1,77	0,59
Širina glave (mm)	9	22,32	28,19	5,87	25,66	2,16	0,72
Duljina kljuna (mm)	9	20,27	27,71	7,44	24,58	2,21	0,73
Širina kljuna (mm)	9	10,79	13,54	2,75	11,70	1,01	0,33
Visina kljuna (mm)	9	11,04	15,88	4,84	12,07	1,50	0,50
Duljina tijela (mm)	9	309	361	52	339,88	20,10	6,70
Duljina tarzusa (mm)	9	40,12	49,13	9,01	45,74	3,03	1,01
Širina tarzusa (mm)	9	8,34	10,54	2,19	9,33	0,68	0,22
Ukupna masa (g)	9	473	651	178	574,11	63,62	21,20
Masa jetre (g)	9	5	9	4	7	1,41	0,47
Masa srca (g)	9	1	2	1	1,77	0,44	0,14
Masa želuca (g)	9	18	24	6	21,77	2,16	0,72
Masa trupa (g)	9	353	507	154	446,11	53,40	17,80
Randman (%)	9	74	80,90	6,90	77,63	1.95	0,65

N - broj uzoraka, xmin – minimalna vrijednost, xmax - maksimalana vrijednost, R – raspon,. x -aritmetička sredina, sta.dev. - standardna devijacija i sta.gr. - standardna pogreška

Tablica 2. Morfometrijske izmjere ženskih jedinki

	N	xmin	xmax	R	x	sta.dev	sta.gr
Raspon krila (mm)	14	472	532	60	508,42	17,51	4,68
Duljina krila (mm)	14	211	250	39	228,64	11,81	3,15
Duljina glave (mm)	14	49,45	57,21	7,75	53,33	2,03	0,54
Širina glave (mm)	14	21,95	27,08	5,12	24,89	1,59	0,42
Duljina kljuna (mm)	14	20,79	28,09	7,30	24,45	1,85	0,49
Širina kljuna (mm)	14	9,34	12,29	2,94	11,09	0,88	0,23
Visina kljuna (mm)	14	8,78	12,17	3,39	10,88	1,11	0,29
Duljina tijela (mm)	14	312	352	40	333,57	14,21	3,79
Duljina tarzusa (mm)	14	39,04	48,03	8,99	43,50	3,40	0,90
Širina tarzusa (mm)	14	8,01	11,12	3,10	8,95	0,87	0,23
Ukupna masa (g)	14	423	569	146	505	45,69	12,21
Masa jetre (g)	14	5	9	4	6,64	1,44	0,38
Masa srca (g)	14	1	3	2	1,35	0,63	0,16
Masa želuca (g)	14	15	22	7	18,42	2,24	0,59
Masa trupa (g)	14	307	451	144	391,07	36,03	9,63
Randman (%)	14	72,57	82,02	9,45	77,46	2,79	0,74

N - broj uzoraka, xmin – minimalna vrijednost, xmax - maksimalana vrijednost, R – raspon,. x -aritmetička sredina, sta.dev. - standardna devijacija i sta.gr. - standardna pogreška

Budući da je primijećena razlika u prosječnim izmjerama mužjaka i ženki sa svrhom utvrđivanja statističke značajnosti razlika napravljen je t-test s pretpostavkom da varijance nisu jednake ($p \leq 0,05$) u programskom paketu SPSS, a rezultati su prikazani u Tablici 3.

Tablica 3. Razina značajnosti razlika utvrđenih t-testom

Morfometrijsko svojstvo	muške jedinke	ženske jedinke	*t* vrijednost	razina značajnosti (*p*)
Visina kljuna, mm	12,07	10,88	-2,35	0,028
Masa srca, g	1,77	1,35	-3,54	0,002
Masa trupa, g	446,11	391,07	-2,96	0,007
Ukupna masa, g	574,11	505	-3,04	0,006

Iz Tablice 3 je vidljivo da je statistički značajna razlika u morfometrijskim svojstvima između mužjaka i ženki utvrđena za visinu kljuna, masu srca, masu trupa te ukupnu masu. Za ostale izmjere nije utvrđena statistički značajna razlika između spolova.

Bernard-Laurent i sur. (2003.) su u južnim francuskim Alpama istraživali razlike morfometrijskih svojstava mužjaka i ženki jarebice kamenjarke pa su mjerili ukupnu masu, duljinu tijela, duljinu krila, duljinu tarzusa i opseg glave. Mužjaci su u svim parametrima imali veće vrijednosti nego ženke. Istraživanje je provedeno na sto i osamdeset jedinki s jednakim brojem mužjaka i ženki. Caglayan i sur. (2011.) su u Turskoj istraživali odnose između određenih tjelesnih parametara i žive mase kako bi se isti koristili u procjeni mase. Mjerenja su izvršena na četrdeset i jednoj ženki i trideset i jednom mužjaku. Razlike masa su postale statistički značajne između 6. i 14. tjedna života. Nakon višetjednog istraživanja zaključili su da se mjerenjem parametara glave i tarzusa može procijeniti masa te da su iste

izmjere lako izvedive i dostupne. Viola i sur. (2019.) su u Italiji istraživali kako uzgoj u zatočeništvu utječe na morfologiju jarebice kamenjarke. Istraživanje je provedeno na trideset i tri jedinke u dvije skupne, prvoj sa šesnaest jedinki s omjerom spolova 1:1 te drugoj sa sedamnaest jedinki u kojoj je nakon smrti tri pilića u prvom tjednu ostalo osam mužjaka i šest ženki. Mjerili su masu tijela, duljinu, širinu i visinu tarzusa, duljinu i širinu glave, duljinu kljuna te duljinu krila. Primijetili su da intenzivan uzgoj nema statistički značajan učinak na morfološka svojstva do 70 dana poslije valjenja. Nakon tog perioda preporuka je ispuštanje jarebica kamenjarki u prirodu jer kasnije ostankom u zatočeništvu razvijaju kraće glave i udove uslijed manjka fizičkog i kognitivnog vježbanja u takvim uvjetima.
Podaci o morfometrijskim svojstvima jarebica kamenjarki općenito, osim što su malobrojni, oni nisu standardizirani. Bernard-Laurent i sur. (2003.) su jedinke, osim po spolu razvrstali na juvenilne i adultne. U istraživanim lovištima Šibensko-kninske županije prosječna masa mužjaka iznosila je 574,11 g, a ženki 505 g. Na području Francuskih Alpa prosječna masa adultnih mužjaka iznosila je 659 g, a adultnih ženki 539 g, dok su juvenile jedinke nešto manje mase - mužjaci su imali masu 532 g, a ženke 475 g (Bernard-Laurent i sur., 2003.). Međutim, Bernard-Laurent i sur. (2003,) nisu testirali razlike između dobi niti za jedno svojstvo. Prema Violi i sur. (2019.) prosječna masa mužjaka nakon 14 tjedana iznosi 489,02 g, a ženke 409,31 g. Çaglayan i sur. (2011.) iznose znatno manje brojke prosječnih masa jarebica kamenjarki starosti 14 tjedana. Mužjaci su težili 435,99 g u prosjeku, a ženke 372 g te su ovi podatci znatno manji u odnosu na naše istraživanje (Tablica 4). Razdoblje razmnožavanja grivne traje od kraja travnja do sredine lipnja, ovisno o nadmorskoj visini (Vavalekas i sur., 2003.), pri čemu inkubacija može trajati od 24 do 26 dana (Madge i sur., 2002.). To bi značilo da jedinke dob od 14 tjedana (3,5 mjeseca) postižu od sredine rujna do kraja listopada, što bi odgovaralo razdoblju lovidbe na području Hrvatske. Respektirajući ovu pretpostavku, a imajući u vidu minimalne i maksimalne vrijednosti masa jedinki iz Hrvatske, čini se da su juvenilne grivne s područja Šibensko-kninske županije veće mase od onih iz Turske, odnosno približno istih masa kao i jedinke iz Italije (Tablica 4), ali i sličnih masa kao i jedinke iz Francuskih Alpa.

Tablica 4. Prosječne tjelesne mase jarebice kamenjarke s različitih dijelova njena areala

	Turska - juvenilne jedinke (Caglayan i sur., 2011.)	Italija – juvenilne jedinke (Violi i sur., 2019.)	Šibensko-kninska županija - adultne i juvenilne jedinke	Francuska - adultne jedinke (Bernard-Laurent i sur., 2003.)
Prosječna masa(mužjaci) (g)	435,99	507,11	574,11	659
Prosječna masa(ženke) (g)	372	427,42	505	539

Budući da ostale mjerene značajke unutar populacije iz Hrvatske nisu pokazale statistički značajne razlike nameće se pitanje da li je ne postojanje razlika u svojstvima između spolova nastala zbog ne razvrstavanja jedinki unutar svakog spola na dobne razrede. Prema Violi i sur. (2019.) intenzitet (režim) uzgoja grivne uglavnom ne utječe na vrijednosti značajki. Unutar svakog tipa uzgoja, u početnim danima života pilića nema razlike u vrijednostima značajki između spolova, no kako pilići rastu tako se počnu javljati signifikantne razlike u ukupnoj masi tijela, duljini i širini tarzusa te duljini i širini glave. Istu zakonitost su dobili i Çaglayan i sur. (2011.), koji su također pratili razvoj značajki pilića grivne do dobi od 14 mjeseci. Štoviše, razlike u prosječnim vrijednostima većine svojstava (ukupna tjelesna masa,

duljina krila, duljina tijela i duljina tarzusa) između spolova u obje dobne kategorije grivni (juvenilne i adultne) su pronašli i Bernard-Laurent i sur. (2003.). Izuzetak je bio jedino opseg glave koji je kod adultnih grivni bio signifikantno viši kod mužjaka nego kod ženki, dok kod juvenilnih jedinki nisu pronađene razlike u vrijednostima tog svojstva između spolova.
Nakon analize i usporedbe vrijednosti parametara grivni iz Šibensko-kninske županije s podatcima iz ostalih istraživanja čini se da se vrijednosti značajki populacije iz Hrvatske razlikuju od populacije iz Francuske, odnosno pokazuju sličnost s populacijama jarebice kamenjarke u Italiji i Turskoj. Naime, jarebice kamenjarke s područja Šibensko-kninske županije imaju veće vrijednosti u svim uspoređenim morfološkim parametrima od jarebica kamenjarki iz Italije i Turske osim duljine tarzusa gdje se poklapaju. Razlog tome je sudjelovanje isključivo juvenilnih jedinki iz umjetnog uzgoja u istraživanjima iz tih zemalja. U Francuskoj se pak uzorak razdvajao na juvenilne i adultne jedinke iz prirodnog uzgoja. Usporedbom s njihovim adultnim jedinkama smo ustanovili da jarebice kamenjarke s područja Šibensko-kninske županije imaju manje vrijednosti morfoloških parametara u odnosu na francusku populaciju osim za duljinu krila gdje vjerojatno došlo do greške pri mjerenju te zbog toga vrijednosti duljine krila uzorka iz Hrvatske imaju veće vrijednosti. Stoga je u daljnjim istraživanjima morfometrijskih značajki jarebice kamenjarke nužno jedinke razdvajati po spolu i dobi.

Zaključak

Prema rezultatima provedenog istraživanja morfometrijskih svojstava jarebice kamenjarke u Šibensko-kninskoj županiji može se zaključiti da postoji statistički značajna razlika između mužjaka i ženki u visini kljuna, ukupnoj masi, masi srca i masi trupa dok za ostala morfometrijska svojstva nije utvrđena statistički značajna razlika.
Rezultati istraživanja se djelomično podudaraju s rezultatima iz dostupne literature, a razlike postoje zbog razlika u načinu kategoriziranja uzorka prema dobi.

Literatura

Anonimus (2010): Pravilnik o uvjetima i načinu lova, nošenju lovačkog oružja, obrascu i načinu davanja lovačke iskaznice, dopuštenja za lov i evidenciji o obavljenom lovu. Narodne novine br. 70/2010.

Anonimus (2018). Zakon o lovstvu. Narodne novine br. 99/2018.

Anonimus (2019). Pravilnik o lovostaju. Narodne novine br. 94/2019.

Bernard-Laurent, A., E.M. Corda, D. Soyez (2003.): Sex differences in body measurements of Rock Partridges *Alectoris graeca* Saxatilis inhabiting the southern French Alps. Avocetta. 27(2): 181-186.

Caglayan, T., K. Kirikci, G. Aytekin, S. Alasahan (2011). Some body measurements and their correlations with live weight in the rock partridge (*Alectoris graeca*). African Journal of Agricultural Science. 6(7): 1857-1861.

Grubešić, M., K. Tomljanović, S. Kunovac(2011). Rasprostranjenost i brojnost jarebice kamenjarke grivne (*Alectoris graeca* Meisner) u Hrvatskoj i Bosni i Hercegovini. Šumarski list. CXXXV (11-12): 567-574.

Janicki, Z., A. Slavica, D. Konjević, K. Severin (2007). Zoologija divljači. Zavod za biologiju, patologiju i uzgoj divljači, Veterinarski fakultet, Zagreb.

Madge S., McGowan P., Kirwan, G.M. (2002). Pheasants, partridges, and grouse – a guide to the pheasants, partridges, quails, grouse, guineafowl, buttonquails and sandgrouse of the World, Princenton University Press, Princenton and Oxford, 488 pp.

Pintur, K. (2010). Tajna je u nogama. Dobra kob. (108): 30-31.

Vavalekas K., Thomaides Ch., Papaevangellou E., Papageorgiu N. (1993). Nesting biology of the Rock Partridge (Alectoris graeca graeca) in northern Greece. Acta Ornithologica. 28(2): 97-101.

Viola, P., R. Primi, F. Gabbianelli, P. P. Danieli, L. Lucentini, A. Amici (2019). Effect of intensive rearing on functional morphology: a case study with the Rock partridge(*Alectoris graeca graeca*). Forestry ideas. 2(58): 275–288.

Morphometric parameters of rock partridge (*Alectoris graeca* M.) in Šibensko-kninska County

Abstract

Rock partridge (*Alectoris graeca* M.) is the only indigenous species of the genus *Alectoris* in Croatia and it is found along the subcoastal, coastal area and the islands. In this study, 23 samples were collected through two hunting seasons, 2019/20 and 2020/2021, in the area of Šibensko-kninska county. Each unit was assigned a sex (14 females and 9 males), followed by measuring morphometric parameters. Statistically significant differences between males and females were found in beak height, body weight, heart weight and carcass weight. After analysing and comparing the values of morphological properties of the Rock partridges from Šibensko-kninska county with data from other studies, it seems that the populations from Croatia differ from the population from France, ie show similarities with the populations of partridge in Italy and Turkey.

Key words: rock partridge, morphometrc traits, sexual dimorphism

IZVORNI ZNANSTVENI RAD

Hlapljivi spojevi multiflorne pčelinje peludi

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Sažetak
Kemijska karakterizacija hlapljivih spojeva pčelinjeg peludi od velike je važnosti za procjenu njezine kvalitete. Hlapljivi spojevi izolirani su metodom mikroekstrakcije vršnih para na krutoj fazi (SPME) koristeći dva različita vlakna različite polarnosti. Dobiveni podaci analizirani su spregnutom tehnikom plinska kromatografija-spektrometrija masa (GC-MS). SPME metodom identificiran je 21 različit spoj. Identificirani spojevi mogu se svrstati u slijedeće kemijske skupine: kiseline, alkoholi, esteri, ketoni i aldehidi.

Ključne riječi: hlapljivi spojevi, pčelinja pelud, GC-MS, SPME

Uvod

Pčelinja pelud ovisi o biljnoj vrsti, razlikuje se po obliku, boji, veličini i težini (Komosinka-Vassev i sur., 2015.). Kemijski sastav hlapljivih spojeva pčelinje peludi u novije vrijeme je od velikog istraživačkog interesa, koji pokriva široke aspekte od fiziologije biljaka do biokemije (Campos i sur., 1997.),te njihovu primjenu pri oprašivanju bilja i u pčelarstvu. Pelud su muške spolne stanice presudne za razmnožavanje biljnih vrsta. Pelud je nosilac genskih svojstava biljke i zato sadrži sve neophodne hranjive tvari za stvaranje i razvoj novog života. Pčelinjoj zajednici pelud je glavni izvor bjelančevina, vitamina i minerala. Pčele peludna zrnca sakupljaju sa cvjetova biljaka, istodobno im dodajući nektar i tvari svojih žlijezda oblikujući male granulice. Veličina tih granulica je otprilike veličine zrna okrugle riže. Pčele te granulice prenose do košnice u „košaricama“ na stražnjem paru nogu. Svaka granula pčelinje peludi sadrži od nekoliko stotina tisuća do preko 2 milijuna peludnih zrnaca. Pelud je, osim za pčele, vrlo vrijedan biološki materijal i za ljudsko zdravlje jer sadrži izvanredno uravnotežene prirodne sastojke: bjelančevine, šećere, vitamine, minerale, enzime, biljne hormone, eterična ulja, vodu te neprobavljiva vlakna u ovojnici (Levaković, 2014.). Cilj ovog rada je bio identificirati hlapljive spojeve multiflorne pčelinje peludi korištenjem spregnute tehnike plinske kromatografije-masene spektrometrije (GC-MS) izorinah mikroekstrakcijom vršnih para na krutoj fazi (SPME) pomoću dva vlakna s različitom polarnošću.

Materijal i metode

Istraživanje je provedeno na jednom skupnom uzorku pčelinje peludi sa dvije lokacije. Multiflorni uzorak peludi je sakupljen 2020. godine sa otoka Šolte (43º37´7.80´´, 16º34´1.97´´) i otoka Čiova (43º49´8.19´´, 16º32´2.67´´), a najzastupljenija pčelinja pelud prema botaničkom porijeklu je: česmina (*Quercus ilex*), maslina (*Olea europea*), bušin (*Cistus spp*.), mlječika (*Euphorba spp*.), dalmatinska kadulja (*Salvia officinalis* L.) i brnistra (*Spartium junceum*) (Prđun i sur., 2021.). Hlapljivi spojevi jednog grama uzorka su izolirane pomoću mikroekstrakcije vršnih para na krutoj fazi (SPME) koristeći vlakna prekriveno sa divinilbenzenom/karboksenom/polidimetilsiloksanom (DVB/CAR/PDMS) (sivo vlakno) i polidimetilsiloksanom/divinilbenzenom (PDMS/DVB) (plavo vlakno) prevlakom (Supelco Co., Bellefonte, PA, SAD) (Kuš i sur., 2018.), te upotrbom magnetske miješalice (Heidolph

MR Her-Standard (100-1400 o/min) s termostatom Heidolph EKT 3001, Njemačka) (Marijanović, 2014.). Analiza izoliranih hlapljivih spojeva provedena je spregnutom tehnikom plinska kromatografija-spektrometrija masa (GC-MS) (Agilent Technologies, Palo alto, Ca, SAD), koristeći plinski kromatograf model 8890, u kombinaciji s masenim detektorom, model 5977E. Separacija komponenti provedena je na kapilarnoj koloni HP-5MS ((5% fenil)-metilpolisiloksan; 30 m × 0,25 mm; debljina sloja stacionarne faze 0,25 µm (Agilent J&W, SAD). Uvjeti rada plinskog kromatografa za HP-5MS kolonu su bili isti kao i u istraživanju Jerković i sur. (2016.). Identifikacija spojeva temeljila se na usporedbi njihovih indeksa zadržavanja (RI), određenih relativno prema retenciji vremena *n*-alkana (C_9-C_{25}), s onim zabilježenim u literaturi (Adams, 2017.) i onima iz *Wiley 9* i *NIST 17* spektralne baze podataka mase (Jerković i sur., 2016.).

Rezultati i rasprava

Izolacija hlapljivih spojeva je provedena na temperaturi koja ne prelazi 40 °C omogućavajući izolaciju hlapljivih spojeva bez stvaranja termičkih artefakata što je bitno za dobivanje pouzdanih rezultata. GC-MS analizom hlapljivih spojeva multiflorne pčelinje peludi identificiran je ukupno 21 spoj. Pelud je zaista jedan od jedinstvenih bioloških proizvoda prirode. To nam dokazuje najinteresantnija harmonija između biljnog i životinjskog carstva, zvana, pčela. S jedne strane, biljke proizvodnjom peludi osiguravaju život pčelama, dok s druge strane, pčele skupljajući nektar pridonose preživljavanju biljnog svijeta kroz proces oprašivanja. Organoleptička svojstva pčelinje peludi se mijenjaju ovisno o botaničkom podrijetlu peluda. Miris peluda je specifičan prema biljnom izvoru, a okus sladak, kiselkast ili ponekad gorak (Bubalo i Rogulja, 2011). Tablica 1. sadrži sastav hlapljivih spojeva multiflorne pčelinje peludi izoliranih uz pomoć sivog vlakna (DVB/CAR/PDMS). Izolirano je 20 spojeva koji sumarno čine 88,8 % ukupnih hlapljivih spojeva. Spoj koji ima prvenstvo s obzirom na najveći udio je butanska kiselina (16,1 %), a osim butanske kiseline kvantitativno značajni spojevi su i nonanal (13,3 %) te metil-heksanoat (13,1 %). Preostali spojevi se nalaze u nešto manjim udjelima to su redom: metil-dekanoat (9,4 %), heksanska kiselina (5,6 %), 6-metil-5-hepten-2-on (4,2 %), butanal (3,8%), metil-oktanoat (3,4 %), heksanal (3,3 %), heptanal (2,1 %), 3-metilbutanal (2,1 %), felandren (1,9 %) te oktan-3-ol (1,9 %). Ostatak spojeva koji je izoliran nalazi se u udjelima manjim od 1,6 %. Hlapljivi spojevi multiflorne pčelinje peludi, točnije kemijski sastav i udio hlapljivih spojeva izoliranih pomoću metode mikroekstrakcije vršnih para na krutoj fazi sa plavim vlaknom (PDMS/DVB) prikazan je u Tablici 1. Pomoću njega je izolirano 19 spojeva koji ukupno čine 90,6 % hlapljivih spojeva uzorka. U uzorku dominira oktan-3-ol (14,1 %) dok ga sa ponešto nižim udjelima slijede nonanal (13,8 %) i (*E*)-heks-2-enal (11,0 %). Redom prema udjelima slijede: metil-heksanoat (10,2 %) i metil-dekanoat (9,9 %), 6-metil-5-hepten-2-on (4,1 %), metil-oktanoat (3,2 %) i butanal (3,2 %). Ostatak spojeva nalazi se u udjelima manjim od 3 %. Usporedbom podatka dobivenih na oba vlakna može se primijetiti kako su izolirani spojevi gotovo identični i kod plavog i kod sivog vlakna, samo u različitim relativnim udjelima. Primjerice oktan-3-ol kod analize sa plavim vlaknom ima udio od 14,1 % dok kod analize sa sivim vlaknom ima udio od 1,9 %. Udio butanske kiseline kod analize sa plavim vlaknom je 2,1% dok je kod analize sa sivim vlaknom njen relativan udio 16,1 %. Izuzetak kod analize sivim vlaknom su spojevi felandren i 2-etilfuran, koji nisu identificirani kod analize sa plavim vlaknom. Također benzil alkohol je identificiran uporabom samo plavog vlakna. Kod plavog vlakna najzastupljeniji spoj je sekundarni alkohol oktan-3-ol, koji se može javiti u dva oblika (*R*) ili (*S*). Ovaj alkohol ima miris po gljivama, a privlači insekte pčela, komaraca… (Afify i sur., 2019.). Metil-heksanoat je masna kiselina metil ester heksanske kiseline, a može se koristiti u formulaciji za okus breskve, kajsije, miris cvijeta jabuke, manga… Također i esteri metil-oktanoat i metil-

dekanoat se mogu naći u hlapljivim spojevima cvijeta jabuke, manga i slično (Pino i sur., 2005.). Svi gore navedeni esteri su također izolirani pomoću sivog vlakna samo sa različitim relativnim udjelima. 6-metil-5-hepten-2-on (4,1-4,2%) je nezasićeni metilirani keton s voćnim mirisom, također privlači insekte. Butanska kiselina (16,1%) i heksanska kiselina (5,6%), koje su izolirane pomoću sivog vlakna u većem udjelu, su sastavni dio ljudskog znoja vrlo neugodnog mirisa (Kanalayavattanakul i Lourith, 2011.). (*E*)-heks-2-enal je izoliran pomoću plavog vlakna (11,0%) u većem postotku nego kod sivog vlakna (1,6%). Ovaj acetat koji potječe od razgradnje linolenske kiseline, nam daje cvjetni miris i miris po pokošenoj travi (Šarolić i sur., 2015.).

Tablica 1. Kemijski sastav i udio najzastupljenijih hlapljivih spojeva multiflorne pčelinje peludi izoliranih pomoću mikroekstrakcije vršnih para na krutoj fazi (SPME)

Red. br.	**Spoj**	**RI**	**Površina pika (%)**	
			A	**B**
1.	butanal	<900	3,8	3,2
2.	3-metilbutanal	<900	2,1	2,1
3.	butanska kiselina	<900	16,1	2,1
4.	2-etilfuran	<900	0,5	--
5.	heksanal	<900	3,3	1,7
6.	(*E*)-heks-2-enal	<900	1,6	11,0
7.	heptanal	904	2,1	1,7
8.	metil-heksanoat	924	13,1	10,2
9.	benzaldehid	969	1,4	2,4
10.	heksanska kiselina	986	5,6	1,9
11.	6-metil-5-hepten-2-on	989	4,2	4,1
12.	oktan-3-ol*	998	1,9	14,1
13.	dekan	1000	1,2	2,0
14.	oktanal	1005	1,5	0,9
15.	felandren	1008	1,9	--
16.	benzil alkohol	1046	--	1,7
17.	(*E*,*E*)-okta-3,5-dien-2-on	1075	1,5	2,9
18.	(*E*,*Z*)-okta-3,5-dien-2-on	1096	1,0	1,7
19.	nonanal	1105	13,3	13,8
20.	metil-oktanoat	1128	3,4	3,2
21.	metil-dekanoat	1327	9,4	9,9
	Ukupno identificirano		*88,8 %*	*90,6 %*

RI *= retencijski indeks na HP-5MS koloni; -- = nije identificirano;* = točan izomer nije identificiran;* ***A*** *= izolirano pomoću SPME metode koristeći vlakno s DVB/CAR/PDMS ovojnicom (sivo vlakno);* ***B*** *= izolirano pomoću SPME metode koristeći vlakno s PDMS/DVB ovojnicom (plavo vlakno).*

Zaključak

Razmatrajući dobivene rezultate analize može se zaključiti slijedeće: dobiveni rezultati pokazuju da su izolirani identični spojevi na oba vlakna, samo sa različitim udjelima, spojevi

2-etilfuran i felandren izolirani su samo uz pomoć sivog vlakna (DVB/CAR/PDMS), a spoj benzil-alkohol izoliran je uz pomoć plavog vlakna (PDMS/DVB). Iz izoliranih spojeva može se zaključiti da su najzastupljenije skupine spojeva: kiseline, alkoholi, esteri, ketoni i aldehidi. Svi ti spojevi sa sobom donose karakteristične mirise. Kako je već rečeno to mogu biti cvjetni mirisi, mirisi voća, ali i trave. Naravno to mogu biti i neugodni mirisi poput mirisa ljudskog znoja. I jedni i drugi mirisi privlače insekte, a upravo je to ono što je potrebno bilju za opstanak. Nije bitno koji je od mirisa privukao insekte, bitno je da dođe do prijenosa peluda.

Napomena
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Literatura

Adams R.P. (2017). Identification of Essential Oil Components by Gas Chromatography/mass Spectrometry, 4.1th ed. Allured Publishing Corporation Illinois.

Afify A., Betz J., Riabinina O., Lahondere C., Potter C. (2019). Commonly Used Insect Repellents Hide Human Odors from Anopheles Mosquitoes. Current Biology. 29(21): 3669-3680.

Bubalo D., Rogulja D. (2011). Pelud, botaničko i zemljopisno porijeklo meda. Raspoloživo: https://www.facebook.com/307003142550/posts/10150523162462551/

Campos M., Merkham K.R., Mitchell K.A., Da Cunha A.P. (1997). An apprroach to the characterization of bee pollens via their flavonoid/phenolic profiles. Phytochemical Anallysis. 8(4):181-185.

Jerković I., Kranjac M., Marijanović Z., Zekić M., A., Tuberoso C. I. G. (2016). Screening of *Satureja subspicata* Vis. Honey by HPLC-DAD, GC-FID/MS and UV/VIS: Prephenate Derivatives as Biomarkers. Molecules. 21(3): 377-388.

Levaković M. (2014). Pelud-ljekovita svojstva i primjena, Diplomski rad. Sveučilište Josipa Jurja Strossmayera u Osijeku, Poljoprivredni fakultet, Osijek.

Kanlayavattanakul M., Lourith N. (2011). Body malodours and their topical treatment agents. International Journal of Cosmetic Science. 33(4): 298-311.

Komosinka-Vassev K., Olczyk P., Kaźmierczak J., Mencner L. Olczyk K. (2015). Bee Pollen: Chemical Composition and Therapeutic Application. Evid. Based Complementary Altern. Med. 297425: 1-6.

Kuś P.M., Jerkovic I., Marijanović Z., Kranjac M., Tuberoso C.I.G. (2018). Unlocking Phacelia tanacetifolia Benth. Honey characterization through melissopalynological analysis, color determination and volatiles chemical profiling. Food Research International. 106(1): 243-253.

Marijanović Z. (2014). Primjena ultrazvučne ekstrakcije otapalom i mikroekstrakcije vršnih para na krutoj fazi za karakterizaciju meda, Doktorska dizertacija. Prehrambeno-tehnološki fakultet Osijek, Osijek.

Pino J.A., Mesa J., Munoz Y., Marti M.P., Marbot R. (2005).Volatile Components from Mango (*Mangifera indica L.*) Cultivars. Journal of Agricultural ans Food Chemistry. 53(6): 2213-2223.

Prđun S., Svečnjak L., Valentić M., Marijanović Z., Jerković I. (2021). Characterization of Bee pollen: Physico-Chemical properties, Headspace Composition and FTIR Spectral Profiles. Foods. 10, 2103: 1-25.

Šarolić M., Gugić M., Friganović E., Tuberoso C.I.G., Jerković I. (2015) Phytochemicals and Other Characteristics of Croatian Monovarietal Extra Virgin Olive Oils from Oblica, Lastovka and Levantinka Varieties. Molecules. 20(3): 4395-4409.

Volatile compounds of multiflorl bee pollen

Abstract
Chemical characterization of volatile compounds of bee pollen is of great importance for assessing its quality. Volatile compounds were isolated by headspace solid phase microextraction (SPME) with two different fibers of different polarity. The obtained data were analyzed by the related technique gas chromatography-mass spectrometry (GC-MS). 21 different compounds were identified by the SPME method. The identified compounds can be classified into the following groups: acids, alcohols, esters, ketones and aldehydes.

Key words: volatiles compounds, bee pollen, GC-MS, SPME

ORIGINAL SCIENTIFIC PAPER

About the Mediterranean scallop *Pecten jacobaeus* (Linnaeus, 1758) in the bay of Valdibora, Croatia

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Abstract
This article reports on the characteristics of the population of the Mediterranean scallop (*Pecten jacobaeus*) in Valdibora Bay on the west coast of Istria, Croatia. Morphometric characteristics, meat index, condition index, gonadosomatic index, vertical distribution and density, and other organisms in the habitat were studied. The study was conducted from January to August 2015. Results indicate that a large proportion of scallops spawned in July. Meat and condition indices were highest in April and March. The depth at which the majority of the scallop population resides was 11 m. The average population density was 0.012 individuals m^{-2}.

Key words: bivalve morphometry, condition index, meat index, gonadosomatic index.

Intorduction

The Mediterranean scallop is an important commercial Mediterranean bivalve species and an integral part of Mediterranean cuisine, collected and consumed since Aristotle's time (Voultisadou et al., 2009). In the Croatian Adriatic, scallops are collected, especially on the western coast of Istria and in the Zadar area (Cetinić & Soldo, 1999; Marguš & Teskeredžić, 2005), but according to the Croatian Ministry of Agriculture - Directorate of Fisheries, 16 kg of Mediterranean scallops were also cultivated in 2015 (https://ribarstvo.mps.hr/default.aspx?id=14). To date, researches have focused on the assessment of age, structure, morphometric characteristics, distribution, reproduction, settlement, larval growth and survival of scallops (Marguš & Teskeredžić, 2005; Cetinić & Soldo, 1999; Onofri & Marguš 1995; Marguš 1990; Piccinetti et al., 1986). These surveys were mostly conducted in estuaries where scallops were collected with dredges. The only recent research was focused on biochemical homeostasis and the activity of defence system in the Northern Adriatic Sea (Topić Popović et al. 2020). In this study, scallops were collected in Valdibora Bay on the west coast of Istria in order to determine some characteristics of the scallop population.

Materials and methods

Samples of Mediterranean scallops and other macrobenthic species were collected at three sites in the northern Adriatic Sea in Valdibora Bay near Rovinj (45°05'30.6 "N, 13°38'20.9 "E, 45°05'40.0 "N, 13°38'22.3 "E, 45°05'39.5 "N, 13°38'04.0 "E). Sampling site 1 is located near the fish processing plant in the southern bay, where it is protected from most winds by the peninsula to the north and the town of Rovinj to the south and southwest. No significant currents were detected during the survey, but the high turbidity made sampling difficult. The nature of the seabed is variable, with an alternation of rocky and sandy bottom. There is no seagrass at sampling site 1. The depth increases and gradually reaches 18 m at 200 m from the coast. Sampling site 2 is located near the beach. During sampling, currents were almost

negligible. The seabed near the coastline is sandy and covered with grass. It reaches a depth of 6-8 m, followed by a zone with a depth of 10-13 m, after which the depth increases abruptly and changes to a muddy bottom. Two hundred m from the shore, the depth reaches 25 m. Sampling site 3 is located in the northern part of the bay. The gravelly seabed in the shallow part of the bay transitions to a sandy bottom, followed by a muddy bottom at depth, which reaches a depth of 22 m 200 m from shore. Occasional currents were noted during the survey.

Samples were collected monthly from January to August 2015 by free diving along a 200 metre transect line. Each scallop was tagged. Their length (l), height (h), and width (w) were measured in the laboratory to the nearest 0.2 mm using a Vernier calliper. The weight of each specimen was measured to the nearest 0.01 g using a digital scale. Subsequently, the weight of the wet soft tissue of the scallops (WMW) and the weight of the gonads were measured individually. The soft tissue of each specimen was dried at 105°C for 48 hours, and then the dried meat weight (DMW) was determined. The total water content of the meat (WC) was calculated using the following formula: WC = 100% - (DMW / WMW - 100%). Meat index (MI) was calculated using the following formula: MI =WMW/WW -100 (Royer et al, 2007). Condition index (CI) was calculated from DMW and shell weight (SW) using the following equation (Mann, 1978): CI = DMW / SW - l000. Gonadosomatic index (GSI) according to the formula: GSI= GW / WMW - 100% (GW= gonad weight) (Connor, 1978). The data obtained were entered into tables created using MS Excel. Descriptive statistical analyses were also performed using MS Excel, including calculations of means, standard deviations, minimum and maximum values presented in figures and tables.

Population density (D) estimates from line transects were calculated using the formula D= n / 2Lμω, where n is the number of detections, L is the total length of transects, and μω is the estimated effective half-width of the strip (Hedley and Buckland, 2004). Because the number of specimens collected is small, data from all three stations were presented together. According to the Croatian Meteorological and Hydrological Service, the water temperature at the Rovinj - Sv. Ivan n/p permanent station varied from 10°C measured in February 2015 to 27°C measured at the end of July 2015, with average monthly temperatures ranging from 10°C in February to 26°C in July. The highest temperature differences were measured in May, when the water temperature increased abruptly.

Results and discussion

During the survey, a total of 137 specimens of Mediterranean scallop were collected from depths ranging from 9 to 18 m. Most specimens were found at 11 m depth. Water content varied only slightly, with both the lowest (69.52%) and highest (95.61%) water content was found in April. The weight of wet tissue showed fluctuations, indicating a constant influx of food, which provides a balanced energy investment in the development of the gonads and the growth of the organism. The highest meat index (40.11) was recorded in May for a 119.81 mm specimen. The lowest meat index (15.6) was also determined in May in a 79.91 mm specimen (Figure 1). The condition index for scallops represents the proportion of meat in the shell (Mann, 1978). In scallops from Valdibora Bay, the condition index was relatively high. The highest condition index was recorded in April at 124.81 for an individual weighing 46.14 g and 78.3 mm long. The lowest value of 22.8 was also recorded in April for individuals weighing 74.8 g and measuring 93.27 mm in length (Figure 2). The highest gonadosomatic indices were determined in March, followed by a gradual decrease in values in June, July, and August (Figure 3). The gonads were clearly visible in all specimens over 5 cm, their size indicated the first sexual maturity of the bivalve (Mattei & Pellizato, 1996; Renzoni, 1991; Valli, 1979). Gonadosomatic indices were lower than the results of Marguš

and Teskeredžić (2005), but in both studies the minimum values occurred in July, possibly coinciding with the beginning of the spawning.

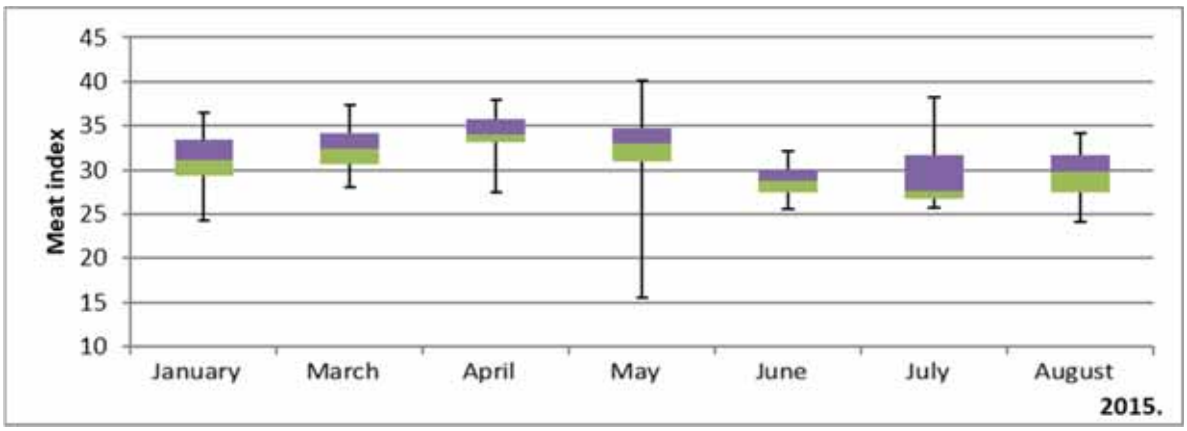

Figure 1. Meat index changes of Mediterranean scallop *Pecten jacobaeus* from the Valdibora Bay, Rovinj

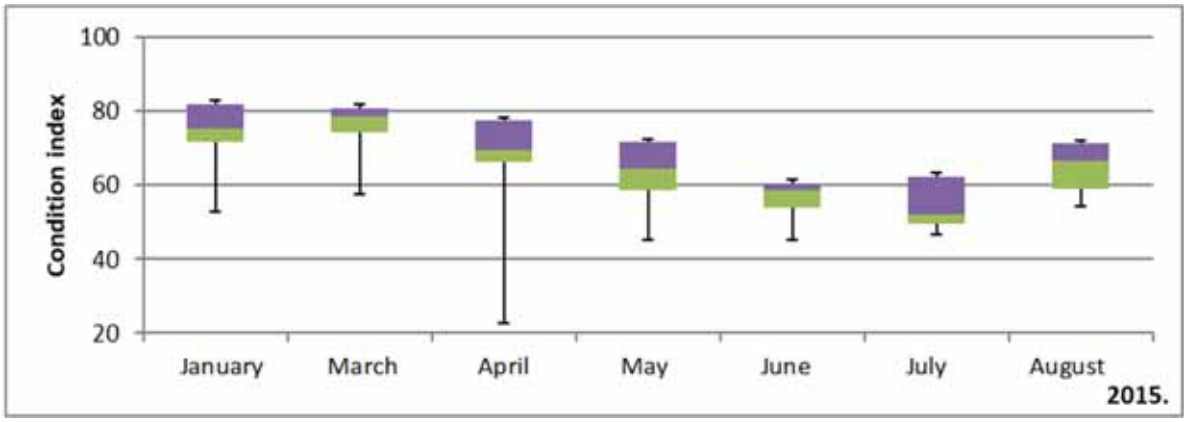

Figure 2. Condition index changes of Mediterranean scallop *Pecten jacobaeus* from the Valdibora Bay, Rovinj

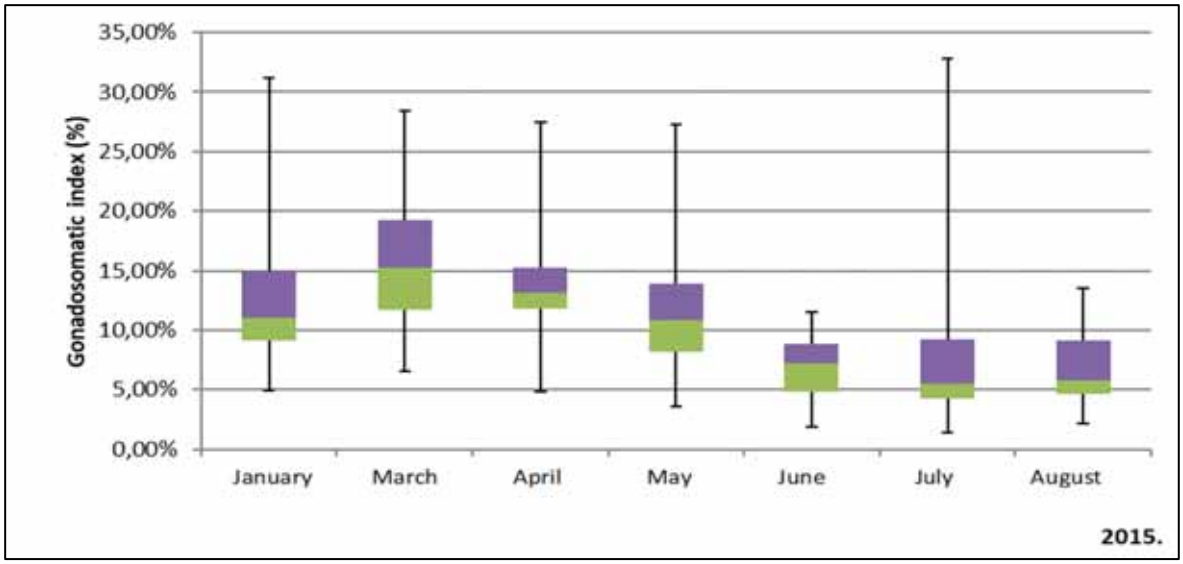

Figure 3. Gonadosomatic index changes of Mediterranean scallop *Pecten jacobaeus* from the Valdibora Bay, Rovinj

The maximum weight of the Mediterranean mussel in May was 136.92 g and the minimum weight in July was 27.28 g. The largest specimen measured in May was 119.81 mm long. The shortest specimen of 49.87 mm was sampled in January. The average morphometric values of the scallops sampled during the study are shown in Table 1. A correlation coefficient for height and length of 0.91 agrees with the values obtained in the study of Mattei and Pellizzato (1996), where a correlation coefficient of 0.98 was calculated. It should be emphasised that they measured 2448 specimens, compared to 137 specimens from this study. We found that the width of Mediterranean scallops varies greatly and that larger specimens may not have wider shells.

Table 1. Average morphometric values of Mediterranean scallop *Pecten jacobaeus* from the Valdibora Bay, Rovinj

Month	Number of specimens	weight (g)	hight (mm)	lenght (mm)	with (mm)
January	18	78.4 ± 22.20	83.58 ± 6.96	94.42 ± 15.39	23.16 ± 2.32
March	20	80.4 ± 8.81	80.77 ± 40	92.46 ± 5.34	23.85 ± 2.32
April	24	63.8 ± 17.78	74.95 ± 80	86.89 ± 11.12	20.54 ± 2.95
May	20	95.5 ± 32.45	86.42 ± 14.80	100.09 ± 17.6	22.95 ± 3.91
June	20	74.4 ± 19.00	82.68 ± 7.71	94.77 ± 9.11	22.5 ± 2.85
July	20	73.1 ± 28.16	81.35 ± 13.11	92.13 ± 14.61	21.8 ± 3.21
August	15	81.5 ± 27.80	85.24 ± 10	95.48 ± 12.47	23.44 ± 40

The total density of the scallop population in the studied area was 0.012 specimens/ m^2. At site 1, the density was 0.0075 specimens/m^2 in March and 0.00916 specimens/m^2 in August. At Site 2, the density in March was 0.00416 specimens/ m^2, and 0,000832 specimens/ m^2 in August, while at Site 3, the density in March was 0.0116 specimens/ m^2 and in August was 0.00333 specimens/ m^2. All scallops were collected at depths ranging from 9 to 18 m. No scallops were found at depths less than 9 m, which is consistent with the results of Katsanevakis (2005), who found no scallops greater than 4.5 cm in length below this depth. In his study, the length ranged from 1.8 to 16 cm, while in Valdibora Bay it ranged from 5 to 12 cm. These data are consistent with the study of Peharda et al. (2003), who found a length range of 7 to 14 cm. Onofri and Marguš (1995) recorded individuals of 16 cm. Since divers frequently collect scallops in the area of our study, the size of the collected individuals is smaller, and the minimum legal length of 10 cm was established.

In addition to the Mediterranean scallop, seven other species that prefer sandy bottoms were found in the study area during the survey. Some of them (the bivalves *Pinna nobilis, Ostrea edulis* and *Aequipecten opercularis*) share the ecological niche of feeding with the Mediterranean scallops, while gastropods *Hexaplex trunculus* and *Cerithium vulgatum* or the scallop *Astropecten irregularis* feed on dead or live bivalve molluscs, and the most common species is the detritus-feeding echinoderm *Holoturia tubulosa*.

Conclusion

The Valdibora Bay of Rovinj is a suitable habitat of Mediterranean scallop which is the most represented bivalve shellfish in the area. All specimens longer than 5 cm collected during the study had clearly visible gonads. According to the results of the gonadosomatic index and meat index, scallops probably mostly spawn in July. Individuals longer than 120 mm have not been reported, maybe due to relative availability to the divers.

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Literature

Cetinić P., Soldo A. (1999). Dredge catches of pilgrim`s scallop (*Pecten jacobaeus* L.) in eastern Adriatic. Folia Universitatis Agriculturae Stetinensis. 192(25): 37-43.

Connor P. M. (1978). Seasonal variation in meat yield of scallop (*Pecten maximus*) from the south coast (Newhaven) of England. The International Council for the Exploration of the Sea, CM Doc. K. 8: 1-4.

Državni hidrometerološki zavod DHMZ (2015). Podatci o temperaturi mora na Jadranu. http://vrijeme.hr/aktpod.php?id=more_n.

Hedley S. L., Buckland S. T. (2004). Spatial Models for Line Transects Sampling. America Statistical Association and the International Biometric Society. Journal of Agricultural Biological and Environmental Statistics. 9(2): 181-199.

Katsanevakis S. (2005). Abundance and spatial distribution of the Mediterranean scallop, *Pecten jacobaeus*, in a marine lake. Fisheries Research. 76: 417-429.

Mann R. (1978). A comparison of morphometric, biochemical and physiological index of condition in marine Bivalve Molluscs. U: Thorp, J., H., Gibbons, J., W. (ur) Energy and environmental stress in aquatic systems. Technical Information Center U.S. Department of Energy. Washington. 484-497.

Maraguš D., Teskeredžić E., Teskeredžić Z., Tomec M. (1993). Reproductive cycle and monitoring of scallop larvae (*Pecten jacobaeus* L.) in plankton from the mouth of the River Krka. Ribarstvo. 48: 43-54.

Marguš D. (1990). The scallop (*Pecten jacobaeus* L.) in the Krka river estuary. Acta biologica Iugoslavica. 22: 69-77.

Marguš D., Teskeredžić E. (2005). Prihvat ličinki, preživljavanje i rast juvenilnih jakovskih kapica (*Pecten jacobaeus*, Linnaeus 1758) u kontroliranom uzgoju u uvali Šarina draga – ušće rijeke Krke. Ribarstvo. 63(1): 1-14.

Marguš D., Teskeredžić E., Modrušan Z., Hacmanjek M. (1992). Rasprostranjenost, gustoća i starosna struktura populacije jakovske kapice (*Pecten jacobaeus* L.) i male kapice (*Chlamys varia* L.) u ušću rijeke Krke. Pomorski zbornik. 30: 599-618.

Mattei N., Pellizzato M. (1996). Apopulation study on three stocks of a commercial Adriatic pectinid (Pecten jacobaeus). Fisheries Research. 26: 49-65.

Ministry of Agriculture - Directorate of Fisheries (2022). Fisheries [Online]. Aquaculture. Available at: https://ribarstvo.mps.hr/default.aspx?id=42 (Accessed: 10 January 2022)

Onofri V., Marguš D. (1995). Biologija jakovske kapice (*Pecten jacobaeus* L.) i mogućnost njezinog uzgoja u području otoka Mljeta. U: Durbešić, P., Benović, A. (eds.) Priopćenje sa simpozija „Prirodne značajke i društvena valorizacija otoka Mljeta“. Ekološke monografije 6, Zagreb. Hrvatsko ekološko društvo. 555-562.

Pagotto G. (1985). La produtzione di Scalopasanta (*Pecten jacobaeus* L.) a Chioggia nel 1984. Nota aggiuntiva allo studio sui dati 1957. CNR. 83.

Pagotto G., Zatta P. (1985). La pesca industriale del *Pecten jacobaeus* nello Alto Adriatico. Pesca, Ambiente, Risorse, Salute. 12: 42-44.

Peharda M., Soldo A., Pallaoro A., Matić S., Cetinić P. (2003). Age and growth of the mediterranean scallop *Pecten jacobaeus* (Linnaeus 1758) in the northern Adriatic Sea. Journal of Shellfish Research. 22(3): 639-642.

Piccinetti C., Šimunović A., Jukić S. (1986). Distribution and abundance of *Chlamys opercularis* (L.) and *Pecten jacobaeus* L. in the Adriatic. FAO Fish Rep/FAO Rapp. PECHES 345: 99-105.

Renzoni A. (1991). Scallops: Biology, Ecology and Aquaculture U: Shumway S., E. (ed.), Developments in Aquaculture and Fisheries Science No. 21. Elsevier, Amsterdam. 777-778.

Royer J., Ropert M., Costil K. (2007). Spatio-temporal changes in mortality, growth and condition of the Pacific Oyster, *Crassostrea gigas*, in Normandy (France). Journal of Shellfish Research. 26(4): 973-984.

Topić Popović N., Beer Ljubić B., Strunjak-Perović I., Babić S., Lorencin V., Jadan M., Čižmek L., Matulić D., Bojanić K., Čož-Rakovac R. (2020). Seasonal antioxidant and biochemicalproperties of the Northern Adriatic *Pecten jacobaeus*. PLoS ONE. 15(3): e0230539

Valli G. (1979.) Biometria e riproduzione in *Pecten jacobaeus* del Golf odi Trieste (Mare Adriatico). Bollettino della Società adriatica di scienze naturali in Trieste. 63: 121-139.

Voultisadou, E., Koutsoubas, D., Achparaki, M. (2009). Bivalve mollusc exploitation in Mediterranean coastal communities: an historical approach. J. Bil. Res. (Thessalon.). 12: 35-45.

O značajkama jakovljeve kapice *Pecten jacobaeus* (Linnaeus, 1758) u uvali Valdibora, Jadransko more

Sažetak

U ovom radu je opisana populacija jakovljeve kapice, *Pecten jacobaeus* u uvali Valdibora, uz zapadnu obalu Istre. Istraživana su morfometrijska svojstva, indeks mesa, indeks kondicije, gonadosomatski indeks, vertikalna raspodjela, gustoća, a zabilježeni su i organizmi s kojima dijeli životno područje. Istraživanje je trajalo od siječnja do kolovoza 2015. godine, dok se je dio istraživanja pomoću transekta proveo u ožujku i kolovozu 2015. godine. Dobiveni rezultati pokazali su da se veliki dio jakovljevih kapica vjerojatno izmrijesti u srpnju, dok je indeks mesa i indeks kondicije najveći u travnju i ožujku. Dubina na kojoj najčešće obitavaju je 11m, a gustoća populacije iznosi 0,012 jedinki m^{-2}.

Ključne riječi: morfometrija školjkaša, indeks kondicije, indeks mesa, gonadosomatski indeks

IZVORNI ZNANSTVENI RAD

Struktura i sezonska dinamika mrežnog zooplanktona na ušću rijeke Neretve (JI Jadransko more)

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Sažetak

Sezonska istraživanja mrežnog zooplanktona na ušću rijeke Neretve provedena su u siječnju, ožujku, srpnju i listopadu 2014. godine na dvije postaje tijekom četiri izlaska. Na obje postaje najveća ukupna brojnost zooplanktona bila je u listopadu (5433 jed. m^{-3} na postaji Ušće i 4058 jed. m^{-3} na postaji Osinj). Među veslonošcima, koji su bili dominantna skupina, najbrojniji su bili ciklopoidi, osim na postaji Ušće tijekom jeseni, kada su dominirali veći rašljoticalci te kalanoidni veslonošci. Dominantne vrste veslonožaca tijekom čitavog istraživanog razdoblja bile su: *Paracalanus parvus*, *Clausocalanus pergens*, *Oithona nana* i *Euterpina acutifrons*. Od ostalih zooplanktonskih skupina veću brojnost imale su ličinke školjkaša, i to na postaji Ušće u srpnju, kada je ova skupina dominirala u ukupnoj zajednici zooplanktona. Holoplankton dominira nad meroplanktonom tijekom čitavog istraživanog razdoblja. Taksonomski sastav zooplanktona uobičajen je za obalne vode južnog dijela istočnog Jadrana.

Ključne riječi: obalni morski ekosustavi, veslonošci, holoplankton, meroplankton, Sredozemno more

Uvod

Zooplankton ima važnu ulogu u morskim hranidbenim lancima jer je ključna karika u prijenosu energije dobivene primarnom produkcijom fitoplanktona na više trofičke razine. Akvatorij ušća rijeke Neretve, koji je karakteriziran unosom slatke vode rijeke Neretve s jedne strane i utjecajem otvorenog mora srednjeg Jadrana kroz Korčulanski i Neretvanski kanal s druge strane, može se smatrati vanjskim dijelom ekološki i gospodarski važnog Malostonskog zaljeva, što ovom području daje posebnu važnost (Viličić i sur., 1998.; Viličić, 2014.). Iako, s obzirom na hidrografske prilike i primarnu produkciju, šire područje Malostonskog zaljeva pripada najvišoj (četvrtoj) zoni produktivnosti u Jadranskom moru (Pucher-Petković i Zore-Armanda, 1973.), rezultati istraživanja zooplanktona od 1940-tih do 1980-tih pokazali su relativno siromašnu zooplanktonsku faunu (Gamulin, 1948.; Buljan i sur., 1973.; Benović, 1977.). Kasnijim istraživanjima obuhvaćene su pojedine veličinske frakcije zooplanktona (Kršinić i Mušin 1980.; Benović i Onofri, 1981.), kao i abundantnije skupine planktonskih organizama (Kršinić 1979.; Regner 1991.). Rezultati tih istraživanja ukazivali su na razliku u kvalitativno-kvantitativnom sastavu populacija i sezonskoj raspodjeli zooplanktona te istaknuli njegovu važnost u održavanju hranidbenih lanaca i usku povezanost s osjetljivim hidrografskim prilikama (Benović i Onofri, 1981.; Vidjak i sur., 2007.). Također, zabilježena je dominacija holoplanktona u odnosu na meroplankton, ali nije posvećena veća pozornost njihovom odnosu unutar zooplanktonske zajednice, koji je u obalnim morskim ekosustavima važan zbog trofičkih odnosa između razvojih stadija uglavnom bentoskih organizama i njihovih holoplanktonskih predatora. Stoga se ukazala

potreba da se u sezonskim istraživanjima zooplanktona detaljnije istraži zastupljenost ovih skupina.

Materijali i metode

Sakupljanje uzoraka na ušću rijeke Neretve obavljeno je na postaji Ušće (N 43°0'47,9''; E 17° 26' 51,8'') i na postaji Osinj (N 43°0'44,7''; E 17°27'45,3''), tijekom četiri sezonska terenska izlaska: 9. 1. 2014., 11. 3. 2014., 16. 7. 2014 i 21. 10. 2014. (Slika 1). Uzorci zooplanktona prikupljeni su standardnom planktonskom mrežom površine otvora 0,25 m^2 i gustoće tkanja 125 µm, horizontalnim potezima dužine 30 m. Osnovni hidrografski parametri, temperatura i slanost, mjereni su sondom WTW Cond 3110 SET 2. Prikupljeni materijal konzerviran je 4 % otopinom formaldehida. Analizirana je 1/16 ili 1/32 svakog uzorka, ali je zbog rjeđih vrsta uvijek pregledan cijeli uzorak. Kvanititativna i kvalitativna analiza sastava zooplanktona u uzorcima je obavljena uporabom stereomikroskopa Olympus SZX9. Sve kvantitativne vrijednosti izražene su kao broj jedinki po metru kubičnom, a dobivene su korištenjem formule za računanje gustoće mrežnog zooplanktona: $N = n * \frac{5*r}{d}$, gdje je *n* broj pronađenih jedinki u uzorku, *d* duljina poteza mrežom, a *r* razrjeđenje uzorka. Zooplankton je određen do različitih taksonomskih razina, od koljena do razreda, sukladno uobičajenom načinu prikaza strukture mrežnog zooplanktona, s izuzetkom podrazreda veslonožaca (Copepoda) koji su, zbog izrazite dominacije i važnosti u ukupnom zooplanktonu, određeni do razine vrste. Analiza rezultata i izrada grafova obavljena je uz pomoć računalnog programa Microsoft Office Excel.

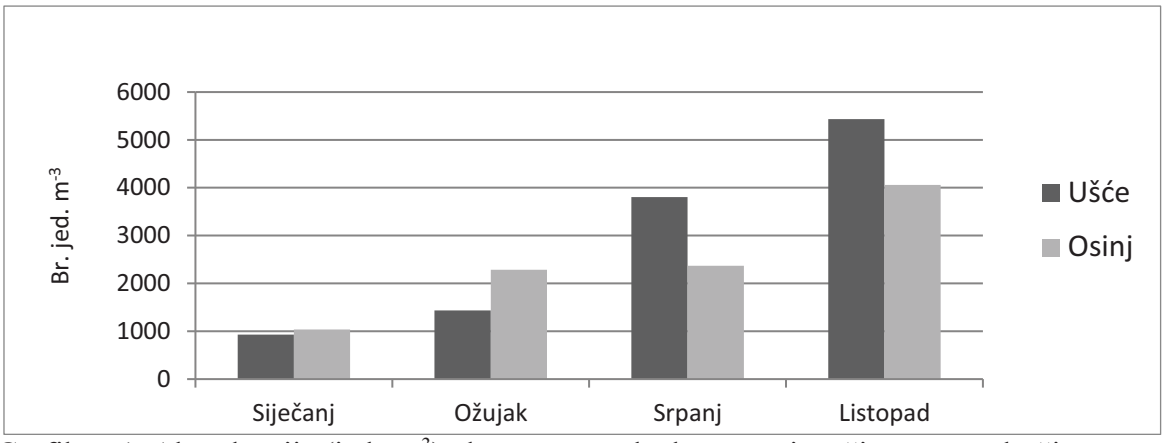
Slika 1. Područje istraživanja s postajama uzorkovanja

Rezultati i rasprava

Najniže površinske temperature mora tijekom istraživanog razdoblja zabilježene su zimi, s minimalnom vrijednosti od 13,4 ºC u siječnju 2014. na obje postaje. Najviše temperature zabilježene su tijekom ljetnog razdoblja, kada je u srpnju izmjereno 25,4 ºC na postaji Ušće, odnosno 24,5 ºC na postaji Osinj. U listopadu 2014. temperature su bile još uvijek znatno visoke, s vrijednostima od 20,8 ºC na postaji Ušće i 22,0 ºC na postaji Osinj.

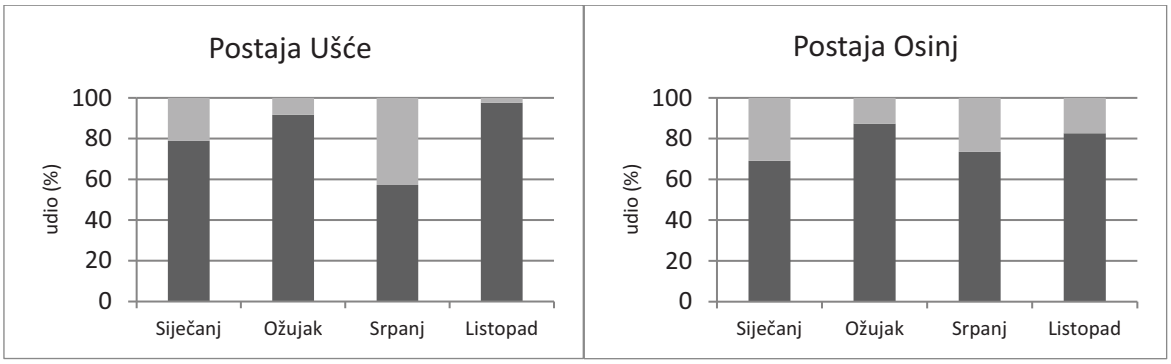
Varijacije slanosti na istraživanom području bile su izraženije između postaja, nego između sezonskih razdoblja. Maksimalna vrijednost slanosti od 35,1 ‰ zabilježena je u ožujku 2014. na postaji Ušće. Istovremeno, na postaji Osinj zabilježena je slanost od 26,5 ‰. Najmanja vrijednost slanosti od 18,9 ‰ nađena je na postaji Osinj u siječnju 2014., dok je na postoji Ušće iznosila 29,5 ‰. U ostalim sezonama vrijednosti slanosti su manje varirale, a u listopadu 2014. na obje postaje zabilježena je identična vrijednost slanosti od 31,4 ‰.

Tijekom četiri sezonska izlaska determinirane su sljedeće zooplanktonske skupine: Appendicularia, Chaetognatha, Cladocera, Copepoda, Ctenophora, Doliolidae, Hydromedusae, Ostracoda, Pteropoda, Siphonophorae, ličinke Bivalvia, Cirripedia, Decapoda, Echinodermata, Gastropoda, Phoronida, Polychaeta i Tunicata te riblja jaja.
Najveće vrijedosti abundancije ukupnog zooplanktona (Grafikon 1) zabilježene su u listopadu (5433 jed. m^{-3} na postaji Ušće i 4058 jed. m^{-3} na postaji Osinj), dok su najmanje vrijednosti na obje postaje nađene u siječnju (928 jed. m^{-3} na postaji Ušće i 1035 jed. m^{-3} na postaji Osinj). Primo i sur. (2009) u trogodišnjoj studiji zooplanktona na ušću rijeke Mondego u Portugalu, najveću abundanciju zooplanktona također pronalaze tijekom jeseni (1102 jed. m^{-3}), a najmanju zimi (21 jed. m^{-3}).
Veslonošci su tijekom cijelog istraživanog razdoblja, s izuzetkom srpnja, dominirali u uzorcima mrežnog zooplanktona, s prosječnim udjelom od 69,4 % u ukupnom zooplanktonu što je sukladno rezultatima istraživanja zooplanktona Neretvanskog kanala, prema kojima je prosječni udio ove skupine u ukupnom zooplanktonu veći od 60 % (Vidjak i sur. 2007.). Tijekom našeg istraživanja veslonošci su imali najniži udio u ukupnom zooplanktonu ljeti, 25,1 % na postaji Ušće i 55,3 % na postaji Osinj. Battuelo i sur. (2016) su u obalnim vodama sjeverozapadne Italije, koje su, kao i ušće rijeke Neretve, pod značajnim antropogenim utjecajima, najmanji udio veslonožaca od 56 % također pronašli tijekom ljetnog razdoblja. Najbrojniji red veslonožaca tijekom našeg istraživanja s prosječnim udjelom od 49 % bili su ciklopoidi, a slijede ih kalanoidi (31,5 %), poecilostomatoidi (14,4 %) te harpaktikoidi (5,2 %). Dominantne vrste veslonožaca tijekom istraživanog razdoblja bile su: *Paracalanus parvus*, *Clausocalanus pergens*, *Oithona nana* i *Euterpina acutifrons*.



Grafikon 1. Abundancija (jed. m^{-3}) ukupnog zooplanktona na istraživanom području

Najveća brojnost kladocera (272 jed. m^{-3}), uz dominaciju vrsta *Penillia avirostris* i *Evadne spinifera*, pronađena je tijekom jeseni na postaji Ušće Primo i sur. (2009.) najveću brojnost kladocera na ušću rijeke Mondego u Portugalu pronalaze također u jesenskom razdoblju (2432 jed. m^{-3}), s izrazitom dominacijom vrste *P. avirostris* (2297 jed. m^{-3}).
Meroplankton je tijekom čitavog istraživanog razdoblja bio manje abundantan od holoplanktona. Prosječni udio meroplanktona u ukupnom zooplanktonu tijekom cijelog istraživanja iznosio je 20,3 %, a kretao se od najnižih 2,4 %, koliko je zabilježeno na postaji Ušće tijekom jeseni, do najviših 42,8 %, koliko je zabilježeno na istoj postaji u ljetnom razdoblju (Grafikon 2). Lonsdale i Coull (1977.) su, također, uz istočnu obalu Južne Karoline pronašli najveću brojnost meroplanktonskih skupina ljeti, a kao razlog navode razmnožavanje mnogih bentoskih skupina tijekom kasnog proljeća i ranog ljeta.



Grafikon 2. Odnos holoplanktona (plavo) i meroplanktona (crveno) u ukupnoj populaciji zooplanktona na istraživanom području izražen u postotnim udjelima.

Među meroplanktontima najbrojniji su bili školjkaši, što se podudara s rezultatima koje bilježe Vidjak i sur. (2007.). Tijekom našeg istraživanja najveće abundancije školjkaša pronađene su ljeti, kada su na postaji Ušće bili dominantna skupina s brojnošću od 1354 jed. m^{-3} i udjelom od 35,7 % u ukupnom zooplanktonu. Ličinke poliheta bilježe tri, sezonski odvojena, maksimuma gustoće populacije (128 jed. m^{-3} na postaji Osinj u ranom proljeću, 91 jed. m^{-3} na postaji Ušće tijekom ljeta i 51 jed. m^{-3} na postaji Ušće tijekom zime). Lonsdale i Coull (1977.) su u North Inlet estuariju u Južnoj Karolini, također zabilježili tri sezonski odvojena maksimuma abundancije ličinki poliheta, kao posljedicu vremenski odvojenog mriješćenja barem tri različite vrste poliheta. Ličinke bodljikaša su na obje postaje u značajnijoj abundanciji pronađene samo u rano proljeće (67 jed. m^{-3} na postaji Ušće i 48 jed. m^{-3} na postaji Osinj). Lonsdale i Coull (1977.) u North Inlet estuariju u Južnoj Karolini ličinke bodljikaša gotovo isključivo pronalaze tijekom ljetnog razdoblja.
Prosječni udio ostalih zooplanktonskih skupina u ukupnom zooplanktonu tijekom cijelog istraživanog razdoblja nije prelazio udio od 1 %, što je u suglasju s rezultatima prethodnih istraživanja (Vidjak i sur. 2007.) u Neretvanskom kanalu.

Zaključci

Rezultati istraživanja strukture i sezonske dinamike zajednice mrežnog zooplanktona na ušću rijeke Neretve u skladu su s dosadašnjim spoznajama o kvalitativnom i kvantitativnom sastavu zooplanktona ovoga područja. Maksimalne vrijednosti abundancije ukupnog zooplanktona zabilježene su tijekom jesenskog razdoblja, dok su minimalne vrijednosti nađene zimi. U svim uzorcima prevladavaju holoplanktontske skupine s izrazitom dominacijom veslonožaca, među kojima su najbrojniji ciklopoidi. Meroplankton, osobito ličinke školjkaša, veću abundanciju u ukupnom zooplanktonu ima tijekom toplijih sezona, od proljeća do rane jeseni.

Literatura

Battuelo M., Brizio P., Mussat Sartor R., Nurra N., Pessani D., Abete M. C. (2016). Zooplankton from a North Western Mediterranean area as a model of metal transfer in a marine environment. Ecological Indicators. 66: 440–451.

Benović A. (1977). Biomasa mrežnog zooplanktona Jadranskog mora, s posebnim osvrtom na neka područja istočne obale. Disertacija. Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, pp. 88.

Benović A., Onofri V. (1981). Mrežni zooplankton Malostonskog zaljeva i Malog mora. U: Roglić J., Meštrov M. (ur.) Zbornik radova savjetovanja Malostonski zaljev prirodna podloga i društveno valoriziranje. JAZU. Znanstveni savjet za zaštitu prirode. Dubrovnik, 120-131.

Buljan M. (1964). Ocjena produktivnosti Jadrana dobivena na temelju njegovih hidrografskih svojstava. Acta Adriatica. 11: 35-45.
Gamulin T. (1948). Prilog poznavanju zooplanktona srednjedalmatinskog otočnog područja. Acta Adriatica. 15: 159-194.
Kršinić F. (1979). The Tintinnids (Ciliata) from the coastal waters of the Southern Adriatic in the year 1975/76. Nova Thalassia. 3: 199-211.
Kršinić F., Mušin D. (1980). Mikrozooplankton Malostonskog zaljeva i Malog mora; Malostonski zaljev – prirodna podloga i društveno valoriziranje, 108-119.
Lonsdale D. J., Coull B. C. (1977). Composition and seasonality of zooplankton of North Inlet, South Carolina. Chesapeake Science. 18(3): 272–283.
Primo A. L., Azeitero U. M., Marques S. C., Martinho F., Pardal, M. A. (2009). Changes in zooplankton diversity and distribution pattern under varying precipitation regimes in a southern temperate estuary. Estuarine, Coastal and Shelf Science. 82: 341–347.
Pucher-Petković T., Zore-Armanda N. (1973). Essai d'evaluation et prognostic de la production en fonction des facteurs du milieu dans l'Adriatique. Acta Adriatica. 15: 1-37.
Regner D. (1991). Long-term investigations of copepods (zooplankton) in the coastal waters of the eastern middle Adriatic. Acta Adriatica. 32: 731-740.
Vidjak O., Bojanić N., Kušpilić G., Ninčević Gladan Ž., Tičina V. (2007). Zooplankton community and hydrographical properties of the Neretva Channel (eastern Adriatic Sea). Helgoland Marine Research. 61: 267–282.
Viličić D., Jasprica N., Carić M., Burić Z. (1998). Taxonomic composition and seasonal distribution of microphytoplankton in Mali Ston Bay (eastern Adriatic). Acta Botanica Croatica. 57: 29 – 48.
Viličić D. (2014). Specifična oceanološka svojstva hrvatskog dijela Jadrana. Hrvatske vode: časopis za vodno gospodarstvo. 90: 297-314.

Structure and seasonal dynamics of net zooplankton at the mouth of the Neretva river (SE Adriatic Sea)

Abstract
Net zooplankton were investigated at two stations located in the Estuary of the Neretva River during four seasonal field work done in January, March, July, and October 2014. Samples were collected by horizontal hauls of standard plankton net with an opening area of 0.25 m^2 and mesh size of 125 µm. The highest densities of the total zooplankton population at both stations were recorded in October (5433 ind. m^{-3} at Ušće station and 4058 ind. m^{-3} at Osinj station). Among copepods, the most dominant zooplankton group, cyclopoids were the most numerous order, except at station Ušće in the autumn period, when large cladocerans and calanoids dominated. The dominant species among copepods were *Paracalanus parvus, Clausocalanus pergens, Oithona nana and Euterpina acutifrons*. The highest quantity of shellfish larvae was found at station Ušće in July, when they dominated the total zooplankton community. The dominance of holoplankton over meroplankton was determined throughout the study period. The taxonomic composition of zooplankton is common in the coastal waters of the southern part of the eastern Adriatic.

Key words: coastal marine ecosystems, copepods, holoplankton, meroplankton, Mediterranean Sea

IZVORNI ZNANSTVENI RAD

Fizikalno-kemijska svojstva meda od žljezdastog nedirka (*Impatiens glandulifera* Royle)

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Sažetak
Žljezdasti nedirak *(Impatiens glandulifera* Royle*)* invazivna je biljna vrsta koja u vrijeme cvatnje izlučuje značajne količine nektara te na taj način omogućava proizvodnju uniflornog meda. Cilj je ovoga rada odrediti fizikalno-kemijska svojstva meda od žljezdastog nedirka. Istraživanje je provedeno na pet uzoraka meda prikupljenog 2020. godine s područja Međimurske županije. Prosječna vrijednost električne provodnosti iznosila je 0,34 mS/cm, HMF-a 15,37 mg/kg, vrijednost dijastaze 40,38 DN, a ph 4,11. Na osnovu boje ovaj med pripada u skupinu svjetlijih vrsta (ekstra svjetlo jantarna) s 41,0 mm Pfunda. Ovo preliminarno istraživanje prikazuje vrijednosti odabranih fizikalno-kemijskih parametera meda od žljezdastog nedirka, međutim potrebna su daljnja istraživanja koristeći suvremene analitičke metode kako bi se dobio potpuni fizikalno-kemijski profil ove vrste meda.

Ključne riječi: med, žljezdasti nedirak, fizikalno-kemijska svojstva

Uvod

Med je jedinstveni prirodni proizvod složene nutritivne vrijednosti. Fizikalno-kemijska i senzorna svojstva meda (nektarni med ili medljikovac) koriste se za određivanje njegove kvalitete, a na njih uvelike utječu vrsta nektara, tip tla, klimatski uvjeti i postupci prije i nakon vrcanja meda (Persano Oddo i sur., 1995.; Gomes i sur., 2010.). Identifikacija fizikalno-kemijskih svojstava meda i kontrola njegove kvalitete ključna je za određivanje njegove prikladnosti za preradu, osiguravanje autentičnosti i povećanje proizvodnih kapaciteta pčelara kako bi se zadovoljila potražnja na domaćem i međunarodnom tržištu (Jones i sur., 2011.). Iako u Europi postoji više od 100 medonosnih biljaka koje bi mogle proizvesti dovoljne količine nektara za proizvodnju specifičnih uniflornih medova (Persano Oddo i sur., 2004.), dosada je samo mali broj njih opisan kao uniflorni. Korištenje alohtonih ukrasnih biljnih vrsta u vrtovima i parkovima utječe na lakše širenje invazivnih vrsta i na taj način omogućuje nastanak populacija koje mogu suzbiti lokalnu floru, a time ujedno ugrožavaju i raznolikost staništa (Horvat i Franjić, 2016.). Žljezdasti nedirak (*Impatiens glandulifera* Royle) je visoka jednogodišnja invazivna biljna vrsta iz obitelji Balsaminaceae, porijeklom sa zapadne Himalaje (Pakistan, Indija). U Europu je uveden kao vrtna ukrasna biljka (Beering i sur., 1993.), ali se snažno proširio i danas se smatra invazivnom vrstom u 23 europske zemlje (Maleš i sur., 2020.). Prvi zapis o žljezdastom nedirku u Hrvatskoj pojavio se krajem 1960-ih i početkom 1970-ih. Iako je zabilježen na više lokacija u Hrvatskoj, danas je najrašireniji na sjeveru zemlje uz obale rijeke Mure na području Međimurske županije i značajan je izvor nektara (19 µL/24 h s 48-53 % šećera) u kasno ljeto (Titze, 2000.; Chittka i Schürkens, 2001.). Ima velike cvatove s ljubičastim, 3-4 cm dugim, zigomorfnim cvjetovima koji cvatu u kolovozu i rujnu (Slika 1a). Kasna cvatnja i značajne količine nektara i peluda privlače pčele u velikom broju (Kortesniemi i sur., 2016.). Iako je

ova biljna vrsta široko rasprostranjena u Europi, uglavnom u obalnom pojasu i svoje stanište pronalazi u blizini rijeka i manjih vodotokova (Hawkins i sur., 2015.), još uvijek nema dostupnih znanstvenih podataka o ovoj specifičnoj vrsti meda. Stoga je cilj ovog preliminarnog istraživanja bio utvrditi neka fiziklno-kemijska svojstva meda od žljezdastog nedirka i na taj način dobiti saznanja o ovoj još neopisanoj vrsti.

Materijal i metode

Ukupno je analizirano pet uzoraka meda od žljezdastog nedirka (Slika 1b). Uzorci meda prikupljeni su tijekom kolovoza 2020. godine od lokalnih pčelara s područja Međimuske županije čije su pčelinje zajednice (*Apis mellifera carnica* Pollman, 1879) bile smještene uz rijeku Muru. Uzorci su bili pohranjeni u staklenkama i držani na tamnom mjestu na 4°C do daljnjih analiza. Određivanjem botaničkog podrijetla analiziranih uzoraka meda prema metodi Von der Ohe (2004) potvrđena je njegova uniflornost.

Slika 1. a) Žljezdasti nedirak *(I. glandulifera* Royle*);* b) med od žljezdastog nedirka

Fizikalno-kemijski parametri određeni su u skladu s Pravilnikom o kakvoći uniflornog meda (2009.) i Pravilnikom o medu (2015.). Sadržaj vlage određen je refraktometrijskom metodom uz pomoć prijenosnog refraktometra Mettler Toledo (Refracto 30 PX) na 20°C, dok je električna provodnost utvrđena uz pomoć prijenosnog konduktometra Mettler Toledo (EL3) u 20% vodenoj otopini meda na 20°C. Sadržaj hidroksimetilfurfurala (HMF) određen je metodom po Whiteu, a rezultati su izraženi u mg/kg meda, dok je aktivnost dijastaze meda određena pomoću Phadebas testa (amilaza) i izražena je kao dijastazni broj (DN). Obje metode provedene su pomoću Shimadzu (Japan) spektrofotometra (UV-1800). pH vrijednost određena je titracijskom metodom pomoću Mettler Toledo pH metra (MP 220). Za određivanje boje meda korišten je Lovibond Honey Color-Pod, a rezultati su izraženi u mm Pfund ljestvice.

Rezultati i rasprava

Rezultati utvrđenih fizikalno-kemijskih svojstava meda od žljezdastog nedirka prikazani su u Tablici 1. Sadržaj vode kretao se od 16,00 % do maksimalnih 17,20 %, a u prosjeku je iznosio 16,50 %. Ovaj parametar u medu uvelike ovisi o tehnologiji pčelarenja i vremenu vrcanja meda. S druge strane, sadržaj vode može se razlikovati između meda ovisno o izvoru nektara ili vremenskim uvjetima na području u kojem se med proizvodi. Stoga se vrijednosti ovog parametra mogu mijenjati iz godine u godinu (Majewska i sur., 2019.). Električna provodnost je fizikalno-kemijski parametar koji je u korelaciji s botaničkim podrijetlom meda i koristi se za identifikaciju meda u kombinaciji s melisopalinološkom analizom. Električna provodnost meda izravno je povezana sa sadržajem njegovih minerala i organskih kiselina (Habib i sur., 2014.; DaSilva i sur., 2016.) i koristi se za razlikovanje nektarnog

meda od medljikovca te za klasifikaciju uniflornog meda. Prema Pravilniku o kakvoći uniflornog meda (2009.) i Pravilniku o medu (2015.) maksimalna granična vrijednost električne provodnosti za medove nektarnog podrijetla je 0,80 mS/cm. Prosječna vrijednost električne provodnosti meda od žljezdastog nedirka iznosila je 0,34 mS/cm (raspon 0,27 mS/cm - 0,45 mS/cm). Ovaj med pripada skupini medova s nižom vrijednosti električne provodnosti, kao što su med od amorfe, kadulje, uljane repice, suncokreta i vriska (Szczesna i sur., 2011.; Svečnjak i sur., 2015.; Juan-Borras i sur., 2017.).

Tablica 1. Deskriptivna statistika fizikalno-kemijskih svojstava meda od žljezdastog nedirka

Uzorak	Voda %	El.provodnost mS/cm	HMF mg/kg	Dijastaza DN	pH	Boja mm Pfund
1	16,70	0,28	17,44	26,80	3,98	36,0
2	16,30	0,40	8,08	43,20	4,24	33,0
3	16,30	0,27	25,97	36,20	4,01	63,5
4	16,00	0,45	2,40	43,60	4,42	32,0
5	17,20	0,28	22,98	52,10	3,91	40,5
Prosjek	**16,50**	**0,34**	**15,37**	**40,38**	**4,11**	**41,0**
Min.	**16,00**	**0,27**	**2,40**	**26,80**	**3,91**	**32,0**
Max.	**17,20**	**0,45**	**25,97**	**52,10**	**4,42**	**63,5**
SD	**0,46**	**0,08**	**9,95**	**9,45**	**0,21**	**13,0**

*SD -standardna devijacija

Hidroksimetilfurfural (HMF) i aktivnost dijastaze jedan su od najvažnijih parametara za određivanje svježine meda (Ruoff i sur., 2007.; Zhu i sur., 2020.), kao i trajanja i uvjeta skladištenja, te imaju tendenciju rasta tijekom procesa zagrijavanja (dekristalizacije) meda i/ili njegovim dugotrajnim skladištenjem. Prosječni sadržaj HMF-a kod meda od žljezdastog nedirka iznosio je 15,37 mg/kg, s rasponom od 2,40 mg/kg do 25,97 mg/kg. Aktivnost dijastaze u prosjeku je iznosila 40,38 DN, s minimalnom vrijednošću od 26,8 DN i maksimalnom vrijednošću od 52,1 DN. pH vrijednost meda je također u korelaciji s njegovim skladištenjem i rastom mikroorganizama koji mogu promijeniti teksturu i stabilnost meda. U ispitivanim uzorcima meda pH vrijednosti kretale su se od 3,91 do 4,42, s prosjekom od 4,11. Prema odgovarajućim vrijednostima Pfundove ljestvice (mm) med se prema svojoj boji može razvrstati u nekoliko skupina: < 9 mm vodeno bijeli, 9–17 mm ekstra bijeli, bijeli 18–34 mm, ekstra svijetlo jantarni 35–50 mm , svijetlo jantarni 51–85 mm, jantarni 86–114 mm i tamno jantarni >114 mm (Pontis et al., 2014.). Prema boji med od žljezdastog nedirka klasificiran je kao ekstra svijetlo jantarni (41,0 mm), s rasponom od 32,0 mm do 63,5 mm te u tom slučaju (>50 mm) može se klasificirati i kao svijetlo jantarni.

Zaključak

U ovom su radu po prvi puta prikazani rezultati fizikalno-kemijskih svojstava meda od žljezdastog nedirka (*I. glandulifera*), invazivne biljne vrste koja ima veliki medonosni potencijal, a koji se očituje kroz mogućnost dobivanja uniflornog meda. Ova vrsta meda ima prosječnu električnu provodnost od 0,34 mS/cm, ph 4,11, a prema boji se svrstava u skupinu svjetlijih vrsta meda (ekstra svjetlo jantarni do svjetlo jantarni) s prosječno 41,0 mm Pfunda. Prosječna vrijednost HMF-a iznosila je 15,37 mg/kg, a vrijednost dijastaze 40,38 DN. Budući da ovaj rad prikazuje preliminarne podatke o fizikalno-kemijskim svojstvima meda od žljezdastog nedirka potrebna su daljnja istraživanja s ciljem određivanja botaničkog podrijetla (peludnog spektra) te ostalih fizikalno-kemijskih svojstava uz pomoć suvremenih analitičkih metoda (HPLC, GC-MS, FTIR-ATR i sl.) kako bi se dobio potpuni kemijski profil ove raritetne vrste meda.

Napomena
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Literatura

Beerling D.J. (1993). The impact of temperature on the northern distribution limits of the introduced species *Fallopia japonica* and *Impatiens glandulifera* in north-west Europe. Journal of Biogeography. 20: 45-53.

Chittka L., Schürkens S. (2001). Successful invasion of a floral market. Nature. 411: 653.

Da Silva P.M., Gauche C., Gonzaga L.V., Costa A.C.O., Fett R. (2016). Honey: Chemical composition, stability and authenticity. Food Chemistry. 196: 309-323.

Gomes S., Dias L.G., Moreira L.L., Rodrigues P., Estevinho L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association. 48 (2): 544–548.

Habib H.M., Al Meqbali F.T., Kamal H., Souka U.D., Ibrahim W.H. (2014). Physicochemical and biochemical properties of honeys from arid region. Food Chemistry. 153: 35-43.

Hawkins J., de Vere N., Griffith A., Ford C.R., Allainguillaume J., Hegarty M.J. (2015). Using DNA Metabarcoding to Identify the Floral Composition of Honey: A New Tool for Investigating Honey Bee Foraging Preferences. PLoS ONE. 10 (8): e0134735.

Horvat G., Franjić J., (2016). Invazivne biljke Kalničkih šuma. Šumarski list, 1-2: 53-64.

Jones S., Jones H., Thrasyvoulou A. (2011). Disseminating research about bee products. A review of articles published in the Journal of Apicultural Research over the past fifty years. Journal of ApiProduct & ApiMedical Science. 3: 105-116.

Juan-Borrás M., Soto J., Gil-Sánchez L., Pascual-Maté A., Escriche I. (2017). Antioxidant activity and physico-chemical parameters for the differentiation of honey using a potentiometric electronic tongue. Journal of the Science of Food and Agriculture. 97: 2215-2222.

Kortesniemi M., Slupsky C.M., Ollikka T., Kauko L., Spevacek A.R., Sjövall O., Yang B., Kallio, H. (2016). NMR profiling clarifies the characterization of Finnish honeys of different botanical origins. Food Research International. 86: 83-92.

Majewska E., Drużyńska B., Wołosiak R. (2019). Determination of the botanical origin of honeybee honeys based on the analysis of their selected physicochemical parameters coupled with chemometric assays. Food Science Biotechnolgy. 28: 1307-1314.

Maleš Ž., Duka I., Bojić M., Vilović T., Mitić B., Hruševar D. (2020). Determination of phenolics and antioxidative activity of two invasive species of the genus Impatiens L. Farmaceutski glasnik. 76 (1): 1-8.

Ministarstvo poljoprivrede, ribarstva i ruralnog razvoja (2009). Pravilnik o kakvoći uniflornog meda. Narodne novine. 122: 15-16.

Ministarstvo poljoprivrede (2015). Pravilnik o medu. Narodne novine. 30: 3–5.

Persano Oddo L., Piazza M.G., Sabatini A.G., Accorti M. (1995). Characterization of unifloral honeys. Apidologie. 26: 453-465.

Persano Oddo L., Piana L., Bogdanov S., Bentabol A., Gotsiou P., Kerkvliet J., Martin P., Morlot M, Ortiz V.A, Ruoff K., Von der Ohe K. (2004). Botanical species giving unifloral honey in Europe. Apidologie. 35: S82–S93.

Pontis J.A., Costa L.A.M.A., Silva S.J.R.D., Flach A. (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. Food Science Technology. 34: 69-73.

Ruoff K., Luginbühl W., Bogdanov S., Bosset J.O., Estermann B., Ziolko T., Kheradmandan S., Amad R. (2007). Quantitative determination of physical and chemical measurands in honey by near-infrared spectrometry. European Food Research and Technology. 225: 415-423.

Szczesna T., Rybak-Chmielewska H., Waś E., Kachaniuk K., Teper D. (2011). Characteristics of Polish unifloral honeys. I. Rape honey (*Brassica napus* L. Var. oleifera Metzger). Journal of apicultural science. 55 (1): 111-119.

Svečnjak L., Bubalo D., Baranović G., Novosel H. (2015). Optimization of FTIR-ATR spectroscopy for botanical authentication of unifloral honey types and melissopalynological data prediction. European Food Research and Technology. 240 (6): 1101-1115.

Titze A. (2000). The efficiency of insect pollination of the neophyte *Impatiens glandulifera* (Balsaminaceae). Nordic Journal of Botany. 20: 33-42.

Zhu M., Zhao H., Wang Q., Wu F., Cao W. (2020). A Novel Chinese Honey from *Amorpha fruticosa* L.: Nutritional Composition and Antioxidant Capacity In Vitro. Molecules. 25: molecules25215211.

Physico-chemical properties of Himalayan balsam honey (*Impatiens glandulifera* Royle)

Abstract

The aim of this study was to determine the physico-chemical properties of Himalayan balsam honey (*Impatiens glandulifera* Royle). The research was conducted on five honey samples collected in 2020 from the area of Međimurje County. The average value of electrical conductivity was 0.34 mS/cm, HMF 15.37 mg/kg, diastase value 40.38 DN, and ph 4.11. According to the color, this honey type belongs to the group of lighter types (extra light amber) with 41.0 mm Pfund. This preliminary study represents the data on selected physico-chemical properties of Himalayan balsam honey, but further analyses using modern analytical methods are required in order to obtain a complete physico-chemical profile of this honey type.

Key words: honey, Himalayan balsam, physico-chemical properties

IZVORNI ZNANSTVENI RAD

Utjecaj sezonskih promjena na zastupljenost u koćarskom ulovu i indeks kondicije oslića, *Merluccius merluccius*, trlje, *Mullus barbatus* i arbuna, *Pagellus erythrinus*

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Sažetak
U radu su istraženi masena zastupljenost tri gospodarski važne vrste riba u demerzalnom ribolovu na istočnoj obali Jadrana, oslića, *Merluccius merluccius* Linnaeus, 1758, trlje, *Mullus barbatus* Linnaeus, 1758 i arbuna, *Pagellus erythrinus* Linnaeus, 1758 te sezonske varijacije njihovog indeksa kondicije na području ribolovne zone G5. Masena zastupljenost trlje i oslića u koćarskim lovinama tijekom 2021. godine bila je između 23 i 35 %, a arbuna između 2 i 9 % ovisno o sezoni. Vrijednosti indeksa kondicije za *M. merluccius* i *P. erythrinus* korespondiraju sa sezonom mrijesta, dok za *M. barbatus* to nije slučaj. S obzirom na komercijalni značaj ove vrste potrebna su daljnja istraživanja kako bi se objasnila ova pojava.

Ključne riječi: *Merluccius merluccius*, *Mullus barbatus*, *Pagellus erythrinus*, indeks kondicije, sezonske promjene

Uvod

Gospodarski najvažnije vrste riba u ribolovu pridnenom povlačnom mrežom koćom na istočnoj obali Jadrana su oslić, *Merluccius merluccius*, trlja, *Mullus barbatus* i arbun, *Pagellus erythrinus* (Sabatella i sur., 2017.; FAO, 2009.). Utjecaji tehničkih čimbenika na ribolov opširno su opisani dok se manje spominje važnost okolišnih čimbenika. Njihovi učinci često su povezani s međusobnom interakcijom više abiotičkih (temperatura, otopljeni kisik, zamućenost, koncentracija hranjivih tvari i dr.) i biotičkih čimbenika koji se značajno mijenjaju tijekom sezona te utječu ne samo na kondiciju ribe, već i na njihovu zastupljenost u ulovu (Rahman i sur., 2021.). Koeficijentom kondicije izražava se opće stanje ili stupanj dobrobiti ribe. Njegove godišnje varijacije pri optimalnim ambijentalnim uvjetima prvenstveno odražavaju stanje spolne zrelosti i stupanj uhranjenosti (Williams, 2000.). Cilj ovog rada bio je utvrditi utjecaj sezonskih promjena na varijacije indeksa kondicije oslića, trlje i arbuna i na njihovu zastupljenost u koćarskom ulovu.

Materijal i metode

Istraživano područje obuhvatilo je ribolovnu podzonu G5 koja se proteže se od točke Sućuraj na otoku Hvaru do uvale Vira na kopnu i nastavlja se kopnom prema jugoistoku do rta Višnjica pa ravnom crtom do uvale Crkvice na poluiotoku Pelješcu. Prema zapadu se proteže obalom Pelješca do rta Lovišće i nastavlja do uvale Veprinova na otoku Hvaru, a od te točke granica ide do početne točke na rtu Sućuraj.

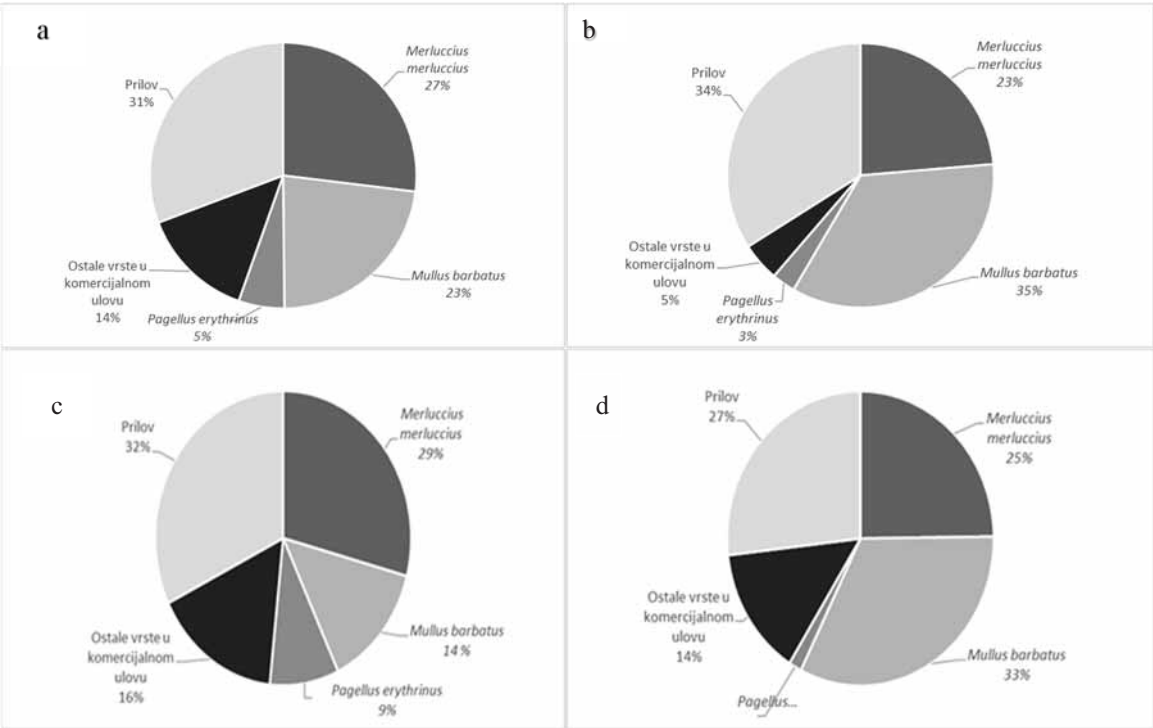
Istraživanje je provedeno povlačnom mrežom koćom u razdoblju od siječnja do studenog 2021. godine, u tri sezone: zima, proljeće i jesen. S obzirom da ribolov koćom nije dozvoljen u cjelokupnom ljetnom razdoblju na ovom području, ta sezona je izostavljena iz uzorkovanja. Nakon svakog podizanja koće izvagan je ukupni ulov, razdvojene su različite riblje vrste i posebno su izvagane te odvojene komercijalne vrste od prilova koji se sastojao od tržišno bezvrijednih vrsta, komercijalnih vrsta nedovoljne mase i oštećenih riba. Pri svakom uzorkovanju je izdvojeno po 20 jedinki oslića, trlje i arbuna, kojima je uz pomoć ihtiometra s preciznošću od 1 mm izmjerena je ukupna dužina (TL), dok je masa jedinki (W) izmjerena vagom s preciznošću od 0,001 g. Na osnovi ukupne dužine i mase jedinke, za sve tri istaživane vsrte izračunat je Fultonov faktor kondicije (CF) po formuli (Ricker, 1975.):

$$CF=\frac{W}{TL^3}*100$$

gdje W masa, a TL ukupna dužina ribe. Za obradu podataka korišten je Microsoft Excel (2016).

Rezultati i rasprava

Tijekom cijelog istraživanog razdoblja na komercijalne vrste riba u ulovu je otpadalo 69 % mase, a na prilov 31% (Slika 1a). U komercijalnom ulovu utvrđeno je 35 vrsta, pri čemu je najzastupljeniji bio oslić s masenim udjelom od 27 %, potom trlja s 23 % i arbun s 5 %. Na ostale komercijalne vrste otpadalo je 14 %. Pri tome su najveći maseni udio imale hrskavičnjače s mekušem, *Mustelus mustelus* i mačkom mrkuljom, *Scyliorhinus stellaris*, kao najzastupljenijim vrstama s masenim udjelom od po 2 %.



Slika 1. Zastupljenost istraživanih vrsta *M. merluccius, M, barbatus* i *P. erithrinus* te postotna zastupljenost komercijalnih vrsta riba i prilova u koćarskom ulovu tijekom cijelog perioda istraživanja (a), zimi (b), u proljeće (c) i jesen (d) u području ribolovne podzone G5, 2021.

Na slikaci 1b, c i d prikazana je zastupljenost istraživanih vrsta, ostalih komercijalno značajnih vrsta te prilova u zimskoj (b), proljetnoj (c) i jesenskoj (d) sezoni. Vidljivo je da se zastupljenost pojedinih vrsta u ulovu mijenjala tijekom različitih sezona. Tijekom zime trlja je bila najzastupljenija, dok je tijekom proljeća njen maseni udio u lovinama opadao, da bi u jesen ponovo narastao. Zastupljenost oslića bila je relativno stabilna u svim istraživanim sezonama krećući se između 23 i 29%. Arbun je od tri istraživane vrste bio najmanje zastupljen u lovinama s najvećom vrijednosti od 9% u proljeće. Zastupljenost prilova, koji sadrži nekomercijalni ulov morskih organizama bila je relativno stalna kroz sve tri sezone i kretala se od 27-34%.

Masa ulovljenih jedinki *M. merluccius* u cijelom periodu istraživanja kretala se od 31,8±9,61 do 150,5±53,15 g. Ukupna dužina svih jedinki ove vrste kretala se od 14,6 do 33,8 cm (Tablica 1). Prosječne mjesečne vrijednosti ukupne dužine i mase bile su najveće tijekom zime, a najmanje u jesen.

CF svih jedinki oslića kretao se u rasponu od 0,47 do 1,15 g/cm³. Najveća prosječna vrijednost CF *M. merluccius* zabilježena je tijekom zime, a najmanja tijekom jeseni (Tablica 1).

Tablica 1. Ukupna dužina, masa i Fultonov faktor kondicije CF (minimum, maksimum, srednja vrijednost (SV) i standardna devijacija (SD)) za *M. merluccius* po sezonama

Sezona	**N**	**Ukupna dužina TL (cm)**			**Masa (g)**			**CF (g/cm³)**		
		Min	Max	SV ± SD	Min	Max	SV ± SD	Min	Max	SV ± SD
Zima	20	22,9	33,8	26,4±3,14	90	330	150,5±53,15	0,55	0,94	0,8±0,11
Proljeće	20	20,3	29,8	25,5±12,89	70	210	140±0,15	0,47	1,15	0,77±0,15
Jesen	20	14,6	20,3	17,28±1,58	18	49	31,8±9,61	0,49	0,69	0,6±0,05

Jedinke *M. barbatus* izmjerene tijekom jeseni imale su najmanju prosječnu masu od 68,9±19,82 g, a u proljeće je zabilježena najveća prosječna masa koja je iznosila 100±29,74 g (Tablica 2). Najmanja prosječna vrijednost ukupne dužine ulovljenih jedinki izmjerena je tijekom zime, dok su tijekom jeseni i proljeća izmjerene vrijednosti bile nešto više i bez većih razlika. Vrijednost CF svih jedinki M. barbatus kretao se u rasponu od 0,79 do 3,04 g/cm³, a zabilježene vrijednosti po sezonama prikazane su u tablici 2.

Tablica 2. Ukupna dužina, masa i Fultonov faktor kondicije CF (minimum, maksimum, srednja vrijednost (SV) i standardna devijacija (SD)) za *M. barbatus* po sezonama

Sezona	**N**	**Ukupna dužina TL (cm)**			**Masa (g)**			**CF (g/cm³)**		
		Min	Max	SV ± SD	Min	Max	SV ± SD	Min	Max	SV ± SD
Zima	20	15,6	20,8	17,57±1,29	60	180	86,4 ±28,50	1,04	3,04	1,62±0,55
Proljeće	20	16	24,1	19,65±1,97	60	170	100±29,74	1,09	1,46	1,29±0,11
Jesen	20	15,2	21,6	19,01±1,62	32	107	68,9±19,82	0,79	1,19	0,97±0,09

Prosječna vrijednost ukupne mase *P. erythrinus* bila je najveća tijekom proljeća i iznosila je 118±25,61 g, dok je tijekom jeseni bila najmanja i iznosila je 80,1±17,44 g. Ukupna dužina svih jedinki kretala se u rasponu od 15,4 do 22,1 cm, a zabilježene vrijednosti ukupnih dužina po sezonama prikazane su u tablici 3.

Tablica 3. Ukupna dužina, masa i Fultonov faktor kondicije CF (minimum, maksimum, srednja vrijednost (SV) i standardna devijacija (SD)) za *P. erythrinus* po sezonama

Sezona	N	Ukupna dužina TL (cm)			Masa (g)			CF(g/cm³)		
		Min	Max	SV ± SD	Min	Max	SV ± SD	Min	Max	SV ± SD
Zima	20	15,4	22,1	18,55±1,72	55	120	82,5±21,24	1,00	1,51	1,28±0,15
Proljeće	20	17,1	22	19,63±9,88	70	150	118±25,61	1,18	1,95	1,44±0,20
Jesen	20	15,7	21,2	19,52±1,63	44	103	80,1±17,44	0,96	1,21	1,06±0,05

CF svih jedinki P. erythrinus kretao se u rasponu od 0,96 do 1,95 g/cm³. Indeks kondicije bio je najmanji tijekom jeseni, a najveći tijekom proljeća (Tablica 3).
Indeks kondicije može varirati ovisno o vrsti, spolu, dostupnosti hrane, temperaturi, onečišćenju te drugim biotičkim i abiotičkim čimbenicima, ali isto tako može varirati zbog porasta gonadosomatskog i hepatosomatskog indeksa (Ricker, 1975.; Chapman 2009.). Sezonske promjene kao što su temperatura, dostupnost hrane i kvaliteta staništa mogu utjecati na period mrijesta ribe, ali isto tako mogu utjecati na indeks kondicije (Chapman 2009.). U ovom istraživanju CF kod *M. merluccius* i *P. erythrinus* bio je najveći u vrijeme mrijesta. Prema dostupnoj literaturi *M. merluccius* se u Jadranu mrijesti od prosinca do veljače (Zorica i sur., 2021.), a *P. erythrinus* u proljeće i početkom ljeta (Jardas, 1996.). Najveći raspon u vrijednostima CF može se uočiti kod *M. barbatus*. Vrijednost indeksa kondicije ne poklapa se u ovom istraživanju s periodom mrijesta *M. barbatus*, najvjerojatnije jer se ženke u jugozapadnom Jadranu mrijeste od travnja do svibnja, dok se spolno zreli mužjaci nalaze tijekom cijele godine (Carbonara i sur., 2015.). Prema nalazima Sieli i sur. (2011.) na sjeverozapadnom Sredozemlju ženke trlje mrijeste od travnja do rujna s vrhuncem u svibnju, a mužjaci pokazuju vrhunac spolne zrelosti u svibnju.

Zaključak

Zastupljenost trlje i oslića u koćarskim lovinama u ribolovnoj podzoni G5 tijekom svih sezona uzorkovanja zadržala se na visokih 23-35 %, za razliku od značajno manje zastupljenosti arbuna. Vrijednosti indeksa kondicije za oslića i arbuna korespondiraju sa sezonom mrijesta, dok za trlju to nije slučaj. S obzirom da se radi o jednoj od komercijalno najvažnijih demerzalnih vrsta potrebno je detaljnije istražiti godišnje varijacije indeksa kondicije u ovisnosti o biotičkim i abiotičkim čimbenicima i njihovu povezanost sa sezonom mrijesta.

Napomena

Istraživanje je provedeno u sklopu Projekata "Ribarsko-znanstvena mreža Grada Ploča" u sklopu Mjere I.3., "Partnerstvo između znanstvenika i ribara za razdoblje 2020.-2022." i „Partnerstvo između znanstvenika i ribara u Općini Gradac" u sklopu Mjere I.3.

Literatura

Carbonara P., Intini S., Modugno E., Maradonna F., Spedicato M. T., Lembo G., Carnevali O. (2015). Reproductive biology characteristics of red mullet (*Mullus barbatus* L., 1758) in Southern Adriatic Sea and management implications. Aquatic Living Resources. 28(1): 21-31.

Chapman E. (2009). Climate change and Fish populations. www.seagrant.unh.edu

FAO Fish. Stat (2009). Capture production. Pristupljeno 24.1.2022.

Jardas I. (1996). Jadranska ihtiofauna. Školska knjiga d.d. Zagreb, str. 543.

Rahman M. M., Fathi A., Broadhurst M. K. (2021). Environmental variables affecting the gillnet catches and condition of Labiobarbus festivus and Osteochilus hasseltii in northern Malaysia. Aquaculture and Fisheries. https://doi.org/10.1016/j.aaf.2021.05.004.

Ricker W. E. (1975). Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada. 191:1-382.

Sabatella E.C., Colloca F., Coppola G., Fiorentino F., Gambino M., Malvarosa L., Sabatella R. (2017). Key Economic Characteristics of Italian Trawl Fisheries and Management Challenges. Frontiers Marine Science. 4:371.

Sieli G., Badalucco C., Di Stefano G., Rizzo P., D'Anna G., Fiorentino F. (2011). Biology of red mullet, *Mullus barbatus* (L. 1758), in the Gulf of Castellammare (NW Sicily, Mediterrane) subject to a trawling ban. Journal of Applied Ichthyology. 27(5): 1218-1225.

Zorica B., Isajlović I., Vrgoč N., Keč V. Č., Medvešek D., Vuletin V., Radonić I., Cvitanić R., Lepen Pleić I., Šestanović M. (2021). Reproductive traits of the European hake, *Merluccius merluccius* (L. 1758), in the Adriatic Sea. Acta Adriatica. 62(2): 183-198.

Williams J. E. (2000). The Coefficient of Condition of Fish. Odjeljak 13 u Schneider James C. (ed.) 2000. *Manual of fisheries survey methods II: with periodic updates*. Michigan. Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.

Influence of seasonal changes on the presence and body condition of hake, *Merluccius merluccius,* red mullet, *Mullus barbatus* and common pandora, *Pagellus erythrinus* in trawl catches

Abstract

The paper investigates the mass representation of three economically important species in trawl catches on the eastern Adriatic coast, European hake, *Merluccius merluccius* Linnaeus, 1758, red mullet, *Mullus barbatus* Linnaeus, 1758 and common pandora, *Pagellus erythrinus* Linnaeus, 1758 as well as seasonal variations of their condition index in the G5 fishing zone. The representation of red mullet and hake in trawl catches, from winter to autumn, remains high at 23-35%, in contrast to the significantly lower representation of common pandora (2-9%). The mass fraction of by-catches was constantly kept at around 30%. The value of the condition index for *M. merluccius* and *P. erythrinus* corresponds to the spawning season, while for *M. barbatus* this was not the case. Given the commercial importance of this species, further research is needed to explain this phenomenon.

Key words: *Merluccius merluccius*, *Mullus barbatus*, *Pagellus erythrinus,* fish body condition, seasonal changes

IZVORNI ZNANSTVENI RAD

Pouzdanost fizikalno-kemijskih metoda u otkrivanju patvorenja pčelinjeg voska

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Sažetak
Dodavanjem patvorina u pčelinji vosak mijenja se njegov sastav, kakvoća i fizikalno-kemijska svojstva. Cilj je ovog rada bio utvrditi pouzdanost fizikalno-kemijskih metoda u otkrivanju patvorenja pčelinjeg voska. Rezultati istraživanja pokazali su kako određivanje kiselinskog broja pokazuje zadovoljavajuću mjernu pouzdanost u otkrivanju patvorenja (R^2=0,996 za parafin; R^2=0,9997 za stearinsku kiselinu), dok je određivanje točke tališta (R^2=0,8969 za parafin; R^2=0,8823 za stearinsku kiselinu) i specifične težine (R^2=0,9824 za parafin; R^2=0,1489 za stearinsku kiselinu) upitne pouzdanosti ovisno o tipu patvorina te navedeni fizikalno-kemijski parametri mogu poslužiti samo kao indikacija patvorenja.

Ključne riječi: pčelinji vosak, patvorenje, parafin, stearinska kiselina, fizikalno-kemijske metode

Uvod

Pčelinji je vosak česta meta patvorenja, a danas na međunarodnom tržištu postoji preko 15 tvari različitog podrijetla kojima se patvori pčelinji vosak (Bogdanov, 2017.). Uz parafin kao najučestaliju patvorinu pčelinjeg voska, u posljednjih se nekoliko godina na tržištu sve češće pojavljuju i druge patvorine (primarno stearin i/ili stearinske kiselina), kao i višestruko patvorenje (Reybroeck i van Nevel, 2018.; Svečnjak i sur., 2018., Tanner i Lichtenberg-Kraag, 2020.). Analitičke metode koje se primjenjuju u otkrivanju patvorenja pčelinjeg voska uključuju klasične fizikalno-kemijske metode te instrumentalne analitičke tehnike (spektroskopske i kromatografske).

Iako se na upitnu pouzdanost fizikalno-kemijskih metoda u otkrivaniju patvorenja pčelinjeg voska sustavno ukazuje (Tulloch, 1973.; Bernal i sur., 2005.; Serra Bonvehi i Orantes Bermejo, 2012., Maia i Nunes, 2013.; Svečnjak i sur., 2015., 2021.), njihova je primjena implementirana u EU zakonsku regulativu vezanu za kriterije kakvoće (čistoće) pčelinjeg voska koji je namijenjen primjeni u farmaceutskoj (Vijeće Europe, 2020.) i prehrambenoj industriji (Uredba Komisije, 2012.), dok se kontrola kakvoće i provjera autentičnosti pčelinjeg voska u sektoru pčelarstva (satne osnove, blokovi voska namijenjeni za njihovu izradu) na EU razini još uvijek ne provode. Na cjelokupnu problematiku patvorenja pčelinjeg voska, uključujući i aspekte pouzdanosti i implementacije analitičkih metoda u otkrivanju patvorenja, nedavno je ukazala i Europska agencija za sigurnost hrane (EFSA, 2021.).

Cilj je ovog rada bio utvrditi pouzdanost primjene fizikalno-kemijskih metoda (određivanje točke tališta, specifične težine i kiselinskog broja) u otkrivanju patvorenja pčelinjeg voska.

Materijal i metode

Pripremljene su smjese pčelinjeg voska i parafina (*Paraffinum solidum*, Ph.Eur. 7,8: PS smjese) te smjese pčelinjeg voska i stearinske kiseline (*Acidum stearicum*, Ph.Eur. 8,1: AS smjese) s različitim udjelima dodanih patvorina koji se kretao od 5 % do 95 % (w/w), slijedeći porast od 5 % dodanih patvorina u pojedinim smjesama. Smjese su homogenizirane

zagrijavanjem na 90 °C kroz 3 sata, ostavljene da se ohlade na sobnoj temperaturi te čuvane na sobnoj temperaturi na tamnom mjestu do analiza

Pripremljene smjese pčelinjeg voska i patvorina analizirane su primjenom fizikalno-kemijskih metoda kako bi se utvrdio tip i intenzitet promjena odabranih fizikalno-kemijskih svojstava patvorenog pčelinjeg voska u ovisnosti o različitoj razini patvorenja parafinom i stearinskom kiselinom. Uzorci autentičnog pčelinjeg voska i patvorina (parafina i stearinske kiseline) te smjesa pčelinjeg voska i patvorina analizirani su sljedećim fizikalno-kemijskim metodama: određivanje točke tališta, specifične težine i kiselinskog broja.

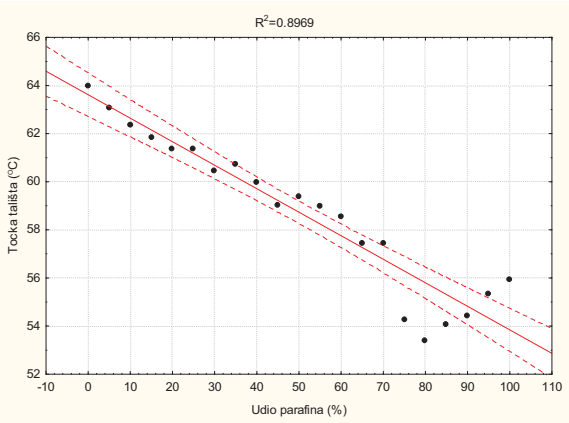
Specifična težina određena je pomoću digitalne precizne analitičke vaga (MS 204 Mettler Toledo, SAD) kojom je određena masa uzorka na zraku i u mediju (apsolutni alkohol), a vrijednost specifične težine uzorka izražena je kao:

$$ST = \frac{\text{gustoća tvari}}{\text{gustoća medija}} \text{ (g/cm}^3\text{)}$$

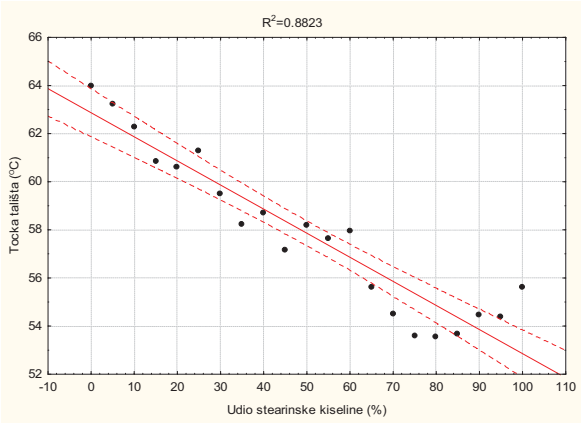
Određivanje točke tališta istraživanih uzoraka provedeno je pomoću digitalnog talištometra (MP 55 Melting Point System, Mettler Toledo, SAD) temeljenom na kapilarnoj metodi za automatsko određivanje točke tališta koji koristi mikroprocesorski sustav povećanja temperature. Određivanje kiselinskog broja provedeno je sukladno metodologiji propisanoj Europskom farmakopejom (Vijeće Europe, 2020.). Kiselinski broj ispitivanih uzoraka određen je potenciometrijskom metodom pomoću digitalnog pH metra za kiselo-bazne titracije (Mettler Toledo, SAD). Analiza i obrada podataka provedena je regresijskim analizama koristeći statistički paket Statistica version 7. (StatSoft Inc.).

Rezultati i rasprava

Obradom rezultata vrijednosti točke tališta uočeno je kako postoji linearan trend pada točke tališta dodatkom parafina i stearinske kiseline u pčelinji vosak, međutim rezultati (koeficijent determinacije R^2) nisu pokazali precizne kalibracijske krivulje u slučaju smjesa pčelinjeg voska s različitim udjelima parafina (Grafikon 1.; R^2=0,8969) niti različitim udjelima stearinske kiseline (Grafikon 2.; R^2=0,8823). Također, treba naglasiti da se u svrhu patvorenja koriste supstance sa širokim rasponom točke tališta (posebice parafini) koje su često istovjetne točki tališta prirodnog pčelinjeg voska (61-65 °C). Stoga točka tališta u nekim slučajevima može poslužiti samo kao indikacija patvorenja, ali ne daje jasan dokaz o patvorenju. Serra Bonvehi i Orantes Bermejo (2012.) u svom istraživanju također dolaze do zaključka kako vrijednost točke tališta pčelinjeg voska unutar raspona koje je predložila Europska farmakopeja ne osigurava autentičnost proizvoda, ali može poslužiti kao indikator patvorenja. Naime, već i male količine patvorina mijenjaju vrijednost točke tališta, odnosno proširuju raspon njezine vrijednosti pa tako pčelinji vosak koji je patvoren parafinom s niskom točkom tališta (54-56 °C) smanjuje točku tališta na < 61 °C kada je udio dodanog parafina veći od 10%. Kako je prikazano na Grafikonu 2., dodavanjem stearinske kiseline pčelinjem vosku, također je uočeno značajno smanjenje vrijednosti točke tališta (> 65 °C), ali regresijski pravac i vrijednost koeficijenta determinacije (R^2=0,8823) ukazuje na određeni stupanj mjerne nesigurnosti.

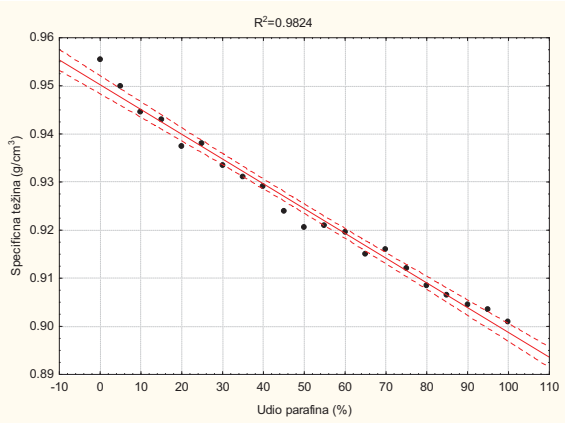


Grafikon 1. Odnos između utvrđene točke tališta i udjela parafina u smjesama pčelinjeg voska i parafina (model jednostavne linearne regresije)

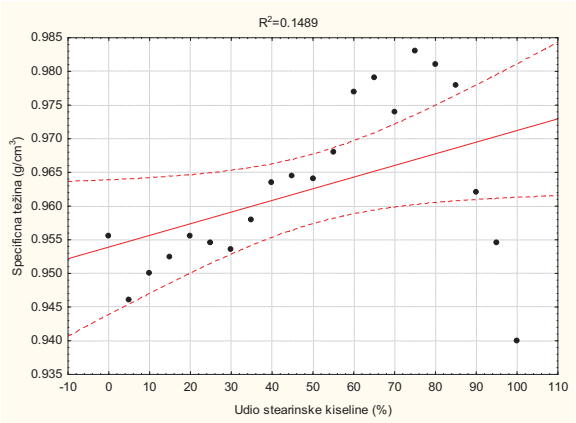


Grafikon 2. Odnos između utvrđene točke tališta i udjela stearinske kiseline u smjesama pčelinjeg voska i stearinske kiseline (model jednostavne linearne regresije)

Rezultati analize specifične težine ispitivanih uzoraka smjesa pčelinjeg voska i patvorina prikazani su na Grafikonu 3. i 4. Obradom rezultata utvrđeno je kako se patvorenje parafinom može utvrditi temeljem ovog parametra; naime, uočen je linearan trend pada specifične težine dodatkom parafina u pčelinji vosak (Grafikon 3.; R^2=0,9824), ali valja istaknuti kako su razlike u vrijednostima specifične težine pčelinjeg voska i parafina neznatne što narušava pouzdanost rezultata. Suprotno tome, u slučaju patvorenja stearinskom kiselinom nije moguće utvrditi patvorenje obzirom da se vrijednosti specifične težine dodatkom stearinske kiseline mijenjaju i značajno variraju, ali ne u ovisnosti o %-tnom (w/w) udjelu stearinske kiseline (Grafikon 4., R^2=0,1489). Tako neke od smjesa pčelinjeg voska i stearinske kiseline imaju vrijednost specifične težine gotovo istovjetnu specifičnoj težini autentičnog pčelinjeg voska, koja prosječno iznosi 0,956 g/cm^3.



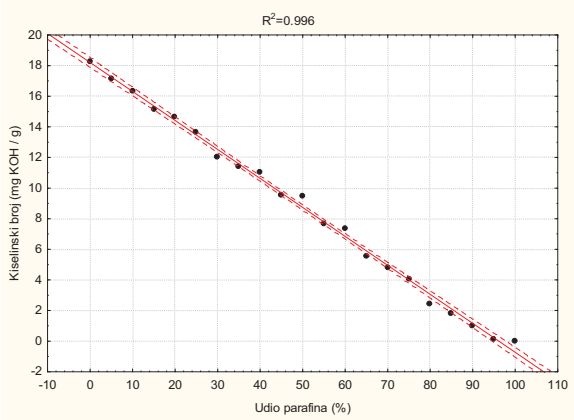
Grafikon 3. Odnos između utvrđene specifične težine i udjela parafina u smjesama pčelinjeg voska i parafina (model jednostavne linearne regresije)



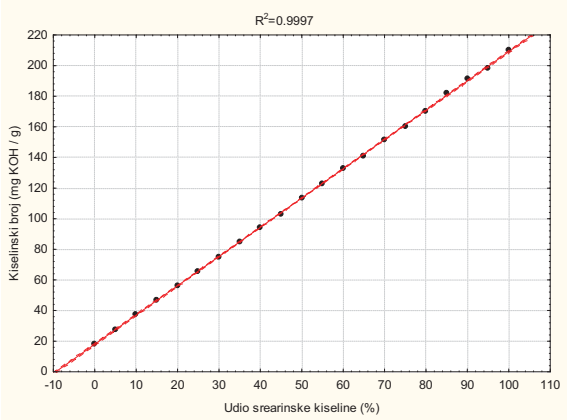
Grafikon 4. Odnos između specifične težine i udjela stearinske kiseline u smjesama pčelinjeg voska i stearinske kiseline (model jednostavne linearne regresije)

Rezultati korelacije između vrijednosti kiselinskog broja i udjela patvorina u smjesama pčelinjeg voska i patvorina (parafina i stearinske kiseline) prikazani su na Grafikonu 5. i 6. Grafikon 5. prikazuje pouzdan linearan trend pada kiselinskog broja dodatkom parafina u

pčelinji vosak (R^2=0,996), a nasuprot tome, trend porasta dodatkom stearinske kiseline (Grafikon 6.; R^2=0,9997). Ovakav je ishod očekivan obzirom da parafin ne sadrži lipidne sastavnice (estere i masne kiseline), dok stearinska kiselina logično povisuje vrijednost ovog parametra. Navedeni rezultati impliciraju kako je kiselinski broj najpouzdaniji fizikalno-kemijski parametar u otkrivanju patvorenja pčelinjeg voska, što je u skladu s rezultatima iz dostupne znanstvene literature (Bernal i sur., 2005.; Maia i Nunes, 2013.; Svečnjak i sur., 2015.).



Grafikon 5. Odnos između kiselinskog broja i udjela parafina u smjesama pčelinjeg voska i parafina (model jednostavne linearne regresije)



Grafikon 6. Odnos između kiselinskog broja i udjela stearinske kiseline u smjesama pčelinjeg voska i stearinske kiseline (model jednostavne linearne regresije)

Zaključak

Rezultati ovog istraživanja pokazali su kako određivanje kiselinskog broja pčelinjeg voska pokazuje zadovoljavajuću mjernu pouzdanost u otkrivanju patvorenja (R^2=0,996 za parafin; R^2=0,9997 za stearinsku kiselinu). Temeljem rezultata analize vrijednosti specifične težine istraživanih uzoraka voska, utvrđeno je kako radi neznatnih razlika u vrijednostima između pčelinjeg voska i parafina / stearinske kiseline nije moguće utvrditi patvorenje, a točka tališta kao parametar kakvoće pčelinjeg voska može poslužiti samo kao indikacija patvorenja. Utvrđivanje patvorenja pčelinjeg voska koristeći isključivo fizikalno-kemijske parametre ne pruža zadovoljavajuće rezultate za potvrdu autentičnosti pčelinjeg voska, stoga se za pouzdanu detekciju patvorina u pčelinjem vosku preporuča kombinacija analiza minimalno dva fizikalno-kemijska parametra uz provjeru rezultata instrumentalnim analitičkim tehnikama (kromatografskim i/ili spektroskopskim) koje ujedno omogućuju pouzdanu kvantifikaciju patvorina u pčelinjem vosku s niskim granicama detekcije (< 3,5 %).

Napomena

Istraživanja neophodna za ovaj rad dio su projekta „Kemijska karakterizacija kontaminiranog pčelinjeg voska i utvrđivanje pozadinskih mehanizama negativnih učinaka kontaminacije primjenom fizikalno-kemijskih i spektroskopskih (NMR, FTIR-ATR, GC-MS) metoda“ kojeg je financirala Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju.

Literatura

Bernal J. L., Jiménez J. J., Del Nozal M. J., Torbio L., Martin M. T. (2005). Physico-chemical parameters for the characterization of pure beeswax and detection of adulterations. European Journal of Lipid Science And Tehnology. 107(3):158-166.

Bogdanov S. (2017). Beeswax: Production, Properties, Composition and Control. Beeswax book: Chapter 1. Bee Product Science: 1-19.

European Food Safety Authority (EFSA). (2020). Risk assessment of beeswax adulterated with paraffin and/or stearin/stearic acid when used in apiculture and as food (honeycomb). EFSA Supporting Publication. 17(5): 1-64.

Maia M., Nunes F. M. (2013). Authentication of beeswax (*Apis mellifera*) by high-temperature gas chomatography and chemometric analysis. Food chemistry. 136(2): 961-968.

Serra Bonvehí J. S., Orantes Bermejo F. J. (2012). Detection of adulterated commercial Spanish beeswax. Food Chemistry. 132(1): 642- 648.

Reybroeck W., Van Nevel J. (2018). Effect of beeswax adulterated with sterin on the development of worker bee brood: results of a field trial. Program & Abstract Book EurBee 8. 8th Congress of Apidology, Ghent, Belgium, p. 115.

Svečnjak L., Baranović G., Vinceković M., Prđun S., Bubalo D., Tlak Gajger I. (2015). An approach for rutine analytical detection of beeswax adulteration using FTIR-ATR spectroscopy. Journal of Apicultural Science. 59(2): 37-49.

Svečnjak, L., Prđun, S., Baranović, G., Damić, M., Rogina, J. (2018). Alarming situation on the EU beeswax market: The prevalence of adulterated beeswax material and related safety issues. Program & Abstract Book EurBee 8. 8th Congress of Apidology, Ghent, Belgium, p. 114-115.

Svečnjak L., Nunes F. M., Garcia Matas R., Cravedi J.-P., Christodoulidou A., Rortais A., Saegerman C. (2021). Validation of analytical methods for the detection of beeswax adulteration with a focus on paraffin. Food control. 120, 107503: 1-13.

Tanner N., Lichtenberg-Kraag B. (2019). Identification of Quantification of Single and Multi-Adulteration of Beeswax by FTIR-ATR Spectroscopy. European Journal of Lipid Science and Tehnology. 121: 1-10.

Tulloch A.P. (1973). Factors affecting values of beeswax and detection of adulteration. Journal of the American Oil Chemist'S Society. 50 (7): 269-272.

Uredba Komisije (2012). br. 231/2012 o utvrđivanju specifikacija za prehrambene aditive navedene u prilozima II. i III. Uredbi (EZ) br. 1333/2008 Europskog parlamenta i Vijeća. Official Journal of the European Union. L83: 1-295.

Vijeće Europe (2020). European Pharmacopoeia 10.2 (Ph. Eur. 10th ed.): Beeswax. white (01/2008:0069), Beeswax yellow (01/2008: 0070). In EDQM -European Directorate for the Quality of Medicine and Healthcare. Cedex, France, 1804-1805.

Reliability of physico-chemical methods in beeswax adulteration detection

Abstract

The addition of adulterants to beeswax changes its composition, quality, and physico-chemical properties. The aim of this study was to determine the reliability of physico-chemical methods in the detection of beeswax adulteration. The results showed that the determination of the acid number shows satisfactory measurement reliability in the detection of adulteration (R^2=0.996 for paraffin; R^2=0.9997 for stearic acid), while the determination of the melting point (R^2=0.8969 for paraffin; R^2=0,8823 for stearic acid), and specific gravity (R^2=0.9824 for paraffin; R^2=0.1489 for stearic acid) reflects questionable reliability depending on the type of adulterant. Therefore, the latter physico-chemical parameters can only serve as an indication of adulteration.

Key words: beeswax, adulteration, paraffin, stearic acid, physico-chemical methods

IZVORNI ZNANSTVENI RAD

Promjene određenih parametara svježine arbuna, *Pagellus erythrinus*, skladištenog na ledu

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Sažetak
Ovim istraživanjem su praćene promjene dielektričnih svojstava i pH vrijednosti kao pokazatelji svježine arbuna skladištenog na ledu u razdoblju od 7 dana. Elektrovodljivost je opadala s vremenom skaldištenja, dok se vrijednost pH nakon početnog pada nije značajno mijenjala do kraja istraživanja. Dobiveni rezultati ukazuju da je tijekom razdoblja istraživanja arbun zadržao zadovoljavajuću kvalitetu mesa.

Ključne riječi: morska riba, svježina ribe, kvaliteta, pH, dielektrična svojstva

Uvod

Svježina je najvažnija odrednica kvalitete neprerađene ribe (Majolini i sur., 2009.). Procesi koji se odvijaju u mesu ribe nakon uginuća utječu na kvalitetu ribe i postepeno dovode do njenog kvarenja (Šimat i sur., 2009.; Milijašević i sur., 2011.). Riba je vrlo podložna kvarenju zbog niskog udjela vezivnog tkiva u mišićima, prisutnosti autolitičkih enzima, povoljnog pH i visokog udjela vode (Franceschelli i sur., 2021.). Brzina kvarenja je različita kod različitih vrsta i ovisi o fiziološkom stanju ribe, sastavu tkiva i metabolitima u tkivu, metodi usmrćivanja, mikrobnoj kontaminaciji te rukovanju i skladištenju nakon smrti (Majolini i sur., 2009.; Yerlikaya i sur., 2015.). Svježina se kao pokazatelj kvalitete može pratiti korištenjem metoda poput procjene senzorskih osobina (izgled, miris, tekstura) te bilježenjem biokemijskih i fizikalnih svojstava ribe (Franceschelli i sur., 2021.).
Cilj ovog rada je bio kontinuiranim mjerenjem dielektričnih svojstava kože i pH vrijednosti mesa, tijekom 7 dana pratiti promjene svježine arbuna, *Pagellus erythrinus* (Linnaeus, 1758), skladištenog na ledu u hladnoj komori.

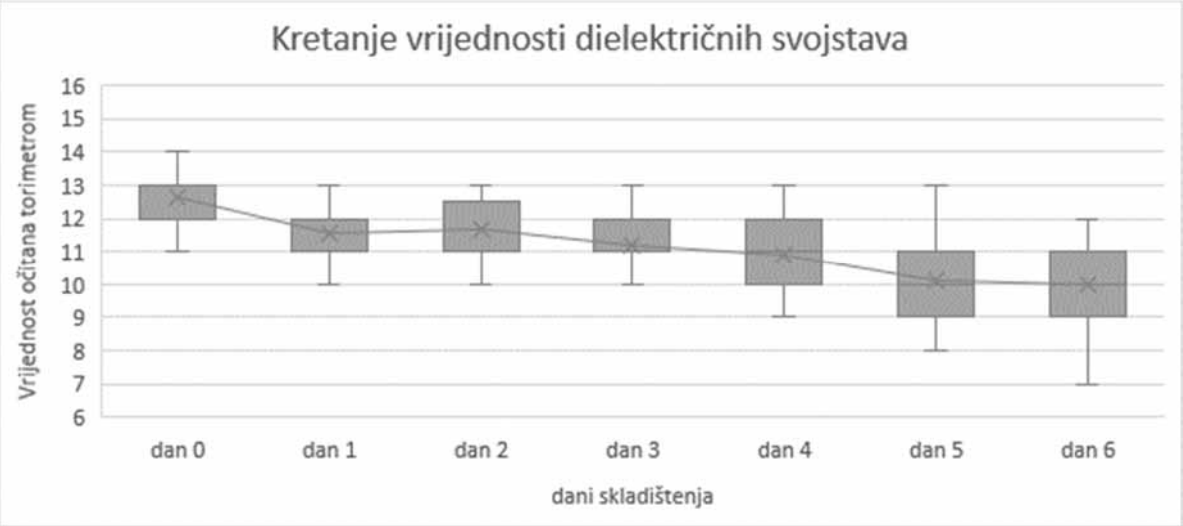
Materijal i metode

Jedinke arbuna su prikupljene koćarenjem u Neretvanskom kanalu tijekom studenog 2021. godine. Sva riba je po ulovu potopljena u mješavinu morske vode s ledom. Iz ukupnog ulova je izdvojeno 27 jedinki arbuna prosječne duljine 19,38±1,37 cm i mase 78,15±15,54 g te su im odmah izmjereni provodljivost kože i pH vrijednost mesa. Riba je veličinski jednoliko raspoređena i položena na ljuskasti led u dvije samodrenirajuće polistirenske kutije te odložena na skladištenje u hladnu komoru konstantne temperature 2 ºC. Ribi su svaki dan u isto vrijeme kroz razdoblje od narednih 6 dana (dan ulova se tretirao kao nulti dan) mjerena dielektrična svojstva i vrijednost pH te je dodavan led po potrebi.
Dielektrična svojstva arbuna su mjerena torimetrom (Distell Fish freshness meter, Model Torrymeter) na način da su elektrode uređaja prislonjene na dorzalnu bočnu stranu tijela, neposredno iza škržnog poklopca i iznad bočne pruge (Distell, 2011.). Na svakoj jedinki je mjerenje izvršeno tri puta za redom te su između svakog mjerenja elektrode prebrisane čistim

papirnatim ubrusom kako bi se smanjila moguća greška pri mjerenju. Bilježene su vrijednosti očitane torimetrom na skali od 0 do 18, pri čemu više vrijednosti označavaju svježiju ribu (Distell, 2011.). Mjerenje pH vrijednosti mesa arbuna je izvršeno digitalnim multiparametarskim uređajem (pH 70 Vio DHS) s ubodnom pH i temperaturnom sondom (XS Instruments). Vrijednosti su mjerene na dorzalnom mišiću ribe iza glave, na način da je tijekom svakog mjerenja sonda ubodena na drugo mjesto, odnosno ni jedno naknadno mjerenje nije provedeno na mjestu uboda prethodnog mjerenja.
Razlike u vrijednostima elektrovodljivosti i pH mesa između svih dana mjerenja su ispitane analizom varijance ponovljenih mjerenja (repeated measures ANOVA) (razina značajnosti 0,01). Nakon toga je proveden Tukey HSD post hoc test za višestruko uspoređivanje parova dana kako bi se odredilo između kojih točno dana postoji značajna razlika u mjerenjima. Svi podaci su obrađeni u programu Microsoft Excel, a statističke analize su provedene u statističkom programu PAST 4.05 (Hammer i sur., 2001.).

Rezultati i rasprava

Praćenjem dielektričnih svojstava arbuna je utvrđen trend pada srednjih vrijednosti očitanih torimetrom kroz razdoblje skladištenja (Grafikon 1). Analizom varijance ponovljenih mjerenja utvrđena je značajna razlika između dana skladištenja ($p<0,01$). Tukey HSD post hoc testom je zabilježen značajan pad torimetrijskih vrijednosti između dana uzorkovanja ribe (nulti dan) i prvog dana skladištenja, te između četvrtog i petog dana skladištenja (Tablica 1).



Grafikon 1. Kretanje vrijednosti dielektričnih svojstava arbuna kroz vrijeme skladištenja

Distell priručnik (2011.) za srodnu ribu iz roda *Pagellus* (*Pagellus coupei*, validno *Pagellus bellottii*) navodi da torimetrijske vrijednosti od 11-13 označavaju vrlo svježu ribu, 7-10 su znak pada svježine, 4-6 gotovo pokvarenu ribu, a vrijednosti ispod 4 označavaju nejestivu ribu. U ovom istraživanju je vrijednost dielektričnih svojstava s početne torimetrijske vrijednosti 12,62±0,62, očitane nulti dan, pala na 10,00±1,08, očitane šesti dan mjerenja. Ove vrijednosti pokazuju da je u razdoblju od 6 dana skladištenja očuvana zadovoljavajuća kvaliteta ribe. Sličan pad u vrijednostima dielektričnih svojstava su opisali i Fagioli i sur. (2009.) u istraživanju provedenom u sličnim uvjetima na drugoj vrsti iz porodice Sparidae, oradi, *Sparus aurata*, i to kroz razdoblje od 16 dana. Jedan od mogućih uzroka usporenog pada torimetrijskih vrijednosti u njihovom istraživanju mogao bi biti utjecaj veće veličine jedinki koje dulje zadržavaju svježinu na ledu (Shawyer i Medina Pizzali, 2003.), no ne treba

isključiti niti fiziološko stanje ribe, način izlova te rukovanje s ribom nakon izlova (Majolini i sur., 2009.; Yerlikaya i sur., 2015.).

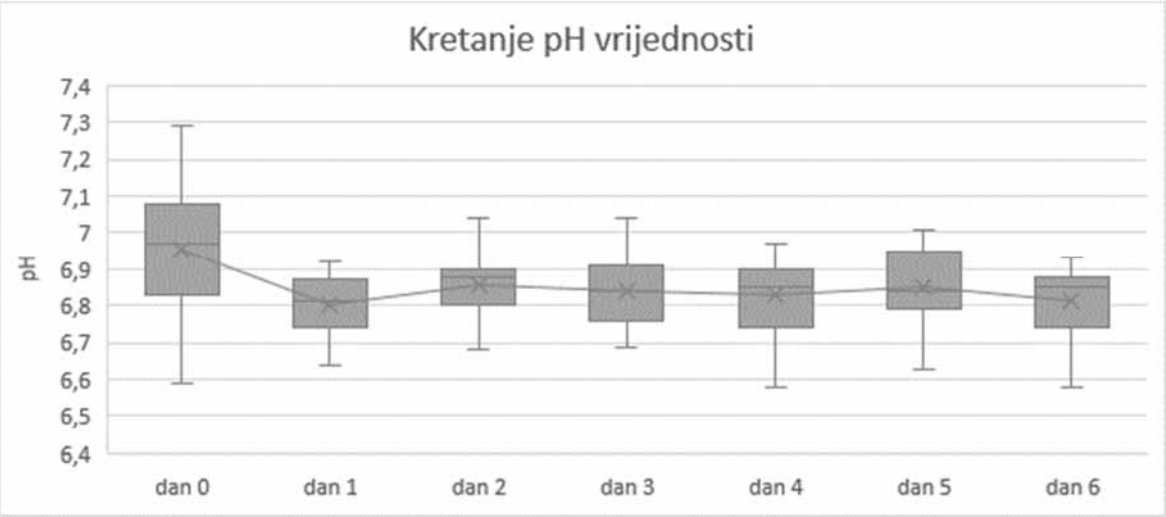
Tablica 1. Dobivene p-vrijednosti Tukey HSD post hoc testa između pojedinih dana mjerenja za pH vrijednosti (podebljano) i za dielektrična svojstva

	dan 0	dan 1	dan 2	dan 3	dan 4	dan 5	dan 6
dan 0		**<0,01***	**<0,01***	**<0,01***	**<0,01***	**<0,01***	**<0,01***
dan 1	<0,01*		**0.035**	**0.323**	**0.808**	**0.106**	**0.100**
dan 2	<0,01*	0.978		**0.963**	**0.601**	**0.100**	**0.134**
dan 3	<0,01*	0.132	0.011		**0.987**	**0.100**	**0.653**
dan 4	<0,01*	<0,01*	<0,01*	0,540		**0.847**	**0.976**
dan 5	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*		**0.312**
dan 6	<0,01*	<0,01*	<0,01*	<0,01*	<0,01*	0.978	

*statistički značajne razlike

Analizom varijance ponovljenih mjerenja je utvrđena statistički značajna razlika izmjerenih pH vrijednosti između dana skladištenja (p<0,01). Tukey HSD post hoc test je pokazao da ta značajna razlika postoji samo između dana ulova ribe (nulti dan) i prvog dana skladištenja, dok se kroz daljnje vrijeme skladištenja pH vrijednost značajno ne mijenja (Tablica 1, Grafikon 2). Neposredno nakon ulova pH vrijednost ribe opada zbog stresom izazvane anaerobne razgradnje mišićnog glikogena i stvaranja mliječne kiseline (Daskalova 2019.). Nakon tog početnog pada vrijednosti pH, ona neko vrijeme ostaje nepromijenjena i kasnije raste pod utjecajem autolitičkih procesa što pogoduje naseljavanju bakterija i daljnjoj bakterijskoj razgradnji koja vodi ka kvarenju ribe (Kyrana i sur., 1997.; Abbas i sur., 2008.). Neki autori ipak napominju da, iako raste s vremenom skladištenja, povišena pH vrijednost nije direktni pokazatelj kvarenja i treba je tumačiti uz dodatne kemijske analize (Yerlikaya i sur., 2015.).

Yerlikaya i sur. (2015.) su ustanovili da se kod arbuna skladištenog na ledu značajan porast vrijednosti pH može uočiti već treći dan mjerenja, no koristili su drugačiju metodu mjerenja pH vrijednosti na mješavini homogeniziranog mesa i vode. Istraživači koji su promjenu pH mjerili direktno na skladištenoj ribi su ustanovili da pH ne pokazuje značajan porast tijekom 10 dana kod brancina, *Dicentrarchus labrax*, (Abbas i sur., 2008.) te u rasponu od 7 do čak 16 dana kod orade (Kyrana i sur., 1997.; Fagioli i sur., 2009.).



Grafikon 2. Kretanje pH vrijednosti arbuna kroz vrijeme skladištenja

Zaključak

Rezultat praćenja promjene dielektričnih svojstava i pH mesa arbuna tijekom 7 dana skladištenja na ledu upućuju da je došlo do određenog pada svježine, no kvaliteta ribe je do kraja istraživanja bila zadovoljavajuća. Pad svježine se manifestirao padom mjerenih torimerijskih vrijednosti dok je pH vrijednost nakon početnog pada ostala nepromijenjena do zadnjeg dana skladištenja, što indicira da u razdoblju istraživanja nije došlo do kvarenja ribe.

Napomena

Istraživanje provedeno za ovaj rad je izvršeno u sklopu Projekta "Ribarsko-znanstvena mreža Grada Ploča" u okviru Mjere I.3. "Partnerstvo između znanstvenika i ribara za razdoblje 2017.-2020.".

Literatura

Abbas K.A., Mohame A., Jamilah, B., Ebrahimian M., (2008). A Review on Correlations between Fish Freshness and pH during Cold Storage. American Journal of Biochemistry and Biotechnology. 4: 416–421.

Daskalova A. (2019). Farmed fish welfare: stress, post-mortem muscle metabolism, and stress-related meat quality changes. International Aquatic Research. 11: 113–124.

Distell (2011). User manual Distell fish freshness meter, Model Torrymeter, version 2.9.

Fagioli P., Badiani A., Bonaldo A., Testi S., Gatta P. (2009). Freshness quality of gilthead sea bream (*Sparus aurata*) reared under different farming conditions. Italian Journal of Food Safety. 1(5):60-4. Dostupno na: https://www.pagepressjournals.org/index.php/ijfs/article/view/ijfs.2009.5.60. Pristupljeno 29.01.2022.

Franceschelli L., Berardinelli A., Dabbou S., Ragni L., Tartagni M. (2021). Sensing Technology for Fish Freshness and Safety: A Review. Sensors. 21, 1373. doi:10.3390/s21041373

Hammer Ø., Harper D.A.T., Ryan P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9pp.

Kyrana V.R., Lougovious V.P., Valsamis D.S. (1997). Assessment of shelf-life of maricultured gilthead sea bream (*Sparus aurata*) stored in ice. International Journal of Food Science and Technology. 32: 339-347.

Majolini D., Trocino A., Tazzoli M., Xiccato G.(2009). Evolution of European sea bass (*Dicentrarchus labrax*) freshness during storage. Italian Journal of Animal Science. 8 (3): 282-284.

Milijašević M., Babić J., Spirić A., Jovanović J., Lakićević B., Borović B., Baltić M. Ž. (2011). Praćenje promena ukupnog broja mezofilnih bakterija i torimetrijske vrednosti u uzorcima sveže pastrmke upakovane u modifikovanu atmosferu i vakuum. Veterinarski glasnik. 65(5-6): 375-384.

Shawyer M., Medina Pizzali A.F (2003). The use of ice on small fishing vessels. FAO Fisheries Technical Paper. No. 436, Rome, FAO: 108 pp

Šimat V., Soldo A., Maršić-Lučić J., Tudor M., Bogdanović T. (2009). Effect of different storage conditions on the dielectric properties of the sea bass (*Dicentrarchus labrax*, L.). Acta Adriatica. 50(1): 5 – 10.

Yerlikaya P., Ucak I., Gumus B., Gokoglu N. (2015). Citrus peel extract incorporated ice cubes to protect the quality of common pandora. Journal of Food Science and Technology. 52: 8350–8356.

Changes in certain freshness parameters of common pandora stored on ice

Abstract
In this study, the values of dielectric properties and pH were monitored as indicators of freshness of common pandora stored on ice for a period of 7 days. Conductivity of the fish decreased with time, while pH values did not change significantly after the initial decrease until the end of the study. The results showed that the common pandora retained satisfactory flesh quality throughout the study period.

Key words: marine fish, fish freshness, quality, pH, dielectric properties

IZVORNI ZNANSTVENI RAD

Utjecaj prihrane na pojavnost nozemoze (*Nosema spp.*) u zajednicama medonosne pčele (*Apis mellifera* L.) u proljeće

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Sažetak

Cilj ovog istraživanja bio je utvrditi utjecaj prihrane pčelinjih zajednica na prisutnost i razvoj nozemoze. U istraživanju je bilo uključeno 19 pčelinjaka: 10 s područja Osijeka te 9 s područja Siska. Na svakom pčelinjaku 30 zajednica podjeljeno je u 3 skupine po 10: dvije su prihranjivane s testnim pogačama (T1 i T2), dok jedna skupina (K) nije prihranjivana. Prosječan broj spora prije početka prihrane iznosio je 3,21 milijuna bez statistički značajnih razlika između skupina. Nakon prihrane prosječan broj spora iznosio je 3,41 milijun bez značajnih razlika između skupina. Iako nisu utvrđene značajne razlike između skupina, može se uočiti trend pada broja spora kod testnih skupina, a porast kod kontrolne skupine.

Ključne riječi: medonosna pčela, nozemoza, prihrana

Uvod

Nozemoza je nametnička bolest srednjeg crijeva odraslih pčela (*Apis mellifera* L.) uzrokovana mikrosporidijama *Nosema apis* i *N. ceranae*. Mikrosporidiji su jednostanični eukariotski organizmi i obligatni unutarstanični nametnici koji stvaraju spore te su sistematski klasificirani pod carstvo gljiva (Michalczyk i Sokół, 2014.). U srednjem crijevu iz spora se razvija nametnik koji napada epitelne stanice srednjeg crijeva i razmnožava se. Napadnuta stanica propada te se ponovno stvaraju nove spore koje ulaze u zdrave stanice ili se izmetom izlučuju van. Ova nametnička bolest raširena je po cijelom svijetu i uzrokuje značajne gubitke u pčelarstvu i gospodarstvu općenito (Tlak Gajger i sur., 2009.). Spore su vrlo otporne; u prirodi mogu preživjeti 2-4 mjeseca, dok se u srednjem crijevu, 5 dana nakon zaraze, može razviti oko 250 milijuna spora. Navedena bolest pčelarstvu nanosi velike ekonomske štete, što se odražava na smanjen prinos meda i ostalih pčelinjih proizvoda, slabljenjem pčelinje zajednice, a ponekad i njenim ugibanjem. Materijalni gubici nastaju zbog kraćeg života odraslih pčela radilica kada su skupljačice, što se ogleda u smanjenju oprašivanja i time gubitaka u poljoprivrednoj proizvodnji, a posebice u voćarstvu (Matašin i sur., 2007.).

Kako bi pčelinja zajednica što bolje iskoristila glavne proljetne paše (uljana repica, bagrem i kadulja), potrebna je što pravilnija podjela rada između radilica u pčelinjoj zajednici, što se može postići u jakim zajednicama, uz dovoljnu opskrbu hranom. Zimske rezerve peludi u pčelinjm zajednicama također imaju važnu ulogu tijekom zime kada matica započinje s uzgojem legla. Dovoljna i uravnotežena hrana osigurava normalan razvoj zdravih pčelinjih zajednica, a nedostatak može ograničiti njihov razvoj, posebno u proljeće kada je podjela rada između radilica u najosjetljivijoj fazi (Puškadija i sur., 2017.). Loši vremenski uvjeti tijekom intenzivnog proljetnog uzgoja legla mogu uzrokovati značajno smanjen unos peludi, a ako se potroše rezerve peludi, uzgoj legla se može drastično smanjiti ili čak obustaviti. Takvi neželjeni scenariji u razvoju legla mogu se spriječiti stimulativnim hranjenjem zajednica u pravo vrijeme. Stoga je cilj istraživanja bio utvrditi utjecaj prihrane različitim pogačama na prisutnost spora nozemoze (*Nosema spp.*) u pčelinjim zajednicama.

Materijal i metode

Istraživanje je bilo provedeno na ukupno 19 pčelinjaka: deset pčelinjaka s područja Osijeka i okolice, a 9 s područja Siska i okolice. Na svakom pčelinjaku bilo je uključeno 30 pčelinjih zajednica podjeljenih u tri skupine po 10. Dvije skupine bile su prihranjivane s dvije različite pogače (skupine T1 i T2), dok treća nije bila prihranjivana i služila je kao kontrolna skupina (skupina K). Prije dodavanja prve pogače krajem veljače 2021. godine iz svih zajednica skupljeni su uzorci živih pčela. Nakon prvog dodavanja pogače, zajednice su svakih 15 dana pregledavane te je po potrebi dodavana nova pogača. Sredinom travnja ponovno su skupljeni uzorci živih pčela. Tijekom veljače skupljeni su uzorci iz 563 košnice, a tijekom travnja iz 556 košnica, odnosno ukupno su bili skupljeni uzorci iz 1119 pčelinjih zajednica. Nakon prikupljanja uzoraka na terenu, uzorci su pohranjeni u zamrzivač do dana provođenja laboratorijske pretrage.

Analiza prisutnosti spora nozemoze provedena je na Fakultetu agrobiotehničkih znanosti Osijek prema standardnim metodama (Human i sur., 2013., Fries i sur., 2013). Utvrđen je broj spora u uzorku te je izračunat broj spora po pčeli množeći utvrđeni broj spora u uzorku s 50.000.

Statistička obrada podataka napravljena je u programu SPSS v26. Osim deskriptivne analize podataka, za utvrđivanje razlika između skupina u utvrđenom broju spora korišten je neparametrijski Kruskal-Wallis test.

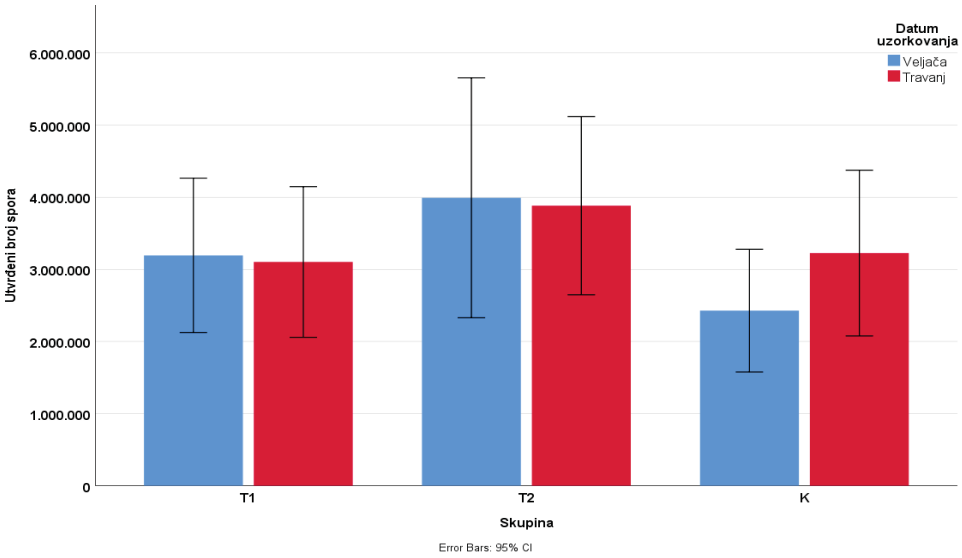
Rezultati i rasprava

U kasnu zimu (veljača) ukupno je analizirano 563 uzoraka (Tablica 1). Prosječan broj spora svih istraživanih skupina iznosio je 3,21 milijun, u rasponu od 2,43 milijuna u skupini K do 3,99 milijuna u skupini T2. Na početku istraživanja nije bilo statistički značajnih razlika ($\chi2$ (2) = 0,358, p = 0,836) između skupina. Nakon prihrane pčelinjih zajednicama testnim pogačama, tijekom sredine travnja ukupno je skupljeno 557 uzoraka pčela. Prosječan broj spora iznosio je 3,41 milijun u rasponu između skupina od prosječnih 3,10 milijuna u skupini T1 do 3,88 milijuna u skupini K (Tablica 1). Između skupina nisu utvrđene statistički značajne razlike ($\chi2$ (2) = 0,761, p = 0,683). Iako nisu utvrđene značajne razlike između skupina, može se uočiti trend pada broja spora nozemoze kod prihranjivanih skupina, dok je kod kontrolne skupine broj spora nozemoza narastao (Grafikon 1).

Tablica 1. Srednja vrijednost i standardna devijacija utvrđenog broja spora u uzorku od 20 pčela (broja spora u uzorku) i izračunatog broja spora po pčeli (broj spora po pčeli) kod tri različite skupine u veljači i travnju.

Mjesec	Skupina	Broj spora u uzorku			Broj spora po pčeli		
		Srednja vrijednost	N	SD	Srednja vrijednost	N	SD
Veljača	T1	63,87	190	149,65	3.193.684	190	7.482.444
	T2	79,85	189	231,86	3.992.593	189	11.592.813
	K	48,55	184	117,02	2.427.717	184	5.851.014
	Ukupno	64,23	563	173,59	3.211.545	563	8.679.830
Travanj	T1	61,6	187	145,55	3.102.659	187	7.264.472
	T2	77,67	189	172,24	3.883.598	189	8.611.919
	K	64,52	180	156,25	3.226.111	180	7.812.248
	Ukupno	68,01	556	158,34	3.407.540	556	7.911.645

U svim pčelinjacima koji su bili uključeni u istraživanje nije bio niti jedan sa svim pozitivnim ili svim negativnim rezultatima. Mortensen i sur. (2018.) proveli su istraživanje sa 75 komercijalnih pčelinjih zajednica u središnjoj Floridi od lipnja do rujna 2015. godine kako bi utvrdili utječe li hranjenje proteinskom pogačom na snagu zajednice i intenzitet širenja nozemoze. Pčele su statistički manje konzumirale pogaču Brood Builder nego li Bee-Pro, Ultra Bee, MegaBee i pelud samoniklog bilja. Tijekom vremena došlo je do smanjenja intenziteta nozemoze u svim tretmanima, što se može usporediti s rezultatima ovog istraživanja, gdje je vidljivo kako je u travnju blago opao utvrđeni broj spora nakon prihrane s pogačom. Iz ovoga se može očitati važnost prihrane pčelinjih zajednica pogačom kako bi se potakao rast i smanjila količina patogena. De Jong i sur. (2019.) istraživali su kako prihrana različitom koncentracijom peludi i različitim dodacima peludi utječu na fiziologiju pčele i zaraženost nozemozom te ublažava li uključivanje male količine peludi negativne učinke konzumiranja proteinskih dodataka. Velika razina nozemoze otkrivena je kod pčela iz zajednica koje su hranjene proteinskim dodatkom s prosječnom vrijednošću od 960.000 spora po pčeli. Kod onih koje su hranjene s peludi imale su prosječno 1.100.000 spora po pčeli, a one koje su hranjene s peludi s proteinskim dodatkom imale su prosječno 216.000 spora. Broj spora u pčelama iz zajednica hranjenih s peludi ili peludi s proteinskim dodatkom nije se značajno razlikovao. Utvrdili su kako su pčele više konzumirale i imale manji broj spora konzumirajući peludnu prehranu nego samo proteinski dodatak ili dodatak pomiješan s peludi.



Grafikon 1. Prosječan broj spora po pčeli prema pojedinim skupinama

Charistos i sur. (2015.) istraživali su kako dodatak prihrani HiveAlive™ utječe na snagu pčelinje zajednice i broj spora nozemoze te su utvrdili kako je njegova uporaba prije i poslije zime povećala broj pčela radilica za 89% i smanjila broj spora za 57% u usporedbi s kontrolnim zajednicama. Jack i sur. (2016.) istraživali su kako razrjeđivanje peludi utječe na zaraženost pčelinjih zajednica nozemozom te su utvrdili značajno više spora kod skupine zajednica koje su dobile veće količine peludi. Pčele su s većom količinom peludi imale značajno veće preživljavanje unatoč većem utvrđenom broju spora. Utvrdili su kako prihrana s većom količinom peludi povećava intenzitet spora, ali i povećava opstanak, odnosno dugovječnost pčela. Matašin i sur. (2007.) su ispitivali prisutnost spora u uzorcima pčela skupljenih tijekom siječnja i veljače te su utvrdili povećanje broja i postotka ukupno

pozitivnih uzoraka od 18. siječnja. do 15. veljače., ali manji broj i postotak pozitivnih uzoraka skupljenih 29. veljače.

Zaključak

Na osnovu provedenog istraživanja te rezultata drugih autora može se zaključiti kako mnogi čimbenici utječu na pojavnost nozemoze u pčelinjoj zajednici. Kako bi se smanjio rizik od zaraze, potrebno je pridržavati se biotehničkih mjera i dobre pčelarske prakse. U ovom istraživanju velik broj spora utvrđen krajem zime postupno je opadao u prihranjivanim skupinama, ali ne i u kontrolnoj skupini. Smanjenje šteta uzrokovanim nozemozom moguće je provođenjem higijenskih mjera kojima se smanjuje širenje bolesti iz izmeta do hrane ili vode, stimulativnom prihranom te odabirom i uzgojem pčela tolerantnih na nozemozu.

Napomena

Istraživanje je provedeno u sklopu projekta „Istraživanje i razvoj inovativne funkcionalne hrane za pčele radi povećanja efikasnosti globalne pčelarske proizvodnje“ financiranog u okviru modaliteta „Povećanje razvoja novih proizvoda i usluga koji proizlaze iz aktivnosti istraživanja i razvoja – faza II“. Projekt je financiran od strane Ministarstva gospodarstva, poduzetništva i obrta Republike Hrvatske.

Popis literature

Charistos L., Parashos N., Hatjina F. (2015). Long term effects of a food supplement HiveAlive™ on honey bee colony strength and *Nosema ceranae* spore counts. Journal of Apicultural Research. 54(5): 420-426.

De Jong W.E., DeGrandi-Hoffman G., Chen Y., Graham H., Ziolkowski N. (2019). Effects of diets containing different concentrations of pollen and pollen substitutes on physiology, Nosema burden, and virus titers in the honey bee (*Apis mellifera* L.). Apidologie. 50: 845-858.

Fries I, Chauzat M.P., Chen Y.P., Doublet V., Genersch E., Gisder S., Higes M., McMahon D.P., Martín-Hernández R., Natsopoulou M., Paxton R.J., Tanner G., Webster T.C., Williams G.R. (2013). Standard methods for Nosema research. Journal of Apicultural Research. 52(1): 1-28.

Higes M., Martín R., Meana A. (2006). *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J Invertebr Pathol. 92(2):93-5.

Human H., Brodschneider R., Dietemann V., Dively G., Ellis J. D., Forsgren E., Zheng H.-Q. (2013). Miscellaneous standard methods for Apis mellifera research. Journal of Apicultural Research. 52(4): 1–56.

Jack C. J., Uppala S. S., Lucas H. M., Sagili R. R. (2016). Effects of pollen dilution on infection of *Nosema ceranae* in honey bees. Journal of Insect Physiology. 87: 12-19.

Matašin Ž., Tlak Gajger I., Petrinec Z. (2007). Značenje dijagnosticiranja nozemoze. Međunarodni pčelarski sajam i znanstveno-stručni skup 4. pčelarski dani / Puškadija, Z. (ur.). Vinkovci: Poljoprivredni fakultet Osijek, str. 98-103.

Michalczyk M., Sokół R. (2014). Nosemosis in honey bees. Polish Journal of Natural Sciences. 23(1): 91-99.

Mortensen A. N., Jack C. J., Bustamante T. A., Schmehl D. R., Ellis J. D. (2018). Effects of Supplemental Pollen Feeding on Honey Bee (Hymenoptera: Apidae) Colony Strength and Nosema spp. Infection. Journal of Economic Entomology. 112(1): 60-66.

Puškadija Z., Spiljak L., Kovačić M. (2017). Late winter feeding stimulates rapid spring development of carniolan honey bee colonies (*Apis mellifera carnica*). Poljoprivreda. 23(2): 73-76.

Tlak Gajger I., Petrinec Z., Pinter Lj., Kozarić Z. (2009). Experimental Treatment of Nosema Disease with „Nozevit“ Phyto-pharmacological Preparation. American Bee Journal. 149: 485-490.

Influence of supplemental feeding with different patty on nosemosis (*Nosema spp.*) in honey bee colonies (*Apis mellifera*) in spring

Abstract
The aim of this study was to determine the impact of feeding bee colonies on the presence and development of nosemosis. In total 19 apiaries were included in the research: 10 from the area of Osijek and 9 from the area of Sisak. In each apiary, 30 colonies were divided into 3 groups of 10: two were fed with test patties (T1 and T2), while one group (K) was not fed. The average number of spores before the start of feeding was 3.21 million without statistically significant differences between groups. After supplement feeding, the average number of spores was 3.41 million with no significant differences between groups. Although no significant differences were found, a declining trend in the number of spores in the test groups and an increase in the control group can be observed.

Key words: honey bee, nosemosis, supplement feeding

Croatian 57 International Symposium on Agriculture 2022

Stočarstvo

07

Animal Husbandry

IZVORNI ZNANSTVENI RAD

Fenotipske odlike lakon ovce u Republici Hrvatskoj

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Sažetak
U Republici Hrvatskoj zadnjih je godina sve veći interes za ovčjim mlijekom i uzgojem mliječnih pasmina ovaca pa tako i za lakon ovcom. Cilj je ovoga rada prikazati fenotipske odlike lakon ovce u uzgojima u Republici Hrvatskoj te ih usporediti s dostupnim istraživanjima. Istraživanje je provedeno na 40 ovaca prosječne dobi 4 godine u trećoj laktaciji. Od fenotipskih odlika utvrđeni su tjelesna masa, tjelesne mjere, indeksi tjelesne razvijenosti te je izračunata korelacija između fenotipskih odlika. Prosječna tjelesna masa ovaca bila je 60,94 kg, visina grebena 68,05 cm, dužina trupa 78,49 cm, a indeks tjelesne kondicije 3,05. Utvrđena je značajna jaka pozitivna korelacija između većine fenotipskih odlika ovaca. Manje odstupanje u fenotipskim odlikama lakon ovaca možemo povezati s procesom prilagodbe, ali i utjecaju hranidbe.

Ključne riječi: lakon ovca, fenotipske odlike, tjelesna masa, tjelesne mjere

Uvod

U Republici Hrvatskoj zadnjih je godina sve veći interes za ovčjim mlijekom i uzgojem mliječnih pasmina ovaca pa tako i za lakon ovcom. Tijekom 2020. godine u RH je otkupljeno 2 820 751 kg ovčjeg mlijeka što je 6,6 % više od otkupljenih količina u 2019. godini. Uzgoji lakona u RH organizirani su u kontinentalnom dijelu bogatom žitaricama s obzirom na potrebu kvalitetnih krmiva pri balansiranju njihovoga obroka u cilju ispoljavanja genetskoga potencijala. Tako prema podacima Hrvatske agencije za hranu i poljoprivredu-HAPIH (2021.) u 2020. godini u RH od ukupnog broja uzgojno valjanih ovaca (48 923) na uzgojno valjane lakon ovce otpada 4,61 %, odnosno 2254 grla. Županije s najvećim brojem uzgojnih grla lakona su Karlovačka (1 106), Virovitičko-podravska (637) te Vukovarsko-srijemska (209). Manji uzgoji su u Bjelovarsko-bilogorskoj i Istarskoj županiji. Primjetan interes za ovom pasminom vidljiv je i kroz povećanje broj ovaca u RH za 45 % (2019. se uzgajalo 1 559 grla kod 5 uzgajivača, a 2020. godine već 2 254 grla kod 10 uzgajivača; HAPIH, 2021.). Lakon ovca (Lacaune) je poznata francuska pasmina nastala križanjem domaćih ovaca s merino pasminama i engleskom mesnatom kratkorunom pasminom South down. Ime je dobila po planinskom masivu Mont de Lacaune (800 m nadmorske visine) smještenom u jugoistočnom dijelu Francuske. Od ukupnog broja ovaca u Francuskoj na lakon mliječnu liniju otpada oko 900 000 grla. Kvalitetnom selekcijom na mliječnost provođen je odabir populacija ovaca s visokim potencijalom za mliječnost. Osim visoke proizvodnje mlijeka, ima naglašenu i proizvodnju mesa. Glavnina uzgoja je u područjima Roquerforta, poznatijem po proizvodnji sireva (osobito poznati punomasni, tvrdi sir Roquefort). Lakon ovca je srednje veličine, snažnije konstitucije. Ima izraženu dubinu i srednje razvijenu širinu trupa. Tjelesna masa ovaca je 55-75 kg, a ovnova 80-100 kg (Antunović i Novoselec, 2021.). Tijelo joj je slabo obraslo bijelom vunom a značajne površine tijela su prekrivene kratkom bijelom dlakom (trbuh, noge, lice, uši i dr.). U laktaciji od 180 do 200 dana daje oko 200 litara mlijeka, s tim da najbolja grla daju i preko 400 litara

mlijeka (Mioč i sur., 2007.). U Francuskoj se kontrola mliječnosti lakona sustavno provodi na 512 000 grla (prosječna mliječnost je 292,6 l mlijeka kroz 168 dana; http://en.france-genetique-elevage.org/Lacaune-dairy-line.html). U RH pri provođenju kontrole mliječnosti kod lakon ovce u 3. laktaciji utvrđena je prosječna dnevna proizvodnja mlijeka od 2 kg, a ukupna proizvodnja mlijeka 400 kg u laktaciji od 197 dana (gdje je razdoblje mužnje trajalo 137 dana; HAPIH, 2021.). S obzirom na visoku mliječnost, ali i odgovarajuće mesne odlike, sve je više tražena i izvožena iz Francuske. Još 1992. godine je i službeno potvrđeno da je u 17 zemalja uvezena germplazma lakon ovce iz Francuske (Barillet i sur., 2001.). U RH su zadnjih godina započeli uzgoji lakon ovce, prije svega u cilju proizvodnje mlijeka, ali i širenju njenih uzgoja u čistoj krvi. Stoga se nameće potreba provesti praćenje fenotipskih i proizvodnih odlika lakon ovce u RH u cilju praćenja procesa prilagodbe. Cilj je ovoga rada prikazati fenotipske odlike lakon ovce u uzgojima u RH i usporediti ih s dostupnim istraživanjima za ovu pasminu provedenim u drugim zemljama.

Materijal i metode

Istraživanje fenotipskih odlika lakon ovce provedeno je na stadu od 200 grla ove pasmine na obiteljskom gospodarstvu Orkić d.o.o. u Gundincima. Odabrano je 40 ovaca u početku laktacije (60. dan), odmah po odbiću janjadi, prosječne dobi 4 godine, u 3. laktaciji. Ovce su hranjene peletiranom krmnom smjesom sa 15 % sirovih proteina u količini 1,00 kg, 600 g/dan smjesom žitarica (1/3 zob i 2/3 ječam) te sijenom lucerne po volji. Također su stočnu sol i vodu imale po volji. Mužnja ovaca je provedena dvokratno, ujutro i navečer. Fenotipske odlike praćene su mjerenjem tjelesne mase, indeksa tjelesne kondicije i uzimanjem tjelesnih mjera nakon završetka jutarnje mužnje. Tjelesna masa je određena stočnom vagom, a tjelesne mjere utvrđene su lydtinovim štapom (visina grebena, dužina trupa, te dubina i širina prsa) i stočnom vrpcom (opseg prsa i cjevanice te dužina i širina glave). Indeks tjelesne kondicije (ITK) proveden je prema Russel i sur. (1991.) s ocjenama od 1 do 5. Sve navedene fenotipske odlike provedene su od strane obučenih djelatnika. Također su izračunati indeks anamorfoznosti (opseg prsa x opseg prsa /visina grebena), tjelesnih proporcija (tjelesna masa / visina grebena x 100), tjelesne kompaktnosti (opseg prsa / dužina trupa x 100) i indeks mišićavosti (opseg prsa / visina grebena x 100). Rezultati istraživanja utvrđeni su deskriptivnom statistikom u programu SAS (9.4), procedurom MEANS te su izraženi kao srednja vrijednost, standardna devijacija (SD), minimalna i maksimalna vrijednost (MIN i MAX), standardna pogreška srednje vrijednosti (SEM) i koeficijent varijacije (CV). Povezanost između varijabli utvrđene su pomoću Pearsonovog koeficijenta.

Rezultati i rasprava

Analizom tablice 1 vidljivo je da je prosječna tjelesna masa lakon ovaca bila 60,94 kg, visina grebena 68,05 cm, dužina trupa 78,49 cm, a indeks tjelesne kondicije 3,05. Prema podacima HPA (2016.) očekivane tjelesne mjere za lakon ovcu su 60-75 kg tjelesne mase te 55-62 cm visine grebena. Marie i sur. (1996.) su u Francuskoj kod dvije linije lakon mliječne ovce (visoka i niska proizvodnja mlijeka) utvrdili veće prosječne tjelesne mase (65,1 i 64,4 kg). Istraživanja s odraslim lakon ovcama u Španjolskoj, Švicarskoj i Bugarskoj (Such i sur., 1998.; Fleish i sur., 2015. i Slavova, 2021.) su također pokazala veću prosječnu tjelesnu masu (70,6, 65-75 i 70 kg). González-García i sur. (2015.) su pri istraživanju s lakon ovcama u laktaciji u Francuskoj utvrdili u ovaca u prvoj laktaciji ili u kasnijim laktacijama prosječnu tjelesna masa 57,9 i 73,7 kg te indeks tjelesne kondicije 2,83 i 2,90 i razlike za obje tjelesne mjere su bile značajne. Utvrđeno manje odstupanje u predmetnom istraživanju u tjelesnim mjerama lakon ovaca vjerojatno je posljedica prilagodbe na nove uvjete uzgoja u RH, ali i utjecaja hranidbe. Naime, Hassoun i sur. (2017.) su u istraživanju u Francuskoj s lakon ovcom utvrdili da se pri poboljšanju obroka značajno povećava i tjelesna masa ovaca.

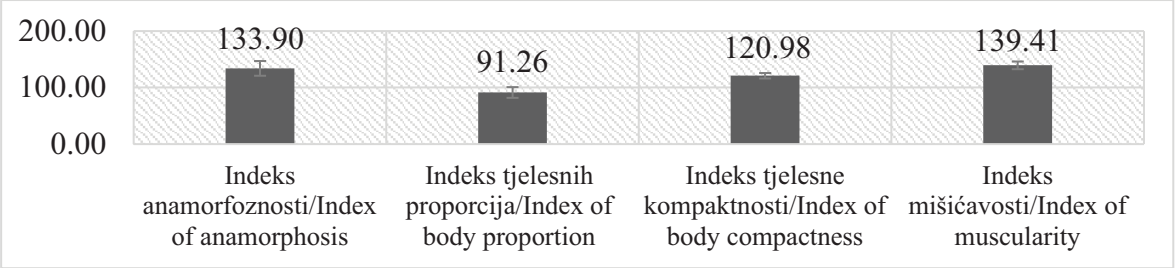
Poznato je da je tjelesna masa također vrijedan selekcijski pokazatelj, iako u mliječnom ovčarstvu ona nema takav značaj kao što je to u mesnom ovčarstvu (Slavova i sur., 2021.).

Tablica 1. Opisna statistika fenotipskih odlika lakon ovaca

Pokazatelj, cm	Srednja vrijednost	SD	SEM	MIN	MAX	CV, %
Tjelesna masa (kg)	60,94	8,91	1,45	46,30	77,50	14,62
Visina grebena	68,05	3,44	0,56	60,00	75,50	5,05
Dužina trupa	78,49	3,52	0,57	71,50	86,50	4,48
Dubina prsa	29,68	2,33	0,38	25,00	35,00	7,85
Širina prsa	20,86	1,89	0,31	16,20	25,00	9,04
Opseg prsa	94,82	5.68	0,92	86,00	110,00	5,99
Opseg cjevanice	8,68	0,43	0,07	8,00	9,50	4,90
Dužina glave	20,04	1,22	0,20	15,50	22,00	6,07
Širina glave	12,97	0,72	0,12	11,50	14,00	5,52
Indeks tjelesne kondicije	3,05	0,37	0,06	2,50	4,00	12,03

SD = standardna devijacija; SEM = srednja standardna greška; Min = minimalna vrijednost; Max = maksimalna vrijednost; CV = koeficijent varijacije (%)

Današnja istraživanja s lakon ovcama podrazumijevaju utvrđivanje njihove prilagodljivosti nakon uvoza, a uključuju i evaluaciju alternativnih sustava. Stoga se promjena fenotipskih odlika ovaca, prvenstveno tjelesne mase i indeksa tjelesne kondicije može koristiti u procjeni tjelesnih pričuva i pogodnosti hranidbe (Friggens i sur., 2007.; Antunović i sur., 2019.). Navedeno daje jasniju sliku uzgajivaču griješi li u hranidbi ovaca i janjadi te što treba poduzeti u sprječavanju utvrđenih pogrešaka (Antunović i sur., 2010.). Određivanje indeksa tjelesne kondicije važno je za kvalitetno upravljanje stadima ovaca te se koristi i kao pokazatelj zdravstvenog statusa i dobrobiti ovaca (Phythian i sur., 2012.). Poznato je da je indeks tjelesne kondicije rasplodnih ovaca za očekivati od 2,75 do 3,5 kada one imaju najveću plodnost i mliječnost (Slavova i sur., 2021.). Casamassima i sur. (2012.) su u Italiji pri hranidbi lakon ovaca s 750 g peletiranom krmnom smjesom u laktaciji od 40 dana utvrdili prosječnu tjelesnu masu od 55,32 kg, te indeks tjelesne kondicije 2,59. U istraživanju s lacon ovcama u Francuskoj Bocquier i Chilliard (1994.) su utvrdili da pri umjerenoj pothranjenosti ne dolazi do većih gubitaka tjelesne mase zbog njihove dobre prilagodbe.
U grafikonu 1 prikazani su indeksi tjelesne razvijenosti lakon ovaca. Indeks mišićavosti je bio najizraženiji (139,41), a indeks tjelesnih proporcija najmanji (91,26).



Grafikon 1. Indeksi tjelesne razvijenosti lakon ovaca

Utvrđena je značajno jaka pozitivna korelacija između većine fenotipskih odlika ovaca (0,42-0,87; Tablica 2). Nisu utvrđene značajne korelacije (od 0,20 do 0,40) između ITK i visine grebena, dužine trupa, širine prsa, opsega cjevanice i dužine glave, kao niti između dužine glave i ostalih tjelesnih mjera te širine glave i dužine trupa, opsega cjevanice i dužine glave te opseg cjevanice i visine grebena. Slične povezanosti u većine fenotipskih odlika u četiri pasmine ovaca u Iranu utvrdili su Shirzeyli i sur. (2013.), a u pasmine Karaya u Turskoj

Yilmaz i sur. (2013.). Sanson i sur. (1993.) su također utvrdili visoku povezanost između tjelesne mase i indeksa tjelesne kondicije ovaca (r =0,89).

Tablica 2. Korelacijski koeficijenti između različitih fenotipskih odlika lakon ovce

	Tjelesna masa	Visina grebena	Dužina trupa	Dubina prsa	Širina prsa	Opseg prsa	Opseg cjevanice	ITK	Dužina glave
Visina grebena	0,72 <0,001								
Dužina trupa	0,78 <0,001	0,48 0,002							
Dubina prsa	0,87 <0,001	0,73 <0,001	0,66 <0,01						
Širina prsa	0,73 <0,001	0,54 <0,001	0,61 <0,001	0,52 <0,001					
Opseg prsa	0,89 <0,001	0,62 <0,001	0,74 <0,001	0,81 <0,001	0,74 <0,001				
Opseg cjevanice	0,70 <0,001	0,382 0,018	0,62 <0,001	0,57 <0,001	0,52 <0,001	0,60 <0,001			
ITK	0,61 <0,001	0,40 0,012	0,40 0,014	0,48 0,002	0,41 0,011	0,51 0,001	0,40 0,013		
Dužina glave	0,42 0,008	0,40 0,013	0,33 0,045	0,33 0,042	0,24 0,151	0,41 0,011	0,38 0,020	0,20 0,239	
Širina glave	0,59 <0,001	0,51 0,001	0,38 0,020	0,57 <0,001	0,47 0,003	0,57 <0,001	0,42 0,010	0,44 0,006	0,40 0,014

ITK- indeks tjelesne kondicije

Zaključak

Analizom fenotipskih odlika lakon ovaca u Republici Hrvatskoj i njihovih korelacijskih koeficijenata utvrđena su manja odstupanja u usporedbi s dostupnim istraživanjima. Navedene promjene se mogu povezati s procesom prilagodbe, ali i utjecajem hranidbe.

Napomena

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Literatura

Antunović Z., Novoselec J. (2021). Ovčarstvo. U. Tehnologija animalne proizvodnje, Ur. Senčić, Đ. Fakultet agrobiotehničkih znanosti Osijek. str. 107-200.

Antunović Z., Marić I., Klir Ž., Mioč B., Novoselec J. (2019). The effect of concentrates on production traits, biochemical parameters and thyroid hormones concentration in Dubrovnik sheep fed forage based-diets. Veterinarski arhiv. 89(4): 505-518.

Antunović Z., Novoselec J., Šperanda M., Domaćinović M., Djidara M. (2010). Praćenje hranidbenog statusa janjadi iz ekološkog uzgoja. Krmiva. 52(1): 27-34.

Barillet F., Marie C., Jacquin M., Lagriffoul G., Astruc J.M. (2001). The French Lacaune dairy sheep breed:use in France and abroad in the last 40 years. Livestock Production Science. 71: 17–29.

Bocquier F., Chilliard Y. (1994). Effects of severe undernutrition on body weight and fat tissue changes in dry Lacaune ewes. Annales de zootechnie, INRA/EDP Sci. 43(3): 300.

Casamassima D., Palazzo M., Martemucci G., Vizzarri F., Corino C. (2012). Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. Small Ruminant Research. 105: 1-8.

Fleisch A., Bollwein H., Piechotta M., Janett F. (2015). Reproductive performance of Lacaune dairy sheep exposed to artifcial long days followed by natural photoperiod without and with additional progestagentreatment during the nonbreeding season. Theriogenology. 83(3): 320-325.

Friggens N.C., Berg P, Theilgaard P, Korsgaard I.R., Ingvartsen K.L., Løvendahl P., Jensen J. (2007). Breed and parity effects on energy balance profiles through lactation: evidence of genetically driven body energy change. Journal of Dairy Science. 90: 5291–305.

González-García E., Tesniere A., Camous S., Bocquie, F., Barillet F., Hassoun P. (2015). The effects of parity, litter size, physiological state, and milking frequency on the metabolic profile of Lacaune dairy ewes. Domestic Animal Endocrinology. 50: 32-44.

HAPIH (2021): Godišnje izvješće za 2020. godinu: Ovčarstvo, kozarstvo i male životinje. Hrvatska agencija za poljoprivredu i hranu. Osijek, 2021. str. 131.

HPA (2016). Godišnje izvješće za 2015. godinu: Ovčarstvo i kozarstvo. Hrvatski savez uzgajivača ovaca i koza, Zagreb str. 130.

Hassoun P., Hardy A., Tesnière A., Legarto J., De Boissieu C. (2017). Feeding strategy of Lacaune dairy sheep: Ewes fed in group according to milk yield FAO-CIHEAM Network on Sheep and Goats FAO-CIHEAM Network on Sheep and Goats. Vitoria-Gasteiz, Spain, 3-5 October 17.

Bocquier F., Barillet F. (1996). Influence du potentiel laitier sur les composantes de l'efficacite alimentaire de brebis Lacaune. Proc. Renc. Rech. Ruminants, INRA, Institut de l'Elevage. 3: 297–300.

Marie, C., Bocquier, F., Barillet, F., (1996): Influence du potentiel laitier sur les composantes de l'efficacite alimentaire de brebis Lacaune. *Proc. Renc. Rech. Ruminants*, INRA, Institut de l'Elevage 3, 297–300.

Mioč B., Pavić V., Sušić V. (2007). Ovčarstvo. Hrvatska mljekarska udruga Zagreb str. 424.

Phythian C.J., Hughe, D., Michalopoulou E., Cripps P.J., Duncan J.S. (2012).Reliability of bodyconditionscoring of sheep for cross-farm assessments. Small Ruminant Research.104: 156-162.

Russel A. (1991). Body condition scoring of sheep. In: Sheep and goat practice. Boden E. (ed.) 44.

Sanson D.W., West T.R., Tatman W.R., Riley M.L., Judkins M.B., Moss G. E. (1993). Relationship of body composition of mature ewes with condition score and body weight. Journal of Animal Science. 71(5): 1112-1116.

SAS® 9.4 (2002-2012). SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.

Shirzeyli F.H., Lavvaf A., Asadi A. (2013). Estimation of body weight from body measurements in four breeds of Iranian sheep. Songklan. Journal of Science and Technology. 35(5): 507-511.

Slavova S. (2021). Estimation of the economic efficiency of Lacaune sheep farms, based on theoretical bio-economic models. Agricultural science and technology. 13(2): 197-204.

Slavova P., Dimova N., Mihaylova M., Slavova S., Laleva S., Popova Y., Miteva D. (2021). Live weight, body condition score, body dimensions, and phenotypic correlations between them in sheep of Bulgarian dairy synthetic population. Agricultural science and technology. 13(2): 141-146.

Such X., Caja G., Pere L. (1998). Comparison of milking ability between Manchega and Lacaune dairy ewes. In:Proc.6th International Symposium Milking of Small Ruminants, Athens, Greece, September 26–October 1.EAAP Pub.95. Pudoc, Wageningen, pp.45–50.

Yilmaz O., Cemal I., Karaca O. (2013). Estimation of mature live weight using some body measurements in Karya sheep. Tropical Animal Health and Production. 45: 397-403.

Official milk recording results 2014 - Institut de l'Elevage & CNB. Raspoloživo: http://en.france-genetique-elevage.org/Lacaune-dairy-line.html

Phenotypic characteristics of Lacaune sheep in the Republic of Croatia

Abstract
In recent years, there has been a rising interest in the Republic of Croatia for sheep's milk and the breeding of dairy breeds of sheep, including Lacaune sheep. The aim of this paper is to present the phenotypic characteristics of Lacaune sheep in breeding in the Republic of Croatia and compare them with available investigations. The study was conducted on 40 sheep with an average age of 4 years in the third lactation. Among phenotypic characteristics, body mass, body measurements, indices of physical development were determined, and the correlation between phenotypic characteristics was calculated. The average body weight of sheep was 60.94 kg, the height at the withers 68.05 cm, the length of the carcass 78.49 cm, and the body condition score 3.05. A significant strong positive correlation was found between most phenotypic characteristics of sheep. Minor deviations in the phenotypic characteristics of Lacaune sheep can be associated with the process of adaptation, but also the impact of feeding.

Key words: Lacaune sheep, phenotypic traits, body weight, body measurements

IZVORNI ZNANSTVENI RAD

Stadij i redoslijed laktacije kao čimbenici odlika mliječnosti alpina koza u intenzivnom sustavu uzgoja

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Sažetak
Cilj rada bio je utvrditi utjecaj stadija i redoslijeda laktacije na proizvodnju i kemijski sastav mlijeka alpina koza u intenzivnom sustavu uzgoja. Utvrđen je značajan utjecaj (P<0,001) stadija laktacije na prosječnu dnevnu količinu mlijeka (DKM) i na analizirane pokazatelje kemijskog sastava mlijeka. Gotovo identičnu DKM koze su proizvele u ranom i srednjem stadiju (3,73 i 3,75 kg), a značajno nižu (2,67 kg) u kasnom stadiju laktacije kada je utvrđen najviši udio mliječne masti (Mm), proteina i suhe tvari bez masti (Sbm). Istraživanjem je utvrđen značajan utjecaj (P<0,001) redoslijeda laktacije na DKM, udio Mm, laktoze i Sbm. Koze su u 4. laktaciji proizvele najvišu DKM s najnižim udjelom Mm vjerojatno uslijed faktora razrijeđenja.

Ključne riječi: kozje mlijeko, laktacija, dnevna količina mlijeka, kemijski sastav mlijeka

Uvod

U intenzivnoj proizvodnji kozjega mlijeka nastoji se maksimalno iskoristiti genetski potencijal koza uz prihvatljive hranidbene troškove, pri čemu zdravlje i dobrobit životinja ne smije biti narušeno. Budući da se kozje mlijeko ponajviše prerađuje u sir, znatnije promjene njegovog kemijskog sastava tijekom laktacije nisu poželjne, ali su neizbježne uslijed djelovanja različitih čimbenika. Količina i kemijski sastav kozjega mlijeka proizvedenog tijekom laktacije variraju ponajviše uslijed genetskog čimbenika, zatim hranidbe, temperature okoline, stadija i redoslijeda laktacije, dobi koze, zdravlja životinja te menadžmenta stada (Guo i sur., 2004.; Mioč i sur., 2008.).
Stadij i redoslijed laktacije dva su značajna fiziološka čimbenika koji izravno utječu na promjene kemijskog sastava kozjeg mlijeka. Stadij laktacije ponajviše utječe na udio mliječne masti u mlijeku koji se smanjuje odmicanjem laktacije, dok se udio proteina istovremeno povećava (Garefa i sur., 1985.). Redoslijed laktacije također utječe na udio mliječne masti i proteina u mlijeku, te količinu proizvedenoga mlijeka. Carnicella i sur. (2008.) su utvrdili nižu proizvodnju mlijeka u prvojarki nego u koza koje su se jarile više puta, pri čemu su najvišu proizvodnju mlijeka utvrdili u koza u 3. i 4. laktaciji.
Iako intenzivni sustavi proizvodnje mlijeka podrazumijevaju kontrolirane uvjete držanja i hranidbe životinja, utjecaj fizioloških čimbenika nije zanemariv. Stoga je cilj ovog istraživanja bio utvrditi utjecaj stadija i redoslijeda laktacije na proizvodnju i kemijski sastav mlijeka alpina koza u intenzivnom sustavu uzgoja.

Materijal i metode

Istraživanje je provedeno na farmi alpina koza u vlasništvu obiteljskog poljoprivrednog gospodarstva koje se bavi proizvodnjom kozjeg mlijeka u mjestu Šemovec u Varaždinskoj županiji. Istraživanjem su bile obuhvaćene 72 uzgojno valjane koze pod nadzorom Hrvatske agencije za poljoprivredu i hranu (HAPIH) što podrazumijeva vođenje sveobuhvatne

evidencije o svakom pojedinom grlu. Koze su tijekom cijele godine držane u staji te je njihov osnovni obrok tijekom cijelog razdoblja istraživanja bio identičan, odnosno sastojao se od sijena djetelinsko-travne smjese i krmne smjese sa 16 % proteina. Sirovinski sastav krmne smjese bio je sljedeći: 30 % kukuruza, 15 % pšenice, 10 % ječma, 6 % zobi i 39 % superkoncentrata sa 27 % sirovih proteina. Voluminozni dio obroka koze su konzumirale po volji, a krmnu smjesu su dobivale u izmuzištu tijekom jutarnje i večernje mužnje u količini od 0,5 kg po obroku.

Kontrola mliječnosti koza provedena je AT metodom, ručnim izmuzivanjem mlijeka (naizmjenično jutarnja i večernja mužnja) od 35. dana laktacije (nakon odbića jaradi) do zasušenja (270.–280. dana laktacije), dok je vremensko razdoblje između mužnji tijekom cijele laktacije bilo 12 h (ICAR Guidelines, 2018.). Količina mlijeka po kozi na kontrolni dan utvrđena je mjerenjem količine namuzenog mlijeka graduiranom menzurom koja je preračunata na masu (kg) korištenjem faktora korekcije (1,030) za gustoću kozjeg mlijeka sukladno odredbama Pravilnika o utvrđivanju sastava sirovog mlijeka (2020.). Dnevna količina mlijeka po kozi procijenjena je množenjem utvrđene količine mlijeka na kontrolni dan x 2 (ICAR Guidelines, 2018.)

Ukupno su prikupljena 503 uzorka mlijeka za laboratorijske analize. Udjeli suhe tvari, mliječne masti, proteina, laktoze i suhe tvari bez masti u mlijeku određeni su metodom infracrvene spektrometrije na instrumentu Milkoscan FT 120, prema HRN ISO 9622:2001. Statistička obrada podataka izvršena je korištenjem statističkog paketa SAS (2015.). Prikupljeni podaci analizirani su primjenom procedure MIXED, korištenjem modela s ponovljenim mjerenjima, koji kao nezavisne varijable uključuje: stadij (rani do 90. dana laktacije, srednji od 91.-180. dana i kasni od 181. dana do kraja laktacije) i redoslijed laktacije (1., 2., 3. i 4.), dok su kao zavisne varijable u predmetni model bile uključene: dnevna količina mlijeka (DKM), udio suhe tvari, mliječne masti, proteina, laktoze, i suhe tvari bez masti.

Rezultati i rasprava

Na temelju rezultata opisnih statističkih pokazatelja prosječne dnevne količine i kemijskog sastava mlijeka alpina koza (Tablica 1.), razvidno je da su prosječne vrijednosti udjela mliječne masti, proteina i suhe tvari bez masti u analiziranim uzorcima sukladne odredbama Pravilnika o utvrđivanju sastava sirovog mlijeka (2020.). Prosječna dnevna proizvodnja alpina koza bila je 3,41 kg mlijeka sa 3,14 % mliječne masti i 2,85 % proteina. Prosječni udio suhe tvari u mlijeku bio je 11,02 %, a suhe tvari bez masti 7,88 %.

Tablica 1. Opisna statistika proizvodnje i kemijskog sastava mlijeka alpina koza (n = 503)

Svojstvo	$\overline{x}$	SD	SE	Min	Max	CV
DKM (kg)	3,41	0,99	0,04	0,82	6,80	29,02
Suha tvar (%)	11,02	0,66	0,03	9,24	14,34	5,99
Mliječna mast (%)	3,14	0,45	0,02	2,09	6,27	14,20
Protein (%)	2,85	0,29	0,01	2,22	4,06	10,29
Laktoza (%)	4,09	0,12	0,01	3,85	4,52	3,05
Sbm (%)	7,88	0,33	0,01	7,00	9,28	4,17

DKM = dnevna količina mlijeka; Sbm = suha tvar bez masti; $\overline{x}$ = srednja vrijednost; SD = standardna devijacija; SE = standardna greška; Min = minimalna vrijednost; Max = maksimalna vrijednost; CV = koeficijent varijacije (%); n = broj uzoraka mlijeka.

Istraživane alpina koze ostvarile su višu prosječnu DKM tijekom laktacije od koza iste pasmine u istraživanju Antolić i sur. (2016.), ali s relativno nižim udjelima mliječne masti i

proteina u mlijeku u usporedbi s rezultatima navedenih autora. Bolja kvaliteta mlijeka u pogledu udjela mliječne masti i proteina povezana je s nižom laktacijskom proizvodnjom mlijeka, što potvrđuju navodi više autora u čijim su istraživanjima alpina koze proizvele manje količine mlijeka, ali s višim udjelima navedenih dvaju sastojaka (Marenjak i sur., 2009.; Memiši i Stanišić, 2014.).

Utvrđen je značajan utjecaj (P<0,001) stadija laktacije na DKM, kao i na sve analizirane pokazatelje kemijskog sastava mlijeka (Tablica 2). Prosječno su najviše mlijeka alpina koze proizvodile sredinom laktacije (3,75 kg dnevno), a najmanje (2,67 kg dnevno) u kasnom stadiju laktacije, što čini razliku veću od 1 kg mlijeka dnevno. Najviše vrijednosti (P<0,001) suhe tvari, mliječne masti, proteina i suhe tvari bez masti utvrđene su u mlijeku proizvedenom u kasnom stadiju laktacije (od 181. dana do zasušenja), a najniže u srednjem stadiju laktacije (od 91. do 180. dana). Rezultat je očekivan budući da su navedeni sastojci mlijeka općenito u obrnuto proporcionalnom odnosu s visinom dnevne proizvodnje mlijeka. Naime, u mliječnih koza, i to zbog sezonske poliestričnosti, viši je udio suhe tvari, mliječne masti i proteina u mlijeku uobičajen za rani i kasni stadij laktacije (Guo i sur., 2001.; Soryal i sur., 2005.; Strzałkowska i sur., 2009.; Mestawet i sur., 2012.). Udio laktoze bio je značajno (p<0,001) najviši tijekom ranog stadija te se smanjivao prema kraju laktacije. Brojni autori također navode najviši udio laktoze u kozjem mlijeku u ranom stadiju, a najniži u kasnom stadiju laktacije (Mioč i sur., 2008.; Vacca i sur., 2010.; Mestawet i sur., 2012.). Fiziološka funkcija laktoze između ostalog je i osmotskog regulatora između krvi i mlijeka u vimenu, u usporedbi s ostalim istraživanim sastojcima mlijeka očekivano je tijekom cijele laktacije ostala prilično ujednačena (CV=3,05 %; Tablica 1).

Tablica 2. Utjecaj stadija laktacije na dnevnu količinu i kemijski sastav mlijeka alpina koza (LSM ± SE)

Svojstvo	Stadij laktacije			Razina značajnosti
	Rani (n=155)	Srednji (n=188)	Kasni (n=160)	
DKM (kg)	3,73^{a}±0,07	3,75^{a}±0,06	2,67^{b}±0,07	***
Suha tvar (%)	11,11^{a}±0,05	10,73^{b}±0,05	11,29^{a}±0,05	***
Mliječna mast (%)	3,21^{a}±0,04	3,00^{a}±0,03	3,24^{b}±0,04	***
Protein (%)	2,72^{a}±0,02	2,70^{a}±0,02	3,03^{b}±0,02	***
Laktoza (%)	4,17^{a}±0,01	4,05^{b}±0,01	4,06^{b}±0,01	***
Sbm (%)	7,88^{a}±0,02	7,73^{b}±0,02	8,05^{c}±0,02	***

DKM = dnevna količina mlijeka; Sbm = suha tvar bez masti; LSM = korigirana srednja vrijednost; SE = standardna greška; ^{a,b,c} = prosječne vrijednosti u istom redu označene različitim slovima značajno se razlikuju; *** (p<0,001); n = broj uzoraka mlijeka.

Redoslijed laktacije fiziološki je čimbenik izravno povezan s količinom i kemijskim sastavom kozjeg mlijeka (Mioč i Pavić, 2002.). Koze neovisno o pasmini u svom proizvodnom ciklusu najnižu DKM proizvode u prvoj laktaciji (Ciappesoni i sur., 2004.; Carnicella i sur., 2008.). Naime, s povećanjem dobi probavni sustav koza u potpunosti je razvijen te najveću laktacijsku proizvodnju ostvaruju koze u 3. i 4. laktaciji (Crepaldi i sur., 1999.). Istraživanjem je utvrđen značajan utjecaj (P<0,001) redoslijeda laktacije na dnevnu količinu mlijeka i udio svih istraživanih sastojaka mlijeka, osim na udio proteina (Tablica 3). Koze u 4. laktaciji ostvarile su za 0,8 kg višu prosječnu dnevnu količinu mlijeka nego prvojarke. Međutim, najveći prosječni udio suhe tvari i mliječne masti utvrđen je u mlijeku prvojarki. Najviši udio laktoze i suhe tvari bez masti utvrđen je u mlijeku alpina koza treće laktacije.

Tablica 3. Utjecaj redoslijeda laktacije na dnevnu količinu mlijeka i kemijski sastav mlijeka alpina koza (LSM ± SE)

Svojstvo (%)	Redoslijed laktacije				Razina značajnosti
	1. (n=85)	2. (n=91)	3. (n=132)	4. (n=195)	
DKM (kg)	$2,84^{a}$±0,10	$3,29^{b}$±0,10	$3,41^{b}$±0,08	$3,68^{b}$±0,07	***
Suha tvar	$11,41^{a}$±0,07	$11,11^{ab}$±0,07	$11,04^{ab}$±0,06	$10,84^{b}$±0,05	***
Mliječna mast	$3,43^{a}$±0,05	$3,21^{ab}$±0,05	$3,09^{b}$±0,04	$3,01^{b}$±0,03	***
Protein	2,80±0,03	2,82±0,03	2,84±0,03	2,80±0,02	NZ
Laktoza	$4,10^{ab}$±0,01	$4,09^{ab}$±0,01	$4,12^{a}$±0,01	$4,07^{b}$±0,01	**
Sbm	$7,88^{ab}$±0,04	$7,89^{ab}$±0,04	$7,94^{b}$±0,03	$7,83^{a}$±0,03	**

DKM = dnevna količina mlijeka; Sbm = suha tvar bez masti; LSM = korigirana srednja vrijednost; SE = standardna greška; ^{a,b,c} = prosječne vrijednosti u istom redu označene različitim slovima značajno se razlikuju; ** ($p<0,01$); *** ($p<0,001$); NZ= nije značajno; n = broj uzoraka mlijeka.

Zaključak

Iako je udio proteina u mlijeku bio nešto niži (što je nepoželjno sa stajališta mljekarske prerađivačke industrije) tijekom ranog i srednjeg stadija laktacije, navedeni nedostatak kompenziran je relativno visokom dnevnom proizvodnjom mlijeka u istome razdoblju. Prvojarke su, očekivano, proizvele najmanje mlijeka u laktaciji s istovremeno najvišim prosječnim udjelom mliječne masti, dok je udio suhe tvari bez masti u mlijeku bio na razini onoga u mlijeku koza 2. i 4. laktacije. Obzirom da je u uvjetima intenzivne proizvodnje mlijeka u Hrvatskoj prvi put provedeno istraživanje mliječnih odlika koza hranjenih prema preporukama NRC-a, prikazani rezultati daju važan doprinos budućem planiranju još uspješnije i isplativije proizvodnje kozjeg mlijeka.

Napomena

Istraživanja neophodna za ovaj rad dio su doktorske disertacije naslova „Koncentracija uree u kozjem mlijeku kao pokazatelj njegove kvalitete i hranidbe koza“, autorice dr.sc. Darije Bendelja Ljoljić.

Literatura

Antolić M., Prpić Z., Vnučec I., Jurković D., Mioč B. (2016). Laktacijske promjene proizvodnje i kakvoće mlijeka alpina koza. Objavljeno u: *Zbornik radova 51. hrvatski i 11. međunarodni simpozij agronoma*, Pospišil M., Vnučec I. (ur.), 351-355. Opatija, Hrvatska: Sveučilište u Zagrebu Agronomski fakultet.

Carnicella D., Dario M., Ayres M.C.C., Laudadio V., Dario C. (2008). The effect of diet, parity, year and number of kids on milk yield and milk composition in Maltese goat. Small Ruminant Research. 77: 71-74.

Ciappesoni G., Přibyl J., Milerski M., Mareš V. (2004). Factors affecting goat milk yield and its composition. Czech Journal of Animal Science. 49(11): 465-473.

Crepaldi P., Corti M., Ciconga M. (1999). Factors affecting milk production and prolificacy of Alpine goats in Lombardy (Italy). Small Ruminant Research. 32: 83-88.

Garefa H.M., Blanco F., Macarra B.J., Subires J. (1985). Lactation curve and milk composition of Malaga goats. Journal of Dairy Science. 81(6): 1492-1507.

Guo M.R., Dixon P.H., Park Y.W., Gilmore J.A., Kindstedt P.S. (2001). Seasonal changes in the chemical composition of commingled goat milk. Journal of Dairy Science. 84: 79-83.

Guo M., Park Y.W., Dixon P.H., Gilmore J.A., Kindstedt P.S. (2004). Relationship between the yield of cheese (Chevre) and chemical composition of goat milk. Small Ruminant Research. (1/2): 103-107.

HRN ISO. (2001). Punomasno mlijeko – Određivanje udjela mliječne masti, bjelančevina i laktoze – Uputstva za rad mid-infrared instrumenata. Hrvatski zavod za norme. Broj 9622. Zagreb, Hrvatska.

ICAR Guidelines (2018). Section 16 – Guidelines for Performance Recording in Dairy Sheep and Dairy Goats. https://www.icar.org/Guidelines/16-Dairy-Sheep-and-Goats.pdf

Marenjak T.S., Poljičak-Milas N., Piršlin J., Beer Ljubić B., Milinković Tur S. (2009). Oxidative stability and quality of raw Saanen and Alpine goats milk. Archiv Tierzucht. 52(6): 637-646.

Memiši N., Stanišić N. (2014). Influence of different growing conditions on production, milk composition and body condition score for alpina goat breed. Biotechnology in Animal Husbandry. 30(4): 635-646.

Mestawet T.A., Girma A., Ådnøy T., Devold T.G., Narvhus J.A., Vegarud G.E. (2012). Milk production, composition and variation at different lactation stages of four goat breeds in Etiopia. Small Ruminant Research. 105: 176–181.

Mioč B., Pavić V. (2002). Kozarstvo. Hrvatska mljekarska udruga, Zagreb.

Mioč B., Prpić Z., Vnučec I., Barać Z., Sušić V., Samaržija D., Pavić V. (2008). Factors affecting goat milk yield and composition. Mljekarstvo. 58 (4): 305-313.

Pravilnik o utvrđivanju sastava sirovog mlijeka (2020). Narodne novine. Broj 136 od 9. studenog.

SAS (2015). SAS Studio University Edition, release: 3.4.

Soryal K., Beyene F.A., Zeng S., Bah B., Tesfai K. (2005). Effect of goat breed and milk composition on yield, sensory quality, fatty acid concentration of soft cheese during lactation. Small Ruminant Research. 58: 275–281.

Strzałkowska N., Jóźwik A., Bagnicka E., Krzyżewski J., Horbańczuk K., Pyzel B., Horbańczuk J.O. (2009). Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation. Animal Science Papers and Reports. 27(4): 311-320.

Stage of lactation and parity as factors of some milk characteristics of Alpine goats in an intensive production system

Abstract

The aim of this study was to determine the influence of stage of lactation and parity on the production and chemical composition of alpine goat milk in an intensive breeding system. A significant influence ($P<0.001$) of lactation stage on the average daily milk yield (DMY) and on the analyzed indicators of the chemical composition of milk was determined. Almost identical DMY was determined in goats in the early and middle lactation stages (3.73 and 3.75 kg, respectively), and significantly lower (2.67 kg) in the late lactation stage when the proportion of milk fat (MF), protein, and solids non-fat (SNF) were highest. The study found a significant effect ($P<0.001$) of parity on DMY, the proportion of MF, lactose, and SNF. Goats produced the highest DMY in the 4th lactation with the lowest proportion MF, probably due to dilution factors.

Key words: goat milk, lactation, daily milk yield, chemical composition of milk

IZVORNI ZNANSTVENI RAD

Kemijski sastav i senzorska svojstva maslaca proizvedenih na tradicionalan i industrijski način

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Sažetak
U proizvodnji hrane od iznimne je važnosti potrošačima osigurati higijenski ispravan proizvod propisanog kemijskog sastava te karakterističnih senzorskih svojstava. Cilj ovog rada bio je utvrditi kemijski sastav i senzorska svojstva maslaca proizvedenih na tradicionalan način na obiteljskim poljoprivrednim gospodarstvima (OPG; n=13) i maslaca proizvedenih na industrijski način (n=19). U gotovo 39 % uzoraka maslaca proizvedenih na OPG-ima utvrđen je manji sadržaj masti od Pravilnikom propisane minimalne vrijednosti, odnosno u 53,85% uzoraka sadržaj vode bio je veći od propisanih 16 %. Maslaci s OPG-a bili su lošije ocijenjeni za sva senzorska svojstva (osobito za okus, izradu i miris) u odnosu na maslace iz industrijske proizvodnje.

Ključne riječi: maslac, kemijski sastav, senzorsko ocjenjivanje, kvaliteta

Uvod

Maslac je mliječni proizvod dobiven procesom bućkanja vrhnja i formiranja maslenih zrna. O njegovoj popularnosti govori podatak da se u Europi godišnje proizvede oko 150 milijuna litara mlijeka od čega se 29,4 % iskoristi za proizvodnju maslaca (Cook, 2018.). Prema Codex alimentarius-u (2011.) maslac je mliječni proizvod dobiven isključivo iz mlijeka i/ili proizvoda od mlijeka, u obliku emulzije vode u ulju. Pritom maslac mora sadržavati minimalno 80% mliječne masti (m.m.), do 16 % vode i do 2 % suhe tvari bez masti. Za proizvodnju maslaca može se koristiti slatko vrhnje dobiveno izdvajanjem mliječne masti iz mlijeka, kiselo vrhnje dobiveno fermentacijom te zakiseljeno slatko vrhnje dobiveno od slatkog vrhnja u koje je naknadno dodana mliječna kiselina (Mallia, 2008.). U vrhnju se nalaze sve komponente okusa i mirisa mlijeka pa je bilo koja neželjena komponenta lošeg okusa sadržana u vrhnju dvostruko izražena u maslacu (Samaržija, 2011.). Neželjeni spojevi u vrhnju uglavnom potječu od loše hranidbe muznih krava ili nehigijenske pohrane sirovog mlijeka. Najkritičnije točke u proizvodnji maslaca su vrijeme proteklo nakon toplinske obrade mlijeka i promjene temperature tijekom njegove pohrane i distribucije (Samaržija, 2011.). U tom smislu, njihove se senzorske karakteristike razlikuju. Primjerice, senzorskim karakteristikama doprinosi tehnološki postupak toplinske obrade vrhnja. Ona se obično provodi u cilju osiguravanja mikrobiološke kvalitete i sigurnosti proizvoda za konzumaciju na način da se unište vegetativne stanice patogenih bakterija i ostalih mikroorganizama (plijesni i kvasci) koji mogu imati negativan učinak na daljnju proizvodnju, uzrokovati kvarenje i skratiti rok trajanja maslaca (Ali i Fischer, 2005.). Povrh toga, toplinsku je obradu potrebno provesti i radi inaktivacije prirodnih enzima, ponajprije lipoproteinskih lipaza prisutnih u mlijeku koje mogu imati negativan učinak na okus maslaca kroz pojavu užeglog okusa (Samaržija, 2011.). Za razliku od industrijskog postupka proizvodnje maslaca, u tradicionalnoj proizvodnji ne koristi se postupak toplinske obrade mlijeka pa je takav maslac ostaje kontaminiran psihrotrofnim bakterijama, kvascima i plijesnima. Psihrotrofne su

bakterije odgovorne za proteolitičku i lipolitičku užeglost maslaca dok su kvasci i plijesni uzročnici diskoloracije i niza promjena u okusu i mirisu maslaca (Samaržija, 2021.). Cilj ovog rada bio utvrditi kemijski sastav i senzorska svojstva maslaca proizvedenih na obiteljskim poljoprivrednim gospodarstavima te ih usporediti s istovrsnim karakteristikama maslaca proizvedenih u kontroliranim industrijskim uvjetima.

Materijal i metode

Istraživanje je započeto u prosincu 2020. godine prikupljanjem ukupno 32 uzorka maslaca od kojih je 13 prikupljeno na tržnicama, a 19 s polica trgovačkih lanaca Grada Zagreba. Uzorci s tržnica potjecali su iz tradicionalne proizvodnje maslaca na OPG-ima, a oni iz trgovačkih lanaca bili su proizvedeni na klasičan industrijski način. Svi industrijski proizvedeni maslaci bili su deklarirani i pakirani u pergament-aluminijske folije ili plastične posudice dok su proizvođači s OPG-a za umatanje proizvoda koristili prozirne filmove ili plastične posudice s poklopcem bez deklaracijskih oznaka.

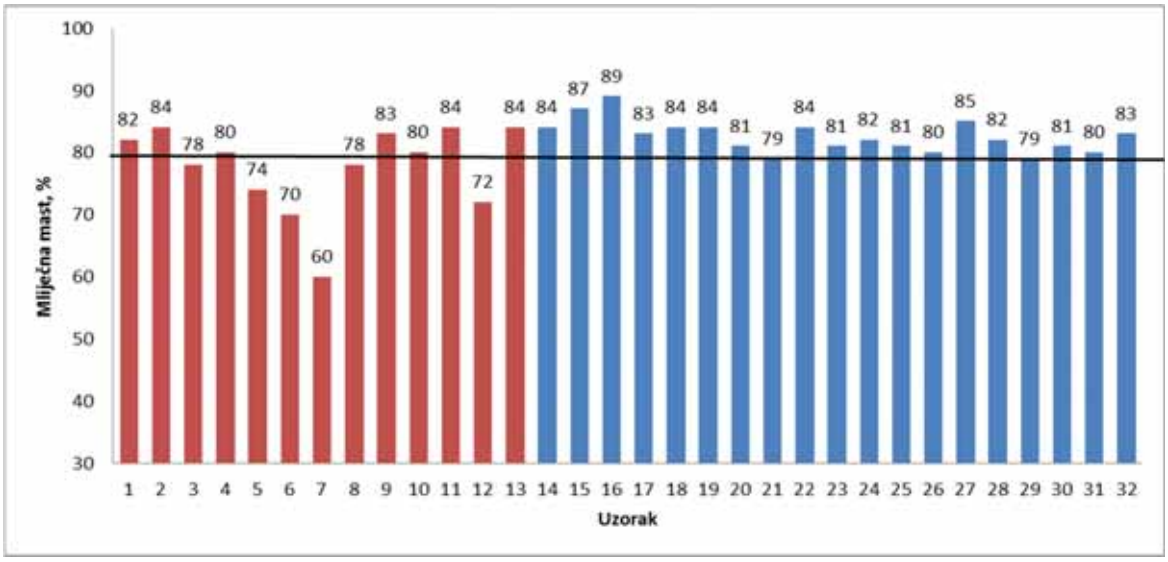
U uzorcima maslaca određen je sadržaj: suhe tvari bez masti prema normi HRN EN ISO 3727-1:2003, vode u skladu s normom HRN EN ISO 3727-1:2003 te mliječne masti butirometrijskom metodom prema Sabadoš (1996.).

Senzorsko ocjenjivanje provela je grupa od 5 ocjenjivača prema normi HRN ISO 22935-3:2009. Normom definirane senzorske karakteristike maslaca koje se ocjenjuju su: izgled (1 bod), boja (1 bod), konzistencija (2 boda), izrada (4 boda), miris (2 boda) i okus (10 bodova). Uzorci u kojima je uočena mikrobiološka kontaminacija u vidu pojave plijesni ili pjega diskvalificirani su odnosno nisu senzorski ocijenjeni.

Klasična statistička obrada podataka nije primijenjena budući da je neprimjereno uspoređivati kvalitetu maslaca proizvedenih u kontroliranim uvjetima i onih za koje uvjeti proizvodnje (toplinska obrada vrhnja, zrenje, postupak ispiranja i gnječenja maslarskog zrna, pakiranje i pohrana), nisu propisani stoga je dat prikaz vrijednosti za svaku grupu maslaca.

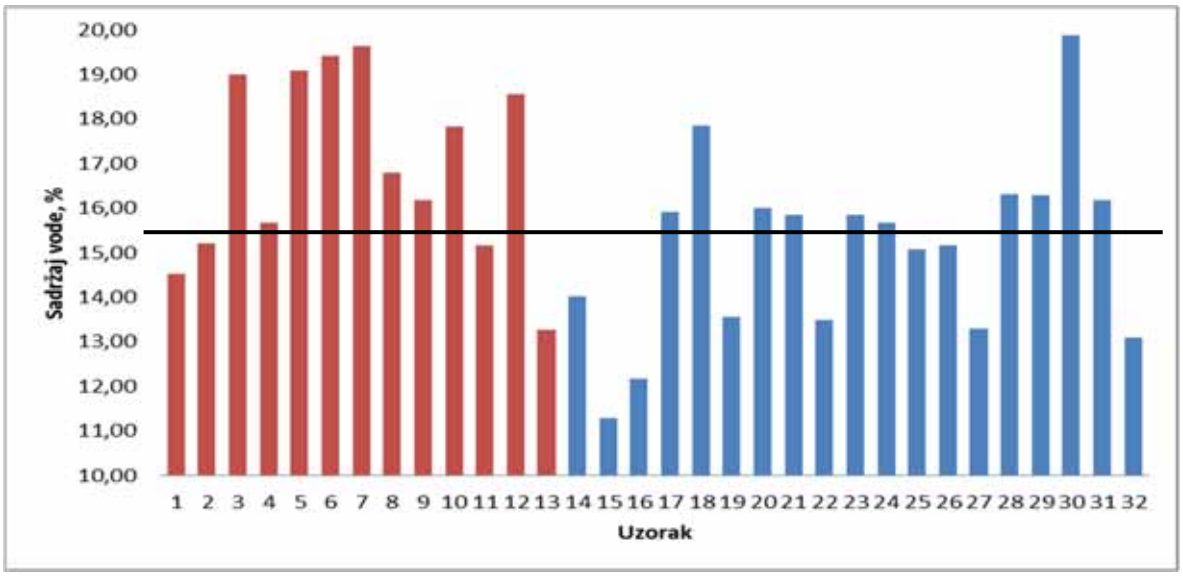
Rezultati i rasprava

Grafikon 1 prikazuje sadržaj mliječne masti u uzorcima maslaca proizvedenih tradicionalno na OPG-u odnosno na industrijski način. Prema Pravilniku o mazivim mastima (NN 41/2012) maslac je proizvod koji sadrži najmanje 80 % mliječne masti i ne više od 90 %. Od ukupno 13 uzoraka tradicionalno proizvedenih maslaca, 61,54 % udovoljavalo je uvjetima koje nalaže Pravilnik u pogledu sadržaja mliječne masti. Od 19 uzoraka iz trgovačkih lanaca, 89,47 % imalo je zadovoljavajući postotak mliječne masti dok je u 10,53 % uzoraka utvrđen manji postotak m.m. od kriterija propisanih Pravilnikom. Mogući razlozi nižeg sadržaja mliječne masti u navedenim uzorcima su loša hranidba muznih krava, loša higijena smještaja i mužnje, nestandardizirano mlijeko, premala količina mliječne masti u mlijeku i vrhnju, neadekvatna tehnologija proizvodnje u pogledu pravilnog i dostatnog gnjetenja kao i patvorenje maslaca dodatkom biljnih masti (Tratnik i Božanić, 2012.).



Grafikon 1. Sadržaj mliječne masti u uzorcima maslaca (crveno obojeni stupci - uzorci maslaca proizvedeni tradicionalno na OPG-u; plavo obojeni stupci - uzorci maslaca proizvedeni industrijski)

Iako prema Codex alimentarius-u (2011.) maslac ne bi smio sadržavati više od 16 % vode, u čak 53,85 % uzoraka maslaca s OPG-a utvrđena je viša vrijednost od propisane (Grafikon 2). Može se pretpostaviti da je to uzrokovano nepotpunom i neadekvatnom obradom maslaca koja prije svega uključuje ispiranje i gnječenje maslarskog zrna kojima se otklanja zaostala mlaćenica, ali s njom i izvjesne mane mirisa i okusa. Sam postupak gnječenja ima za cilj sjedinjavanje zrna maslaca u homogenu smjesu te reguliranje količine vode i njenu pravilnu raspodjelu u maslacu. Ovaj postupak traje onoliko dugo koliko je potrebno da se postigne željeni postotak vode u maslacu odnosno dok se izradom maslaca preostala voda u obliku finih kapljica dispergira u masu maslaca. Time se osigurava propisanih 16 % vode u finalnom proizvodu (Samaržija, 2011.). Zaostala mlaćenica u maslacu sadrži laktozu koja služi kao hrana mikroorganizmima uzročnicima kvarenja. Stoga je od iznimne važnosti osigurati što temeljitije ispiranje i istiskivanje mlaćenice te time osigurati bolju kvalitetu i trajnost maslaca.



Grafikon 2. Sadržaj vode (%) u uzorcima maslaca (crveno obojeni stupci - uzorci maslaca proizvedeni tradicionalno na OPG-u; plavo obojeni stupci - uzorci maslaca proizvedeni industrijski)

Osim izgleda, boje, okusa i mirisa, senzorske karakteristike maslaca uključuju konzistenciju te izradu. Maslac bi trebao biti bez sjaja ili masnog odsjaja (Kashaninejad i sur., 2016.). Karakterizira ga blijedo žuta do žuta boja i plastična tekstura koja podrazumijeva slabu mazivost na temperaturi hladnjaka.

Tablica 1. Senzorska svojstva maslaca proizvedenih na tradicionalan i industrijski način

Ocjena	Tradicionalno proizveden maslac						
	Izgled	Boja	Konzistencija	Izrada	Miris	Okus	Ukupno
$\bar{x}$	0,69	0,89	1,39	2,97	1,06	6,33	13,33
Min	0,5	0,5	0,75	2,0	0,5	3,0	9,25
Max	1,0	1,0	1,75	4,0	1,5	8,0	16,5
	Industrijski proizveden maslac						
$\bar{x}$	1,0	1,0	1,78	3,68	1,63	8,5	17,59
Min	1,0	1,0	1,25	3,0	1,5	5,0	11,0
Max	1,0	1,0	2,0	4,0	2,0	9,75	19,75

$\bar{x}$ = srednja vrijednost; Min = minimalna vrijednost; Max = maksimalna vrijednost

Maslaci s OPG-a bili su lošije ocijenjeni za sva senzorska svojstva u odnosu na maslace iz industrijske proizvodnje (Tablica 1). Najveće razlike u ocjenama utvrđene za okus, izradu i miris maslaca, a utvrđena je razlika i u boji maslaca. U tom smislu, maslaci proizvedeni na tradicionalan način bili su bljeđi, odnosno s nedovoljno izraženim intenzitetom karakteristične žute boje maslaca. Rezultat ne čudi budući da na boju maslaca u najvećoj mjeri utječe veličina globula mliječne masti, vrsta ambalaže, način i vrijeme pohrane (Chudy i sur., 2020.) te temperatura. Naime, gotovo 40 % maslaca proizvedenih tradicionalno imali su niži sadržaj masti od propisanih 82 % te su čuvani u neadekvatnoj ambalaži poput prozirne folije ili običnih plastičnih posudica. Osim toga, na boju maslaca utječe i manipulacija prilikom proizvodnje. Intenzivnom obradom, odnosno mehaničkom manipulacijom dolazi do stvaranja manjih kapljica vode koje uvjetuju blijedu boju maslaca (Kashaninejad i sur., 2016.).

Okus, kao najvažnije senzorsko svojstvo u ukupnoj ocjeni sudjeluje sa 10 bodova. Uz činjenicu da su 3 uzorka maslaca proizvedenih na tradicionalan način diskvalificirana zbog pojave plijesni i ružičastih naslaga na površini te nisu ulazili u prosjek za svojstvo okusa, ostalih 10 uzoraka ostvarili su svega 6,33 boda, a oni industrijski proizvedeni 8,5 bodova. Kao česta zamjerka odnosno pogreška okusa maslaca s OPG-a bila je užeglost, metalan okus ili okus po kartonu. Nepoželjne arome maslaca mogu biti rezultat lipolize, oksidacije masti, mikrobne aktivnosti te djelovanja svjetlosti (Mallia i sur., 2008.; Deeth, 2011.).

Senzorska ocjena konzistencije maslaca proizvedenih na tradicionalan način u prosjeku je iznosila 1,39 boda, a industrijskih 1,78 boda. Općenito, konzistencija i mazivost najvažnije su fizikalne karakteristike maslaca koje visoko koreliraju. Najvažniji čimbenici koji utječu na te dvije fizikalne karakteristike jesu sadržaj masti, omjer između čvrstih i tekućih masti, podrijetlo masti i temperatura (Glibowski i sur., 2008.; Ceylan i Ozcan, 2020.). Kod maslaca je poželjna glatka i blago čvrsta te plastična tekstura, kao i da maslac pruža otpor pri rezanju što je i bilo karakteristično za industrijski proizvedene maslace dok su oni tradicionalno proizvedeni imali rahlu, mrvičastu odnosno rastresitu konzistenciju.

Zaključak

Za proizvodnju kvalitetnog maslaca osim kvalitetne sirovine u pogledu higijenske kvalitete i kemijskog sastava mlijeka od iznimne je važnosti voditi računa o svim fazama proizvodnje maslaca. To se prije svega odnosi na proces obrade zrna maslaca u cilju dobivanja proizvoda vrhunske kvalitete i poželjnih senzorskih karakteristika. Naime, eventualno zaostala

mlaćenica u značajnoj mjeri može utjecati na pojavu pogrešaka maslaca i formiranje neželjenih aroma gotovog proizvoda. S obzirom na utvrđena odstupanja od Pravilnika u pogledu kemijskog sastava maslaca proizvedenih na tradicionalan način, potrebno je više educirati proizvođače, ali i provoditi učestalije kontrole ispravnosti njihovih proizvoda.

Literatura

Ali A.A., Fischer R.M. (2005). Implementation of HACCP to Bulk Cream and Butter Production Line. Food Reviews International. 21(2): 189–210.

Ceylan O., Ozcan T. (2020). Effect of the cream cooling temperature and acidification method on the crystallization and textural properties of butter. LWT. 132: 109806 .

Chudy S., Bilska A., Kowalski R., Teichert J. (2020). Colour of milk and milk products in CIE L*a*b* space. Medycyna Weterynaryjna. 76(2): 77-81.

Codex Alimentarius (2011). Codex Standard for Butter, Codex Standard 279-1971, Milk and Milk Products. World Health Organization and Food and Agriculture. Organizacija Ujedinjenih Naroda, Rim. 36-37.

Cook E. (2018). Farm production, Milk. U: Agriculture, forestry and fishery statistics (ur. Cook, E.). Eurostat, 62-63.

Deeth H.C. (2011). Milk Lipids: Lipolysis and Hydrolytic Rancidity. Encyclopedia of Dairy Sciences, 721–726.

Glibowski P., Zarzycki P., Krzepkowska M. (2008). The Rheological and Instrumental Textural Properties of Selected Table Fats. International Journal of Food Properties. 11(3): 678-686.

HRN EN ISO 3727-1:2003 Maslac - Određivanje sadržaja vode, nemasnih krutina i masti- 1. dio: Određivanje sadržaja vode (Referentna metoda).

HRN ISO 22935-3 (2009). Mlijeko i mliječni proizvodi - Senzorske analize -- 3. dio: Upute o metodi za ocjenu sukladnosti sa specifikacijom proizvoda za senzorska svojstva određena bodovanjem. Hrvatski zavod za norme: Zagreb, Hrvatska.

Kashaninejad M., Razavi S.M.A., Mazaheri Tehrani M., Kashaninejad M. (2016). Effect of extrusion conditions and storage temperature on texture, colour and acidity of butter. International Journal of Dairy Technology. 70(1): 102–109.

Mallia S., Escher F., Schlichtherle-Cerny H. (2008). Aroma-active compounds of butter: a review. European Food Research and Technology. 226: 315–325.

Pravilnik o mazivim mastima. (2012). Narodne novine. Broj 41 od 30. ožujka.

Samaržija D. (2011). Fermentirana mlijeka, vrhnje i maslac, Agronomski fakultet Sveučilišta u Zagrebu.

Samaržija D. (2021). Mikroorganizmi uzročnici kvarenja sirovog mlijeka i mliječnih proizvoda. U: Mljekarska mikrobiologija. Hrvatska mljekarska udruga, Zagreb. 130-164.

Tratnik Lj., Božanić R. (2012). Maslac. U: Mlijeko i mliječni proizvodi. (ur. Tratnik, Lj., Božanić, R.). Hrvatska mljekarska udruga, Zagreb. 425-438.

Chemical composition and sensory properties of traditionally and industrially produced butters

Abstract
In food production, it is extremely important to provide consumers a hygienically safe product of the prescribed chemical composition and characteristic sensory properties. The aim of this study was to determine the chemical composition and sensory properties of butter produced in the traditional way on family farms (n = 13) and industrially produced butters (n = 19). In almost 39% of butter samples produced on family farms, a lower fat content was found than the minimum value prescribed by the Regulations, i.e. in 53.85% of samples, the water content was higher than the prescribed 16%. Butters from family farms were rated lower for all sensory properties (especially for taste, production, and smell) compared to butters from industrial production.

Key words: butter, chemical composition, sensory evaluation, quality

STRUČNI RAD

Ponašanje krava na mliječnoj farmi – primjer mliječne farme ZZ Livač

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Sažetak
Cilj ovog rada je bio promatrati i opisati ponašanje mliječnih krava u staji slobodnog načina kretanja. Kroz razdoblje od 2006. do 2017. godine ukupno je promatrano 270 krava, od čega je bilo 160 holstein i 110 grla simentalske pasmine. Dobiveni rezultati su pokazali kako krave imaju značaj broj obrazaca ponaša, te je vrlo zahtjevno utvrditi jedan zajednički obrazac. To potvrđuje pretpostavku kako je svako grlo individua za sebe. Najbolji izbor za procjenu ponašanja bi bio pojedinačni pristup svakoj životinji. Istraživanja su pokazala kako na ponašanje mliječnih krava u staji sa slobodnim kretanjem ima sam farmer, koji ustvari i najviše radi s njima. Međutim, ukoliko se kravama osiguraju etološko pravilni uvjeti držanja i postupanja, one će imati bolju proizvodnost. Posebno je važno obratiti pozornost na nepravilne reakcija farmera jer one mogu dovesti do potencijalnih problema u proizvodnji.

Ključne riječi: krava, ponašanje životinja, proizvodnja mlijeka

Uvod

U suvremenom stočarstvu proizvodnja mlijeka je najvažnija i najzahtjevnija proizvodnja. Kako bi se postigla odgovarajuća razina proizvodnja, kravama je potrebno osigurati optimalne uvjete za uzgoj. Danas se velika pozornost upravo pridaje načinu držanja, hranidbi, njegi, te pravilnom odnosu prema životinjama. Sve se to radi zbog postizanja optimalnih uvjeta, a koji će se odraziti na razinu proizvodnje mlijeka, odonosno profit (Hultgren, 2001.). Procjenu ponašanja životinja treba pratiti u različitim uvjetima i situacijama (Watters i sur, 2021.) jer je to najbolji način za određivanje što je najbolje za grla i kako treba postupati s njima u cilju postizanja veće proizvodnosti.

Ponašanje životinja je teško generalizirati, jer svako je grlo jedinka za sebe koja se ponaša različito u određenim okolnostima. Teško je dati opću ocjenu ponašanja, ali se mogu izvući određeni zaključci koji će pomoći boljem razumijemo takvog ponašanja. Ponašanje se mijenja tijekom različitih fizioloških stadija životinje, kao i tijekom različitih sezonskih utjecaja (Nikkhah i Kowsar, 2012.). Ipak, najveći utjecaj na ponašanje životinja ima sam čovjek i njegovo ophođenje prema životinjama (Ekesbo i sur., 2018.).

Krave su vrlo socijalne životinje koje njeguju jake i dugotrajno povezane odnose. Kod goveda se uglavnom govori o integracijskom tipu socijalnog ponašanja koji podrazumijeva roditelje, potomstvo, seksualno udruživanje, grupe koje stvaraju životinje istog ranga i udruživanje na osnovu čimbenika životnog okruženja (Vučinić, 2006.). U prirodi životinje imaju mogućnosti slobodnog kretanja i usvajanja socijalnih odnosa, dok su na farmama životinje jedan dio ili cjelokupni život prinuđene provesti u određenim skupinama (Howery i sur., 1996.). Broom i Leaver (1978.) su u svome istraživanju došli do zaključka kako jedinke koje od rođenja rastu zajedno u odrasloj dobi mnogo lakše stvaraju zajednicu. Također, autori navode kako se hijerarhija unutar jedne skupine povećava u objektima gdje hranidba i smještaj nisu na odgovarajućoj razini.

Goveda u svojoj komunikaciji koriste i vizualne signale, poput određenog položaja glave ili tijela (Vučemilo i sur. 2019.). Krave se brzo navikavaju na uobičajene zvukove iz okoliša

odnosno fame. Međutim, pri iznenadnoj buci i nepoznatim zvukovima mogu pokazati veliko uznemirenje. Smiruju ih ugodna glazba i zvukovi niske frekvencije, te mogu upamtiti zvukove i povezati ih s prethodnim iskustvom (Ebinghaus i sur., 2018.). Smatra se kako su goveda sposobna raspoznati do 70 jedinki u stadu. Ukoliko se radi o većim skupinama izraženo pojačano agresivno ponašanje može biti posljedica međusobnog ne prepoznavanja (Matković, 2019.). Agresivno ponašanje uključuje prijetnju, kao što je spuštanje glave kako bi se istaknuli rogovi, te može dovesti i do fizičkog napada. Posebnost kod goveda je ta što krave i bikovi iskazuju agresiju, ali to se iskazuje na različite načine (Tucker, 2014.).
Krave se društvene životinje i vole živjeti u skupinama. Ovo je naročito izraženo tijekom hranjenja, jer tada je karakterističan dolazak do hranidbenog stola u skupinama. S druge strane, kada počnu da jedu tada ne vole da budu preblizu susjedne krave, jer može doći do izražaja hijerarhijski poredak, pri čemu jedinke koje su nižeg ranga izbjegavaju biti u blizini dominantne. Dominantne krave uvijek imaju prioritet tijekom hranidbe, iako često ove jedinke nisu najproduktivnije. Zbog toga je vrlo važno osigurati dovoljno hranidbenog prostora po životinji. Agresivno ponašanje posebno je opasno za bređe ili svježe oteljene krave (Mijić i Bobić, 2011.). U nepovoljnim proizvodnim uvjetima i dominantne krave su također pod stresom iz razloga što moraju održati hijerarhijski poredak. Ne preporučuje se miješati stado s različitim pasmina i životinjama različite starosne dobi jer se tada javljaju nestabilnosti u društvenim odnosima, kao i dodatno vrijeme za ponovnu uspostavu hijerarhijskih struktura (Tucker, 2014.).
U svakom stadu postoji životinja koja će se isticati i predvoditi kretanje grupe. Nije utvrđena povezanost između dominacije i vodstva. Najčešće je vođa stada srednje rangirana, najiskusnija i najstarija jedinka. Hijerarhijski promatrano, one životinje koje se zadnje kreću u stadu su i nisko na hijerarhijskoj ljestvici (Eksebo i Gunnarsson, 2018.). Cilj ovog rada je promatrati i opisati ponašanje mliječnih krava u staji slobodnog načina kretanja. Pretpostavka rada je kako je zahtjevno pronaći jedan zajednički obrazac ponašanja mliječnih krava u proizvodnim uvjetima, te da se treba promatrati svaka životinja pojedinačno.

Materijal i metode rada

Praćenje ponašanja krava je provedeno na mliječnoj farmi Zemljoradničke zadruge Livač pokraj Banja Luke, Bosna i Hercegovina. Vremensko razdoblje promatranja je bilo od 2006. do 2017. godine. Ukupno je promatrano 270 krava čiji je pasminski sastav bio 160 grla holstein i 110 grla simentalske pasmine. U proizvodnim objektima krave su imale slobodan način kretanja i uzgoja. Ležanje i odmor krava je bio u liga boksovima, izgnojavanje blatnog dijela hodnika je bilo putem skrepera, hranidba je bila putem hranidbenog stola bez uklještenja glave i vrata, te su krave slobodno prilaze istome. Mužnja krava se obavljala u stacionarnom izmuzištu sustava "riblja kost", čiji je kapacitet bio 2 x 6 mjesta sa čekalištem kapaciteta 36 grla. U ovom sustavu mužnje također se obavljala i priprema krava za mužnju (pranje i dezinfekcija vimena prije i poslije mužnje), dok se hranidba koncentriranom hranom nije obavljala. Mužnja krava se radila dvokratno u jutro i navečer s razmakom od 12 sati.

Rezultati i rasprava

Na promatranoj farmi krave su imale slobodan način kretanja unutar objekta pri čemu je svako grlo ima svoje ležište s prostirkom od slame. Mogle su se uočiti različita ponašanja životinja koja su bila pod utjecajem različitih čimbenika: pasmina životinje, starost, konstitucija, rogovi, te bređost. U ZZ Livač su promatrana grla dvije pasmine i to simentalska i crveni holštajn. Goveda su bila različite starosti. Matično podrijetlo životinja je bilo iz Švicarske i Njemačke. Podrijetlo životinja je imalo utjecaja na neke različite odlike u ponašanju. Važno je naglasiti kako su grla uvezena kao bređe junice s različitih farmi i

uvjeta držanja, što je bio jedan od pokazatelja različitosti. Bređe junice koje su uzgajane na istoj farmi, bile se bliže jedna drugoj, kako tijekom hranidbe, tako isto i kasnije kada su odlazile na mužnju. Važna značajka je također kako su u izmuzištu zauzimale mjesta jedna do druge u lameli. Ovakvo ponašanje upućuje na zaključak kako je između ovih junica, odnosno prvotelki, bio uspostavljen bolji odnos u odnosu na druge skupine. Također je bilo vidljivo ponašanje međusobne zaštite takvih jedinki.

Prilikom hranjenja, veću agresivnost su pokazivala simentalske krave, koje su bile robusnije, odnosno veće konstitucije i tjelesne mase. Važno je naglasiti kako je jedan dio simentalskih krava imao rogove što im je također davalo određene prednost u hijerarhiji u odnosu na holštajn bezrožna grla. Rogate krave najčešće su zauzimale najbolja mjesta na hranidbenom stolu, a to se posebno ogledalo prilikom podjele koncentrata. Nakon što bi pojele svoj dio predviđenog obroka često puta bi znale potisnuti bezrožnu kravu i prijeći na njeno mjesto uz konzumaciju njezinog obroka.

U grupi se obično drže i visoko bređa grla, kod kojih je jače izražen materinski osjećaj i za njih možemo reći kako na taj način štite jedna drugu od nasrtaja agresivnijih krava. Agresivnost kod simentalske pasmine prema drugim grlima polako splašnjava s porastom bređosti. To je i razumljivo jer tada više pozornosti posvećuju sebi, odnosno priključuju se grupi visoko bređih krava koje se drže zajedno.

Dolazak na mužu je problematičan kod prvotelki nakon teljenja kada ih treba tjerati u izmuzište. Mužnja za njih je nepoznanica i sigurno izaziva određenih strah. Kod starijih grla, mužnja ne predstavlja problem. Nakupljanje mlijeka u vimenu kod visokoproizvodnih krava dodatno ih tjera na mužnju. Ostala grla kreću prema izmuzištu na zvižduk radnika ili udaranjem dlana od dlan.

Grla podrijetlom iz istog stada najčešće idu zajedno na mužnju, pri čemu su isto tako zajedno i dok borave u čekalištu. Visoko produktivnija grla prva ulaze u prostor za mužnju, dok krave s manjom proizvodnjom mlijeka idu zadnje na mužnju. Ono što je bitno napomenuti da se visokobređe krave nalaze među prvima na mužnji, dok krave koje nisu bređe najčešće zaostaju na mužnji. Uzrok može biti u različitoj hormonalnoj aktivnost, pritisku mlijeka u mliječnim alveolama i veličini ploda u maternici. Krave koje prve ulaze od samog početka i zauzimaju prva mjesta u izmuzištu tijekom čitave laktacije se smještaju na ista mjesta, dok grla koja se obično nalaze na sredini nikada neće prva ući u izmuzište.

Svi ovi oblici ponašanja krava tijekom mužnje su pojedinačne značajke grla. Međutim, bez obzira na ove navedene značajke, muzač treba uvijek se glasom javiti, kako bi ga krava prepoznala i locirala. Takvim pristupom muzača, krava neće biti iznenađena kada muzač započne s pripremnim radnjama za mužnju (pranje, čišćenje vimena, te stavljanje muznih čaša na sise). U suprotnom, grlo se mogu uznemiriti, te refleksno udarati muzača nogom ili repom. Mužnja se mora obavljati s blagim pristupom kravi kako ne bi izazvali njezin strah i nagle pokrete. Također je pri mužnji uočeno ukoliko se u grupi nalaze jedna ili više plašljivih krava, one tada postaju mirnije za mužnju. Isto tako zanimljivo je i ponašanje jedne plašljive krave koja će se umiriti tijekom mužnje ako se nalazi između dvije mirnije krave.

Ukoliko muzači prave nagle pokrete ili ako su nervozni tijekom mužnje krava, takva reakcija će se prenijeti i na same životinje. Nakon pravilno završene mužnje životinje same izlaze iz izmuzišta i oslobađaju mjesta za sljedeća grla. Uplašena grla kod kojih mužnja nije kvalitetno i potpuno obavljena teže izlaze iz izmuzišta što upućuje na zaključak kako je došlo do zadržavanja mlijeka u vimenu.

Zaključak

Zaključno se može reći kako pojedinačne značajke grla imaju veliku ulogu u ponašanju tijekom boravka u staji, na hranidbenom stolu ili u izmuzištu. Najbolje bi bio pojedinačni pristup svakoj životinji, što je u farmskim uvjetima najčešće nemoguće Najvažniji čimbenici

ponašanja krava u staji su pasmina, bređost, stadij laktacije, rogatost, i sl. Međutim, presudnu ulogu na ponašanje životinja u staji ipak ima sam farmer. Sve njegove reakcije i oblici ponašanja tijekom svakodnevnih poslova na farmi, on će prenositi i na svoje životinje.
Važno je naglasiti kako je bitno kvalitetno odraditi sve pripremne radnje mužnje, kao i sam proces mužnje bez nekih nagli pokreta i sa uobičajenom rutinom. Isto tako, ako grlima osiguramo odgovarajuće uvjete smještaja, njege, hranidbe, tada može poprilično kontrolirati reakcije ponašanja grla. Kod nekih grla, bez obzira na sve ne možemo utjecati i reaktivno umanjiti, njihov temperament može biti nekontroliran. Zaključno se može reći kako su visokoproduktivna grla ipak "lakša" za držanje i mirnija za razliku od niže produktivnih.

Literatura

Abdouli H., Rekik, B., Haddad-Boubaker A. (2008). Non-nutritional factors associated with milk urea concentrations under Mediterranean conditions. World Journal of Agricultural Sciences. 4(2): 183-188.

Arunvipas P., Dohoo I.R., Van Leeuwen J.A., Keefe G.P. (2003). The effect of non-nutritional factors on milk urea nitrogen in dairy cows in Prince Edward Island, Canada. Journal of Veterinarian Medicine. 59: 83-93.

Broom D. M., Leaver J. D. (1978). The effects of group-housing or partial isolation on later social behaviour of calves. Animal behavior. 26: 1255-1263.

Ebinghaus A., Ivemeyer S., Knierim U. (2018). Human and farm influences on dairy cows´ responsiveness towards humans – a cross-sectional study.

Ekesbo I., Gunnarsson S. (2018). Farm animal behaviour: Characteristics for assessment of health and welfare. 2. izdanje. CABI Publishing, Wallingford, UK.

Hultgren J. (2001). Observational and Experimental Studies of the Influence of Housing Factors on the Behaviour and Health of Dairy Cows. Doctoral thesis Swedish University of Agricultural Sciences Uppsala.

Howery L.D., Provenza F.D., Banner R.E., Scott C.B. (1996). Differences in distribution patterns among individuals in a cattle herd. Applied Animal Behaviour Science. 49: 305–320.

Matković K. (2019). Društveno ponašanje goveda. Mljekarski list. 56, 38-41.

Mijić P., Bobić T. (2011): Neke značajke u ponašanju goveda na mliječnoj farmi. VII. Savjetovanje uzgajivača goveda u Republici Hrvatskoj, Vukovar, hotel „Lav“, 10. i 11. studenoga, 2011. Ur: Bulić, Vesna. Hrvatska poljoprivredna agencija.

Nikkhah A., Kowsar R. (2012). Seasonal and group effects on dairy cow behavior in large yards. Turkish Journal of Veterinary and Animal Sciences. 36(2): 123-129.

Soriano F.D., Polan C.E., Miller V.N. (2001). Supplementing pasture to lactating Holsteins fed a total mixed ration diet. Journal of Dairy Science 84: 2460-2468.

Tucker C. B. (2014). Ponašanje goveda. U: Ponašanje domaćih životinja, prema 2. engleskom izdanju: uvodni tekst. (Pavičić, Ž., K. Matković, ur.), Veterinarski fakultet Sveučilišta u Zagrebu, Zagreb, str. 151-160.

Vučinić M. (2006.). Ponašanje, dobrobit i zaštita životinja. Univerzitet u Beogradu, Fakultet veterinarske medicine, Beograd.

Vučemilo M., Pavičić Ž., Matković K., Ostović M. (2019): Dobrobit farmskih životinja i konja. U: Dobrobit životinja. (Pavičić, Ž., M. Ostović, ur.), Naklada Slap, Jastrebarsko, str. 109-170.

Yoon J.T., Lee J.H., Kim C.K., Chung Y.C., Kim C.H. (2004). Effects of milk production, season, parity and lactation period on variations of milk urea nitrogen concentration and milk components of Holstein dairy cows. Asian-Australian Journal of Animal Science. 17: 479-484.

Watters J. V., Krebs B. L. Eschmann C. L. (2021): Assessing Animal Welfare with Behavior: Onward with Caution. J. Zool. Bot. Gard. 2: 75–87. https://doi.org/10.3390/jzbg2010006

Behavior of dairy cows in free-range stables

Abstract
The aim of this study was to observe and describe the behavior of dairy cows in a free-range stable. During the period from 2006 to 2017, a total of 270 cows were observed, of which 160 were Holstein and 110 Simmental cattle. The obtained results showed that cows have a significant number of behavioral patterns, and it is very difficult to determine one common pattern. This confirms the assumption that each throat is an individual in itself. The best choice for behavioral assessment would be an individual approach to each animal. Research has shown that the farmer himself, who actually works with them the most, is responsible for the behavior of dairy cows in the free-range barn. However, if the cows are provided with ethologically correct housing and handling conditions, they will have better productivity. It is especially important to pay attention to improper reactions of farmers because they can lead to potential problems in production.

Key words: cows, animal behavior, milk production

IZVORNI ZNANSTVENI RAD

Informiranost upravitelja mliječnih farmi o programima križanja mesnih i mliječnih pasmina goveda

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Sažetak

Istraženo je poznavanje upravitelja mliječnih farmi o programima križanja mesnih i mliječnih pasmina goveda. Upravitelji holštajn farmi upoznatiji su s tehnologijom križanja i skloniji postavljanju programa križanja u odnosu na upravitelje simentalskih farmi. Križanje je barem jednom primijenjeno u 16,6 % farmi, uglavnom kao rezultat poznavanja programa križanja (62,9 %). Da su upoznati s križanjem smatra 36,9 % upravitelja, 15,8 % smatra da križanje može doprinijeti profitabilnosti farme, a spremno na uvođenje programa križanja je samo 9,4 % upravitelja. Rezultati istraživanja ukazuju da upravitelji mliječnih farmi nisu upoznati s obilježjima programa križanja u mliječnim farmama.

Ključne riječi: mliječne farme, programi križanja, informiranost upravitelja farmi

Uvod

Križanje u mliječnom govedarstvu nije široko prihvaćena tehnologija, a interes za križanjem započinje nedavno (Penasa, 2009.). Superiornost holštajn pasmine u proizvodnji mlijeka potaknula je njeno globalno širenje (López – Villalobos, 1988.). Unatoč širenju holštajna kao najmlječnije pasmine goveda, interes za križanjem rapidno raste, jer se sve više postavlja pitanje širih aspekata uporabe svih pasmina mliječnih goveda (McAllister, 2002.).

Križanjem mliječnih pasmina sa bikovima mesnih pasmina uzgaja se telad boljih svojstva trupova i kvalitete mesa od čistokrvne teladi (Forrest, 1980., 1981.; Drennan i Murphy, 2006.). Križanjem, mliječne farme doprinose smanjenju deficita teladi za tov, koja već sada čini značajan udio na tržištu, ali su studije o tržišnoj vrijednosti mliječne teladi na tržištu mesa vrlo rijetke (Dal Zotto, 2007.).

Nove tehnologije u mliječnim farmama mogu doprinijeti boljoj dohodovnosti proizvodnje mlijeka (Grupp, 2001.). Primjenom križanja s mesnim pasminama se doprinosi dohodovnosti cjelokupnog mliječnog poslovanja i to na način da se proizvodnja mlijeka kao glavnog proizvoda učini rentabilnijom kroz povećanje vrijednosti teleta koje je sporedan proizvod. Povećanje vrijednosti teleta kao nusproizvoda umanjuje ukupan trošak proizvodne jedinice (krave) i na taj način proizvedenu količinu mlijeka čini ekonomski dohodovnijom.

Edukacija i informiranje je značajan preduvjet napretka u svim segmentima življenja, pa tako i proizvodnji mlijeka. U slabije razvijenim zemljama u kojima je stočarstvo, posebice mliječno govedarstvo, značajno za društvenu i ekonomsku strukturu sela, utvrđeno je da upoznavanje proizvođača mlijeka s tehnologijom križanja pasmina goveda zbog većih prihoda rezultira većim interesom za proizvodnju mlijeka i samozapošljavanjem u mliječnom sektoru (Kumar i Triathi, 2011.)

Cilj rada je bio utvrditi stupanj informiranosti upravitelja mliječnih farmi o mogućnosti programa križanja mesnih i mliječnih pasmina goveda na mliječnim farmama, uz hipotezu da većina upravitelja mliječnih farmi nije dovoljno upoznata.

Materijali i metode

Informiranost upravitelja mliječnih farmi o tehnologiji križanja mliječnih krava s bikovima mesnih i kombiniranih pasmina istražena je anketiranjem 1717 upravitelja mliječnih farmi u sustavu kontrole mliječnosti (pet i više krava) tijekom ožujka 2015. godine na prostoru Grada Zagreba i 18 županija. Prema podacima Hrvatske poljoprivredne agencije (2016.) na dan 31. prosinca 2015. godine u sustav kontrole mliječnosti bilo je uključeno 56,4 % mliječnih krava (98567) od ukupnog broja krava u Republici Hrvatskoj (174805). Anketiranih 1717 mliječnih farmi činilo je 31,3 % od ukupno 5480 farmi u kontroli mliječnosti, a veličina populacije muznih krava anketiranih farmi od 29372 grla je imala udio od 29,8 % u ukupnom broju krava u sustavu kontrole mliječnosti. Prosječna veličina stada muznih krava anketiranih farmi je iznosila 17,1 grlo, a što je gotovo identično prosječnoj veličini mliječnih stada od 17,5 krava svih farmi pod kontrolom mliječnosti u u 2015. godini. Anketni upitnik izrađen je u Zavodu za menadžmentu i ruralno poduzetništvo Agronomskog fakulteta Sveučilišta u Zagrebu. U analizi prikupljenih podataka anketirane farme su podijeljene prema pasminskoj strukturi.

Rezultati i rasprava

Rezultati anketiranja upravitelja mliječnih farmi ukazuju na slabu informiranost o potencijalima križanja mesnih i mliječnih pasmina (Tablica 1). Samo 36,9 % upravitelja izjavilo je da su upoznati s križanjem, njih 34,8 % da je djelomično upoznato, a 28,3 % da nije upoznato. Najviše upoznatih s križanjem je upravitelja farmi u kojima se nalazi više pasmina, što se može objasniti da na istim farmama u praksi frekventnije provodi križanje. Sa stajališta jednopasminskih farmi, upravitelji holštajn farmi su najviše upoznati s programom križanja (38,9 %), a što je očekivano obzirom da je istima križanje od većeg interesa od upravitelja simentalskih farmi čija telad postižu veću prodajnu cijenu zbog boljih mesnih i tovnih svojstava. Dobro su informirani (42,2 %) i upravitelji farmi čija stada čine holštajn i simentalske krave, jer se na istim farmama zasigurno frekventnije javlja križanje holštajna kao mliječne i simentalske kao kombinirane pasmine goveda. Upravitelji čija matična stada čine holštajn i simentalske krave, a koji u stadu imaju veći udio holštajn krava su više upoznati s križanjem (45,9 %), a u slučaju obrnutog omjera ove dvije pasmine upravitelji takvih farmi su najslabije informirani (27,2 %). To potvrđuje da je upraviteljima holštajn farmi križanje poželjnije zbog veće prodajne i tovne vrijednosti križanog teleta. Slaba upoznatost upravitelja mliječnih farmi s prednostima i nedostacima križanja se može vjerojatno objasniti i slabom primjenom praksi, jer je križanje primijenjeno barem jednom u samo 16,9 % istraženih farmi.

Samo 15,8 % upravitelja je smatralo da bi se križanjem mesnih bikova i mliječnih krava povećala profitabilnost farmi kroz povećanje vrijednosti teleta (Tablica 1). Najviše upravitelja (48 %) je smatralo da bi se križanjem možda povećala profitabilnost, dok njih 36,2 % je smatralo da križanje ne može doprinijeti profitabilnost farme. Prema pasminskoj strukturi na farmama, najmanje upravitelja simentalskih farmi (13,8 %) je smatralo se križanjem povećava profitabilnost farme, dok je najviše upravitelja farmi smeđe pasmine smatralo je da je to možda moguće (63,6 %). Najviše upravitelja u farmama s jednakim omjerom holštajn i simentalskih krava (50 % : 50 %) je smatralo da križanje može doprinijeti profitabilnosti farme (25,2 %), da to nije moguće smatrali su upravitelji (37,7 %) u kojima je veći udio holštajn krava, a da je to možda moguće upravitelji u kojima je veći udio simentalskih krava (51,9 %). Moguće objašnjenje za to je da se u farmama s većim udjelom simentalskih krava frekventnije javljalo križanje ove dvije pasmine i odgovor na anketno pitanje vjerojatno je temeljen na iskustvu uzgoja i prodaje križane teladi. U kategoriji farmi ostalih pasmina najveći je broj upravitelja smatrao da se križanjem može povećati profitabilnost farme (32,6 %).

Najviše upravitelja (63,5 %) ne želi primijeniti križanje mliječnih krava s mesnim bikovima, slijede upravitelji koji su spremni prihvatiti križanje uz detaljno upoznavanje, dok je najmanje upravitelja koji su spremni uvesti program križanja u farmu (Tablica 1). Prema pasminskoj strukturi, najviše upravitelja (18,2 %) farmi smeđe pasmine želi ući u program križanja, dok bi se upravitelji farmi holštajn (25,7 %) i simentalske pasmine (25,9 %) podjednako odlučili za ulazak u program križanja nakon detaljnog upoznavanja s tehnologijom. Unutar skupine dvopasminskih stada holštajn i simentalskih krava, upravitelji u kojima je jednak omjer krava ove dvije pasmine najskloniji su postavljanje programa križanja u svojim stadima. U odnosu na sve pasminske strukture, najviše upravitelja ostalih farmi je koji žele ući u program križanja (17,4 %) i koji to žele nakon upoznavanja s tehnologijom (37,0 %), a što se također može objasniti već provođenjem križanja u farmama čija stada čine krave različitih pasmina.

Tablica 1. Upoznatost proizvođača mlijeka s križanjem mesnih i mliječnih pasmina na mliječnim farmama prema pasminskoj strukturi

Pasminska struktura		Broj farmi	Da li ste upoznati s križanjem mesnih i mliječnih pasmina goveda?			Smatrate li da bi se križanjem povećala profitabilnost farme?			Da li bi ste željeli ući u program križanja mliječnih krava s mesnim bikovima?		
			Upoznat sam	Nisam upoznat	Djelomično sam upoznat	Da	Ne	Možda	Da	Da, ali bi se morao detaljnije upoznati s tehnologijom	Ne
Holštajn		144	38,9	32,6	28,5	16,7	37,5	48,5	9,0	25,7	65,3
Simentalac		839	32,7	30,0	37,3	13,8	39,0	47,2	8,1	25,9	66,0
Smeđa pasmina		11	36,4	54,5	9,1	18,2	18,2	63,6	18,2	18,2	63,6
Holstein : Simentalac	>50% : <50%	135	45,9	24,4	29,6	17,8	30,4	51,9	11,9	28,1	60,0
	50% : 50%	123	41,5	22,8	35,8	25,2	24,4	50,4	13,8	35,0	51,2
	<50% : >50%	419	39,1	27,2	33,7	14,3	37,7	48,0	8,8	26,7	64,4
	Ukupno:	677	42,2	24,8	33,0	17,0	33,8	49,2	11,5	29,9	58,5
Ostale pasmine		46	50	10,9	39,1	32,6	19,6	47,8	17,4	37,0	45,7
Sveukupno:		1717	36,9	28,3	34,8	15,8	36,2	48,0	9,4	27,1	63,5

Nastavno na upite upraviteljima istaknute u Tablici 1, a kako bi se dobila informacija na temelju kojih informacija upravitelji farmi koji smatraju da se križanjem ne može povećati profitabilnost farme i zbog čega ne žele primijeniti program križanja bikovima. Na upit da navedu što smatraju nedostacima križanja mesnih i mliječnih pasmina goveda, od 1717 ispitanih upravitelja više od polovice (51,8 %) nije moglo navesti nedostatke križanja na kojima bi temeljili svoj stav. Najvećim nedostatkom križanja 12,1 % upravitelja navelo je visoku stopu remonta mliječnih stada, odnosno da se njegovom primjenom ne može uzgojiti dovoljan broj ženskih rasplodnih grla za remont. Teža teljenja zbog krupnije teladi kao nedostatak navelo je 7,3 % upravitelja, a njih 2,4 % tom nedostatku pridodaje otežan remont stada. Da se križanje smanjuje obim proizvodnje mliječne farme je smatralo 4,9 % upravitelja, križanje je smatralo neprihvatljivim 4,3 % upravitelja objašnjavajući da se bave isključivo proizvodnjom mlijeka i ne tove telad, dok 3 % upravitelja jednostavno ne zanima križanje. Ostale nedostatke koje su upravitelji istaknuli su različiti i zastupljeni su u značajno manjem udjelu (manje od 2 %). Suprotno, 1,9 % upravitelja smatra da križanje nema nedostataka. Temeljem odgovora na kojim nedostacima temelje svoj stav, razvidno je da upravitelji mliječnih farmi nisu upoznati s programima križanja.

Od istraženih 1717 mliječnih farmi, križanje u svrhu uzgoja križane teladi je primijenjeno samo na 272 farme (16,6 %). Primjena križanja na mliječnim farmama u najvećem udjelu je rezultat poznavanja programa križanja, jer je 62,9 % upravitelja istaknulo bolja tovna i mesna svojstva, veću prodajnu cijenu, lakšu prodaju i bolju otpornost križane teladi. Na drugom mjestu s udjelom od 29,3 % je skupina različitih razloga od kojih je najzastupljeniji

znatiželja (15,1 %), prelazak na tov zbog niske cijene mlijeka, popravljanje mesnih osobina holštajn pasmine krava, te da krave nisu ostajale bređe s bikom svoje pasmine i da je bolja oplodnja krava. Skupina drugih razloga odražava nepoznavanje križanja i razloga zbog kojeg bi bilo primijenjeno. Na trećem mjestu (7,7 %) su razlozi iz skupine utjecaja veterinara (preporuka, odluka, pogreška ili neraspolaganje sjemenom bikova istih pasmina kao mliječne krave. Jedan od odgovora upravitelja s udjelom od čak 11,7 %, da je bolja oplodnja krava i da krava nije ostajala bređa u umjetnoj oplodnji s bikom iste pasmine te je na preporuku veterinara osjemenjivanje učinjeno sjemenom bika mesne pasmine. Istraživanja uspješnosti umjetne oplodnje u mesnih pasmina su rijetka, a posebice koja kompariraju stupanj koncepcije u korištenju sjemena bikova mesnih i mliječnih pasmina. McWhorter i sur. (2020.) uspoređuju stupanj koncepcije mliječnih krava u oplodnji sjemenom angus i holštajn bikova i ne utvrđuju razliku, jer je uspješnost u oplodnji iznosila 53 % angus i 55,3 % holštajn bikovima, uz napomenu da se bikovi mesnih pasmina koriste često u rasplodu zdravstveno problematičnih i manje vrijednih mliječnih krava te da je potrebno provesti opsežnije istraživanje.

U križanju u 272 farme u 64,3 % su korišteni bikovi belgijsko plave pasmine, a isti su u kombinaciji sa simentalskim bikovima korišteni u 6,3 % i u kombinaciji sa šarole bikovima u 2,2 % križanja. Samo simentalske bikove u križanju koristilo je 16,5 % farmi. Kao treći po zastupljenosti u križanjima na mliječnim farmama su bikovi šarole pasmine, dok su bikovi ostalih mesnih pasmina (angus, limuzin i hereford) korišteni u samo 3,7 % križanja. Promatrano kroz sve tipove mliječnih farmi, gotovo polovica križanja primijenjena je u farmama koje uzgajaju krave holštajn i simentalske pasmine (48,5 %). Sa stajališta farmi koje uzgajaju jednu pasminu, križanje je najviše primijenjeno u simentalskim farmama (32,7 %).

Zaključak

Istraživanjem je potvrđena hipoteza da upravitelji mliječnih farmi nisu dovoljno upoznati s mogućnostima i prednostima primjene križanja krava mliječnih s bikovima mesnih pasmina. Više od polovice ispitanih upravitelja farmi (51,8 %) nije moglo dati mišljenje o križanju jer nije upoznato s mogućnostima uporabnog križanja, a 63,5 % upravitelja ne bi željelo primijeniti navedeno križanje. Slab interes za informiranje o križanju i još manju primjenjivanost u praksi, može se objasniti podatkom da samo 15,8 % upravitelja farmi smatra da križanje može doprinijeti profitabilnosti mliječnih farmi, njih 28 % smatra da je to možda moguće, dok 36,2 % smatra da križanje ne može doprinijeti profitabilnosti farme. Očekivano, upravitelji simentalskih farmi najmanje su zainteresirani za križanje obzirom da simentalac kao kombinirana pasmina ima dobre rezultate i u proizvodnji mlijeka i uzgoju tovne teladi koja je tražena na tržištu.

Literatura

Dal Zotto R., De Marchi M., Carnier P., Cassandro M., Gallo L., Brittante G. (2007). Effect of crossbreeding on market value of calves from dairy cows. European Association for Animal Producition, August 24, 2007, Dublin, Ireland.

Drennan M.J., Murphy B. (2006). A comparison of the productivity of suckler cows of different breed compsition. End of Project Report. Teagasc, Grange Beef Research Centre, Dunsany, Co., Meath. RMIS No. 4936.

Forrest R.J. (1980). A comparison on growth and carcass characteristics between Holstein-Friesian steers and Simental x Holstein (F1) crossbreds. Canadian Journal of Animal Science. 60: 591-598.

Forrest R.J. (1981). A comparison of the growth, feed efficiency and carcass characteristics between purebred Holstein-Friesian steers and Limousin x Holstein (F1) steers and heifers. Canadian Journal of Animal Science. 60: 515-521.

Grupp T. (2001). An economical revolution – Pure breeding with Fleckvieh and crossbreeding with Fleckvieh x Holstein Friesian. Bayern – Genetik GmbH. www.fleckvieh.de/Fleckviehwelt/Word/FVN_2001/seite8+9.pdf, 18.12.2013.

Hrvatska poljoprivredna agencija (2016). Govedarstvo - Godišnje izvješće 2015. Križevci

Kumar R., Tripathi H. (2011): Profitability of Cross Breeding among the Dairy Farmers. Indian Research Journal of Extension Education. 11(1): 32-38

López-Villalobos, N. (1998): Effects of crossbreeding and selection on the productivity and profitability of the New Zealand dairy industry. PhD Thesis. Massey Univ., Palmerston North, New Zealand.

McAllister A.J. (2002): Is crossbreeding the answer to question of dairy breed utilization? Journal of Dairy Science. 85: 2352-2357.

McWhorter T.M., Hutchison J.L., Norman H.D., Cole J.B., Fok G.C., Lourenco A.L., VanRaden P.M. (2020). Investigating conception rate for beef service sires bred to dairy cows and heifers. Journal of Dairy Science. 103: 10374-10382.

Penasa M. (2009): Crossbreeding effects in dairy cows. Dottorando. Universita' Degli Studi Di Padova. Scuola Di Dottorato Di Ricerca in Scienze Animali. Indirizzo: Genetica, biodiversità, biostatistica e biotechnologie ciclo XXI.

Awareness of dairy farm managers about crossbreeding programs for meat and dairy cattle breeds

Abstract

The knowledge of dairy farm managers about crossbreeding programs for meat and dairy cattle breeds was investigated. Holstein farm managers are more familiar with crossbreeding technology and more inclined to set up crossbreeding programs on farms compared to Simmental farm managers. Crossbreeding was applied at least once in 16.6% of farms, mainly as a result of knowledge of crossbreeding programs (62.9%). 36.9% of managers believe that they are familiar with crossbreeding, 15.8% believe that crossbreeding can contribute to the profitability of the farm, and only 9.4% of managers are ready to introduce a crossbreeding program. The results of the research indicate that dairy farm managers are not familiar with the characteristics of crossbreeding programs in dairy farms.

Key words: dairy farms, crossbreeding programs, informed farm managers

STRUČNI RAD

Stočarstvo u permakulturi

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Sažetak
Cilj rada je prikazati značaj stočarske proizvodnje u sustavu permakulture. Permakultura podrazumijeva harmoniju ljudi i krajolika koja na održivi način osigurava potrebnu hranu, energiju, sklonište i ostale materijalne i nematerijalne potrebe. Stočarstvo kao jedna od primarnih ljudskih djelatnosti podrazumijeva uzgoj stoke u svrhu proizvodnje hrane i drugih sirovina. Pored navedenog razvoj sekundarnih djelatnosti, posebice prehrambene i tekstilne industrije zahtijeva sve veća ulaganja i specijalizaciju stočarske proizvodnje pri čemu se narušava utjecaj na okoliš i dobrobit životinja. Istovremeno, permakultura predstavlja mogućnost održive proizvodnje uz brigu za okoliš i životinje.

Ključne riječi: okoliš, održivost, izvorne pasmine, stočarska proizvodnja

Uvod

Permakultura predstavlja široku paletu stvaranja samoodrživih sustava prateći uzorke iz prirode, te je sljedeći korak u evoluciji iskorištavanja prirodnih resursa jer na vrlo jednostavan način dopušta iznimno učinkovito korištenje krajolika, biljaka i životinja koristeći njihovu prirodnu komplementarnost (Morel i sur., 2019.). Permakultura predstavlja rezultat shvaćanja ljudi da trenutni model obrađivanja zemlje i uzgoja životinja nije dugoročno održiv, zbog čega sve više ljudi pokazuje interes za prihvaćanje samoodrživog modela koji im pruža permakultura. Glavna razlika u odnosu na ostale sustave je alternativni pristup poljoprivredi (Krebs i Bach, 2018.). Razlog zašto permakultura još nije općeprihvaćena od strane poljoprivrednika često jest strah od promjene. Holmgren (2002.), naime, pojašnjava da kao što mnogi ljudi kad ih uhvati glavobolja uzmu tabletu protiv bolova, tako se i većina poljoprivrednika u slučaju bilo kakvog problema u poljoprivrednoj proizvodnji okreće kemikalijama kao privremenom rješenju.
Stočarska proizvodnja igra veliku ulogu u permakulturnom sustavu, jer se dobivaju visokokvalitetni proizvodi, a osim toga stoka se koristi i u obradi te gnojenju tla. Stoga, perad, svinje, konji, goveda, ovce, koze i mnoge druge životinje mogu biti izrazito korisne u permakulturnom sustavu. Za permakulturu su najpogodnije robusne, izdržljive i stare pasmine domaćih životinja koje su prilagođene boravku na otvorenom, a u nekim slučajevima pogodne čak mogu biti i divlje životinje koje ispunjavaju iste zahtjeve. Većina starih, domaćih pasmina su postale jako rijetke te permakultura ujedno pomaže u promociji i zaštiti istih.

Govedarstvo u permakulturi

U permakulturi goveda se uglavnom drže na pregonskim pašnjacima i na taj način zemlja nikada nije previše iskorištena te tlo i vegetacija uvijek imaju dovoljno vremena za oporavak. Sustav pregona kod držanja goveda je važan jer korištenje goveda na istoj površini kroz duže vrijeme može uzrokovati trajno zbijanje tla. Na permakulturnoj farmi Seppa Holzera

različite vrste domaćih životinja, kao i različite pasmine goveda formiraju mala stada gdje životinje mogu razviti svoju hijerarhijsku vezu (Holzer, 1998.). Istovremeno, na farmi jedino bivoli bivaju izolirani iz grupa te ostaju blizu vode, čak imaju svoje omiljeno jezero. Holzer (1998.) pritom ističe da je vrlo bitno obratiti pažnju na društvene odnose između životinja. Poznato je da životinje formiraju prirodnu hijerarhiju unutar skupina pa prilikom formiranja skupina goveda skupinu treba formirati na način da u svakoj skupini postoji dominantni bik. Primjerice, nije dobro staviti u istu skupinu dvije muške jedinke istovjetnih konstitucijskih i kondicijskih odlika pa je preporuka da suparnički bikovi budu znatno slabiji kako bi se spriječile bilo kakve borbe među suparnicima. Također, trebaju se planirati i pregoni dovoljne veličine kako bi suparnici imali dovoljno prostora za međusobno izbjegavanje i bijeg. Vizualne prepreke u obliku šumovitih područja unutar ili između dvaju pregona pomažu u smanjivanju agresivnog ponašanja te omogućuju lakše izbjegavanje suparničkih jedinki (Holzer, 1998.).

Osim navedenoga, treba imati u vidu činjenicu da se goveda ne smije držati tako da ih se može vidjeti sa svih strana (kao u zoološkom vrtu). Vrlo je važno da goveda imaju mjesta za bijeg i da ih ljudi što manje uznemiravaju. Zato ograde oko područja u kojem se goveda drže moraju biti vrlo dobro osmišljene. Goveda znaju preskakati ogradu tako da ograde normalne visine najčešće nisu najbolja rješenja. Stoga, Holzer (1998.) navodi da se najbolji rezultati u držanju goveda ostvaruju sa 2 metra visokom električnom ogradom.

Za zaštitu od hladnoće i vremenskih nepogoda na Holzerovoj farmi najboljima su se pokazale nastambe izgrađene od balvana u koje se goveda mogu skloniti. Obzirom da se permakulturni sustav koji je osmislio Holzer (1998.) nalazio u hladnom planinskom predjelu, bilo je potrebno zaštititi stoku od hladnoće. Kako je na raspolaganju imao veliku količinu balvana iste je ugradio u teren tako da je iskoristio zemlju koja daje izolaciju i pomaže u održavanju temperature prihvatljive za goveda u vrijeme kada su vanjske temperature zraka vrlo niske. U uvjetima držanja goveda na kamenitom području (kao što je, primjerice, krš), gdje su visoke temperature tijekom gotovo cijele godine, mogle bi se koristiti nastambe od kamena. U takvim nastambama ostvaruje se dobra ventilacija uz održavanje ugodne temperature zraka i osiguranje dovoljnih površina hlada za goveda kojima duga izloženost previsokim temperaturama (i izravnom sunčevom osvjetljenju) može naškoditi.

Svinjogojstvo u permakulturi

Svinje su vrijedni i korisni „radnici" u permakulturnom držanju, poboljšavaju tlo rovanjem, oru gornji sloj zemlje i prozračuju tlo. Lako ih je precizno usmjeriti pri obradi tla, raspršivanjem hrane na određenim mjestima. Na taj način se može pripremiti neobrađena zemlja za sjetvu, a mogu se obraditi veće, kao i manje površine. Na površinama koje su teško obradive, svinje su nezamjenjive kao živi plugovi. Voćnjaci su idealni za rad svinja jer, za razliku od koza i ovaca, svinje ne oštećuju voćke. Opasnost pojavljivanja gljivica i plijesni koje se stvaraju na otpalom voću, u velikoj se mjeri može izbjeći pravovremenim puštanjem svinja u voćnjak. Ako su ispravno usmjerene, svinje mogu biti i veliki pomagači u suzbijanju brzorastućih korova (Holzer, 1998.). Svinje, dakle, mogu poslužiti kao dobar primjer prirodnih ciklusa u permakulturnom sustavu. Naime, svinje pripremaju i gnoje tlo, biljke rastu bujnije i zdravije, voće bačeno vjetrom i korijenje zaostalo u tlu služe im za hranu. Istovremeno, puževi i neželjeni insekti i ličinke bivaju pojedeni te služe za osiguranje hranidbenih potreba svinja. Svinje na otvorenom su zdravije, manji je trošak hranidbe, jer dobivaju manje koncentriranih krmiva nego svinje držane u industrijskim sustavima. Jedan od najvećih troškova čini ograđivanje površina na kojima se drže svinje. Idealno zemljište za držanje svinja je po mogućnosti pošumljeno, dobro drenirano te koje je suho u vlažnim sezonama. Blato i mokra zemlja pogoduju razvoju različitih insekata (crvi, gujavice…), a u takvim uvjetima može doći do razvoja različitih parazitarnih bolesti, najčešće u mlade

prasadi. Zimski smještaj svinja može biti neophodan samo u jako hladnim klimatskim zonama, dok najčešće zaštićena zemljišta i suhi ležaj zadovoljavaju potrebe svinja. Prema Holzer-u (1998.) svinje su neodvojivi dio permakulture budući da se slobodnim držanjem i korištenjem pregona smanjuje potreba za ljudskim radom, kao i potrebna količina hrane za životinje. Također uzgojem svinja na ovaj način dobiva se visokokvalitetna hrana (svježe svinjsko meso, suhomesnati proizvodi), kao i mlada prasad koja može biti namijenjena prodaji.

U permakulturi nema svestranije i korisnije životinje nego je svinja, iako su one izgubile dosta starih osobina tijekom godina pod utjecajem selekcije na povećanje prinosa mesa po grlu. Stoga, suvremene (visoko selekcionirane) pasmine svinja nisu prikladne za uzgoj na otvorenom, jer teško preživljavaju zimu te često dolazi do ozljeda papaka uslijed držanja na teškom (nepristupačnom) terenu. Osim toga, suvremene pasmine nemaju više toliko razvijen nagon za rad. Iz navedenih razloga na permakulturnim farmama se drže isključivo stare, najčešće izvorne pasmine svinja. One ispunjavaju sve potrebne kriterije te su u permakulturi mnogo su vrjednije i korisnije od današnjih plemenitih pasmina svinja (Holzer, 1998.).

Peradarstvo u permakulturi

Korištenje peradi u permakulturi pronalazi svoju inspiraciju izravno iz prirode. Naime, prirodna ili divlja jata peradi rijetko prelaze od 20 do 30 jedinki od kojih su 2 do 3 mužjaci, a ostalo ženke. Nepotrebni mužjaci se protjeruju ili usmrćuju i obično postanu žrtve ptica grabljivica ili ostalih predatora. Veća jata od 40 do 60 jedinki će se razdvojiti sama od sebe i formirati dva jata. Lake pasmine su odlični tragači za hranom i pasmine kao hamburg i leghorn mogu pronaći većinu, pa čak i svu hranu na slobodnoj ispaši sa živicama i pašnjacima. Teške pasmine dobro traže hranu, ali trebaju dodatnu ishranu, posebice zimi i to većinom u obliku žitarica, grahorica, mlinskih nusproizvoda i mekinja. Sve pasmine jedu kućni i vrtni otpad, otpale voćke i sjemenke te love i jedu kukce u polju. Na dobro organiziranom pašnjaku može se očekivati da perad dobije 65 % hrane u obliku kukaca, beskralježnjaka, zelenila i žitarica, odnosno uzgajivač bi trebao pribaviti 30 do 35 % hrane, i to koncentratnih krmiva (Mollison, 1998.).

Populacije od 120 do 180 jedinki po hektaru su dovoljne da očiste otpalo voće i pognoje voćnjak. Jato te veličine se može rotirati u 12 do 16 pregona po jednom hektaru svakih 18 mjeseci. Kada unište sav korov, površinske lukovice, sjeme korova i trave, mogu se koristiti kao „kokošji traktor“ za čišćenje tla od trajnih korova. Kako se pregoni prazne, kokoši se sele, tlo se kalcificira i ratarske kulture se zasađuju. Prije povratka kokoši na istu površinu, mogu se usijavati djetelinsko-travne smjese kojima se daje dva do tri mjeseca da sazriju tako da kokoši zapravo oru i pripremaju tlo za vlastitu ispašu. Na zemljištu se zaokružena ishrana može postići opskrbom sa sjemenom bujnih biljaka, bobičastog i bujnog voća i zelenom masom djetelinsko-travnih smjesa zajedno s kukcima i njihovim ličinkama. Neki farmeri na zemljištu namjerno gomilaju izmet gdje kukci polažu svoja jajašca čije ličinke kasnije jedu kokoši (Mollison 1998.).

Uzgoj peradi dobro se slaže s mješovitim uzgojem voćnjaka, mliječnih krava i nekih žitarica. U ovakvom okolišu pronalaze puno otpada, nalaze crve iz izmeta i čiste voćnjak od otpalog voća. Može se reći da niti jedan voćnjak, jabuka i agruma zapravo ne uspijeva kako treba bez peradi kao čistača (Mollison, 1998.).

Patke se oduvijek drže zajedno s kokošima, ali im je potrebno osigurati močvarne biljke, puževe i vodu za brčkanje. One također daju oko 200 jaja godišnje, relativno su otporne na bolesti i dobri su tragači za hranom. Guske su tradicionalno dobri mali ispašivači voćnjaka, zamjenjuju kosilice i traktore u kontroli visine travnjaka. Dakle, prilikom planiranja držanja peradi u permakulturi dobro je isplanirati biljni sustav koji ima koristi od peradi, a ujedno služi kao hrana životinjama (Mollison, 1998.).

Mali preživači u permakulturi

Pažljivo kontrolirana ispaša je jedan od bitnih alata koji može izmijeniti količinu i kontinuitet vegetacije u svrhu smanjivanja potencijala nastanka požara. Tradicionalno su se za suzbijanje vegetacije u požarno rizičnim područjima koristili strojevi, ali takve metode narušavaju strukturu tla i potiče erozija, a usto je njihova upotreba skupa. Još jedna popularna metoda je bilo preventivno nagaranje, ali tu postoji velika opasnost da se vatra izmakne kontroli i zbog toga je korištenje malih preživača za ciljano napasivanje najpogodnija i najjeftinija metoda. Takav način suzbijanja ne samo da smanjuje kaloričnost vegetacije, nego ujedno služi kao način hranidbe stoke.

Napasivanje ovaca i koza, osobito u mediteransko pašnjačko šumskim zajednicama pridonosi funkcioniranju tih ekosustava, čime se pospješuje protok hranjivih tvari i povećava njegova iskoristivost. Zbog nižih zimskih temperatura i nedostatka vlage tijekom ljeta, razgradnja organske tvari je niska, što rezultira nakupljanjem organskih ostataka na površini tla što predstavlja trajnu opasnost za izbijanje požara (Mioč i Pavić, 2002.; Garibović i sur., 2006.). Primjerice, koze se intenzivno koriste u Kaliforniji i Arizoni na teško pristupačnim mjestima, gdje uklanjaju vegetaciju bez uznemiravanja korijena ili uzrokovanja erozije tla. Koze, naime, imaju prirodnu sposobnost za probavu iglica crnogorice, zato se mogu koristiti za suzbijanje gustog crnogoričnog raslinja koje predstavlja veliki rizik za nastanak požara. Ovce su se koristile širom svijeta za smanjivanje rizika od požara u šumama, u tim slučajevima te ovce postaju aktivni dijelovi agro-šumskih sustava u kojima smanjuju kompeticiju između viših i nižih oblika vegetacije, a ujedno smanjuju mogućnost požara. Ovce su pogodnije za suzbijanje niskog raslinja, a koze za drvenaste kulture. Koristeći poznavanje prehrambenih navika malih preživača može se napraviti specifičan plan kontrole vegetacije za pojedino područje.

Hrvatske izvorne pasmine domaćih životinja

Izvorne pasmine domaćih životinja su ključan dio uspješnog permakulturnog stočarstva zbog svoje otpornosti i prilagođenosti uzgoju na otvorenom. Veliki broj hrvatskih izvornih pasmina različitih vrsta domaćih životinja predstavlja veliko bogatstvo i ogroman potencijal glede njihovog korištenja u permakulturi.

U permakulturne sustave, općenito, treba uvoditi pasmine koje su otporne i dobro prilagođene određenom uzgojnom području., a osobito uzeti u obzir njihovu prilagođenost klimi u kojoj se planira sustav. Tako, primjerice, Holzer (1998.) navodi da je za formiranje svojih stada u hladnom austrijskom planinskom području koristio bizone, bivole i škotska goveda.

U Republici Hrvatskoj se uzgajaju tri izvorne pasmine goveda, i to: buša, istarsko govedo i slavonsko-srijemski podolac (Barać i sur., 2011.) koje bi se, obzirom na svoje odlike, lako mogle koristiti za formiranje permakulturnih sustava u različitim agroklimatskim područjima Hrvatske.

U permakulturne sustave lako bi se moglo uključiti i hrvatske izvorne pasmine svinja: crnu slavonsku, turopoljsku i banijsku šaru koje odlikuje izuzetna otpornost i odlična prilagodljivost na slobodni način uzgoja.

Glavne odlike prilikom odabira malih preživača za permakulturni sustav su otpornost, sposobnost samostalnog hranjenja na ekstenzivnom pašnjaku i održavanje vegetacije u pogodnim odnosima gdje ima niskog i visokog raslinja. Naime, visoko raslinje može nadvladati nisko što se može spriječiti korištenjem ovaca i koza. Obzirom na činjenicu da se u Hrvatskoj uzgaja čak devet izvornih pasmina ovaca (Barać i sur., 2011.), kao i tri izvorne pasmine koza, osobito je velik potencijal razvoja permakulturnog stočarstva utemeljenog na korištenju izvornih pasmina ovaca i koza. Pritom gotove sve hrvatske izvorne pasmine ovaca i koza su, zahvaljujući izraženim odlikama otpornosti, adaptabilnosti, sposobnosti dugih

pješačenja, kao i učinkovitog iskorištavanja ekstenzivnih pašnjačkih površina, (Barać i sur., 2011.) vrlo pogodne za uzgoj u permakulturi.

Zaključak

Uzgoj stoke u permakulturnim sustavima daje priliku za revitalizaciju starih, autohtonih pasmina domaćih životinja koje, ne samo da imaju veliku tradicijsku i kulturološku vrijednost, nego su iznimno pogodne za držanje na otvorenom. Uzgojem u permakulturi stoka gnoji tlo iz kojega raste kvalitetna hrana, a sve se to događa u mirnom okruženju gdje stoka nije pod stresom, uz minimalna ulaganja, dok koristi razrađenog sustava permakulture mogu biti višestruke. S tim u vezi može se zaključiti da Hrvatska ima veliki potencijal za razvoj stočarstva u permakulturi zahvaljujući očuvanosti brojnih izvornih pasmina različitih vrsta domaćih životinja.

Literatura

Barać Z., Bedrica Lj., Čačić M., Dražić M., Dadić M, Ernoić M., Fury M., Horvath Š., Ivanković A., Janječić Z., Jeremić J., Kezić N., Marković D., Mioč B., Ozimec R., Petanjek D., Poljak F., Prpić Z., Sindičić M. (2011). Zelena knjiga izvornih pasmina hrvatske. Ministarstvo zaštite okoliša i prirode, Državni zavod za zaštitu prirode, Zagreb.

Holmgren D. (2002). Permaculture: Principles & Pathways Beyond Sustainability. Holmgrem design services, Hepburn.

Holzer S. (1998). Sepp Holzer's permaculture : a practical guide to small-scale, integrative farming and gardening. Chelsea Green Publishing, Vermont.

Garibović Z., Pavić V., Mioč B., Prpić Z., Vnučec I. (2006). Važnost ovčarstva u hrvatskim priobalnim područjima. Agronomski glasnik. 68(6): 509-522.

Krebs J., Bach S. (2018). Permaculture—Scientific Evidence of Principles for the Agroecological Design of Farming Systems. Sustainability 10: 3218.

Mioč B., Pavić V. (2002). Kozarstvo. Hrvatska mljekarska udruga, Zagreb.

Mollison B. (1988). Permaculture: A Designers' Manual. Tagari publications.

Morel K., Léger F., Sass Ferguson R. (2019). Permaculture. In: Fath, Encyclopedia of Ecology, 2nd edition, 4: 559–567. Oxford: Elsevier.

Animal husbandry in permaculture

Abstract

The aim of this paper is to show the importance of livestock production in the permaculture system. Permaculture means the harmony of people and landscape that provides the necessary food, energy, shelter, and other material and immaterial needs in a sustainable way. Livestock production, as one of the primary human activities, involves breeding of livestock for the purpose of producing food and other raw materials. In addition, the development of ancillary activities, especially the food and textile industries, requires increasing investments and livestock specialization, which impairs the impact on the environment and animal welfare. At the same time, permaculture represents the possibility of sustainable production with respect for the environment and animals.

Key words: environment, sustainability, indigenous breeds, livestock production

IZVORNI ZNANSTVENI RAD

Usporedba računske i direktne metode određivanja kolesterola lipoproteina niske gustoće u plazmi krava Holstein pasmine

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Sažetak
Cilj ovoga rada bio je usporediti vrijednosti kolesterola lipoproteina niske gustoće (LDL-K) određene direktnom (putem komercijalnog kita) i računskom metodom (putem Friedwaldove jednadžbe) kod krava Holstein pasmine na dvjema farmama (A i B). Ovim istraživanjem utvrđena je značajna i jaka povezanost LDL-K vrijednosti dobivenih direktnom i računskom metodom. Korelacija rezultata LDL-K dobivenih putem direktne i računske metode na objema farmama iznosila je iznad 0,97. Međutim, korelacija vrijednosti LDL-K između korištenih metoda razlikovala se ovisno o dnevnoj proizvodnji mlijeka (DKM). Kod krava čija je DKM bila ispod 40,5 kg korelacija je iznosila 0,94 dok je kod krava čija je DKM bila iznad 40,5 kg korelacija je iznosila 0,87. Dobiveni rezultati pokazali su da je Friedwaldova jednadžba dovoljno precizna metoda za računsko određivanje LDL-K kod mliječnih krava Holstein pasmine.

Ključne riječi: mliječne krave, kolesterol, lipoproteini, Friedwaldova jednadžba

Uvod

Zlatni standard za određivanje kolesterola lipoproteina niske gustoće (LDL-K) je putem ultracentrifugiranja i beta-kvantifikacije, no ova je metoda iznimno skupa i nepraktična (Nauck i sur., 2002.). Druge direktne metode uključuju korištenje gotovih kitova, što također zahtijeva financijska sredstva. Kao alternativa direktnom određivanju LDL-K razvijene su različite jednadžbe (Miller i sur., 2010.). Od navedenih jednadžbi, iako je definirana prije pedeset godina, danas se za izračun LDL-K najčešće koristi Friedwaldova jednadžba (Friedwald i sur., 1972.). Ona za izračun koristi pokazatelje izmjerene u krvnom serumu ili plazmi kao što su: ukupni kolesterol (CHOL), trigliceridi (TGC) i kolesterol lipoproteina visoke gustoće (HDL-K).
Cilj rada bio je usporediti rezultate LDL-K dobivene direktnom i računskom metodom (putem Friedwaldove jednadžbe) kod krava Holstein pasmine.

Materijal i metode

Uzorci krvi uzorkovani su na dvjema farmama mliječnih krava Holstein pasmine. Na farmi A uzorci su prikupljeni od 24 životinje, a na farmi B od 28 životinja. Životinje su bile u 2., 3. ili 4. laktaciji, a prosječna dnevna količina mlijeka odabranih krava s farme A bila je 43,5 kg, a s farme B bila je 39,3 kg mlijeka.
Krv je uzorkovana iz repne vene u epruvete s litij heparinom te je unutar 2 sata centrifugirana, a plazma je spremljena na -80 °C do daljnjih analiza. Na automatskom biokemijskom analizatoru Beckman Coulter AU400 (Beckman Coulter, SAD) pomoću kitova Beckman Coulter (Beckman Coulter, SAD), određene su koncentracije CHOL-a,

TGC-a, HDL-K i LDL-K. Princip određivanja LDL-K zasniva se na homogenom reagensu gdje se LDL zaštiti od enzimskih reakcija kojima se razgrade svi ostali lipoproteini, a onda se putem CHO/PAP sustava kvantificira LDL-K (Miki, 1999.) Pomoću Friedwaldove formule (LDL-K = CHOL - HDL-K - (TGC/2,2) računski je izračunata koncentracija LDL-K. Putem statističkog programa STATISTICA (TIBCO Software Inc., SAD) i Shapiro–Wilk testa provjerena je normalnost distribucija promatranih pokazatelja. Usporedba rezultata direktnog i računskog određivanja LDL-a provedena je putem Pearsonove korelacije.

Rezultati i rasprava

Pokazatelji varijabilnosti vrijednosti LDL-K u plazmi krava Holstein pasmine na dvije farme prikazani su u Tablici 1. Pokazatelji povezanosti direktno i računski određenog LDL-a u plazmi krava Holstein pasmine s obzirom na farmu, broj laktacije i dnevnu proizvodnju mlijeka prikazani su na Grafikonu 1.

Određivanje kolesterola i drugih pokazatelja metabolizma lipida često se koristi u humanoj medicini s obzirom na utvrđenu povezanost količine lipida u plazmi i kardiovaskularnih bolesti (Martins i sur., 2015.). Kod mliječnih krava važna metabolička bolest vezana za promet lipida je omašćenje jetre (Kessler i sur., 2014.). Omašćenje jetre nastaje obično u prijelaznom razdoblju (početak laktacije) kada zbog negativnog energetskog balansa i velike mobilizacije neesterificiranih masnih kiselina (NEFA) kapacitet jetre nije dostatan za pakiranje triglicerida u čestice lipoproteina iznimno niske gustoće (VLDL). Lipoproteine čine centralno smješteni hidrofobni lipidi (trigliceridi i kolesterilni esteri) te periferno smješteni apolipoproteini, fosfolipidi i slobodni kolesterol. Nakon što tkiva izuzmu dio triglicerida iz VLDL-a, naposljetku nastaju čestice LDL-a koje dostavljaju kolesterol potrebitim tkivima.

Kvaliteta direktnog određivanja LDL-K putem kitova s homolognim reagensima pokazala se upitnom kada su u plazmi prisutne iznimno visoke koncentracije triglicerida (Miller i sur., 2010.). Takvu situaciju nemamo kod goveda s obzirom na iznimno niske koncentracije triglicerida u plazmi u odnosu na humanu plazmu kod pacijenata s hiperlipidemijom. Smanjenje koncentracije triglicerida u krvi krava događa se nakon teljenja, poglavito kod onih životinja kod kojih se razvija sindrom omašćenja jetre (Van den Top i sur., 2005.). Problem niskih koncentracija triglicerida u plazmi mogao bi se pokazati u slučaju korištenja Friedwaldove formule, s obzirom da je utvrđena njezina nepreciznost kod ljudi s vrlo niskim koncentracijama triglicerida manjim od 1,13 mmol/L (Ahmadi i sur., 2008.). Prosječna vrijednost triglicerida kod krava u našem istraživanju bila je 0,126 mmol/L, što je daleko ispod niskih koncentracija u ljudi. Contois i sur. (2011.) navode da se Friedwaldova formula ne može koristiti kod pacijenata koji nisu natašte, s obzirom da ne uzima u obzir kolesterol nastao u hilomikronima nakon obroka. Razlika u fiziologiji probave kod ljudi i krava kao preživača te s obzirom na hranidbu *ad libitum* uvjetuje to da mliječne krave nikada nisu natašte.

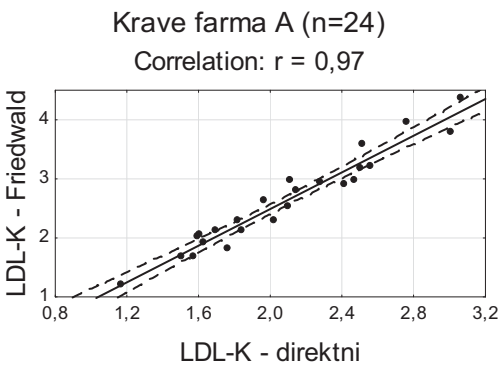
U našem istraživanju, korištenjem Pearsonove analize, utvrdili smo značajnu i jaku korelaciju vrijednosti LDL-K dobivenih direktnom metodom određivanja i računski pomoću Friedwaldove jednadžbe. Korelacija rezultata direktne i računske metode na obje farme (A i B) prelazila je preko 0,97. Ako u obzir uzmemo vrijednosti LDL-K u svih krava (farma A i B) korelacija pada na 0,91 Uzmemo li u obzir samo krave koje su u drugoj laktaciji, korelacija između direktne i indirektne metode iznosi 0,90, što je slično kao kod krava 3. i 4. laktacije (0,91). S obzirom na razred laktacije korelacija između metoda razlikuje se kod krava čija je dnevna proizvodnja mlijeka ispod 40,5 kg (0,94 u odnosu na one dnevne proizvodnje mlijeka veće od 40,5 kg (0,87). Medijan proizvodnje mlijeka promatranih krava bio je 40,5 kg pa smo sukladno tome napravili i razrede radi usporedbe dviju metoda

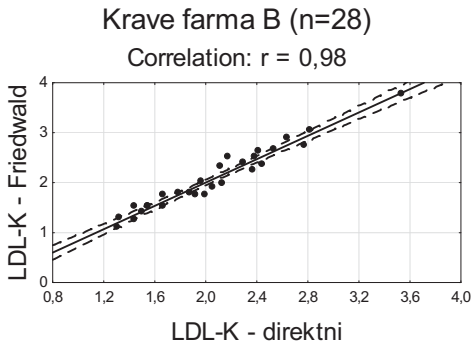
određivanja LDL-K. Velika proizvodnja mlijeka stvara rizik od negativnog energetskog balansa i veće mobilizacije NEFA-e odnosno povećanog rizika od omašćenja jetre, a time i nižih vrijednosti triglicerida. Sukladno tome i adekvatnost korištenja Friedwaldove formule se smanjuje kod krava s vrlo visokom proizvodnjom mlijeka.

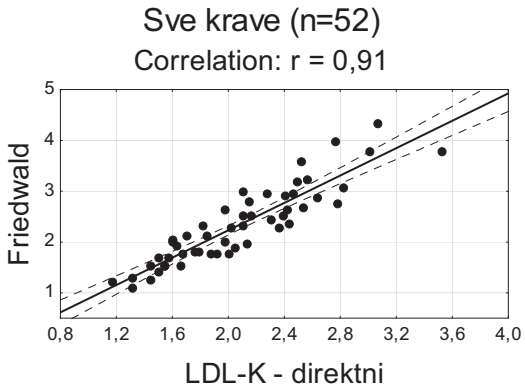
Tablica 1. Pokazatelji varijabilnosti direktno i računski određenih lipoproteina niske gustoće u plazmi krava Holstein pasmine na dvije farme

		$\bar{x}$	Medijan	Min	Max	SD
Farma A	LDL-K-direktni	2,09	2,06	1,17	3,06	0,50
(n=24)	LDL-K-Friedwald	2,63	2,58	1,21	4,35	0,79
Farma B	LDL-K-direktni	2,06	2,03	1,31	3,53	0,53
(n=28)	LDL-K-Friedwald	2,07	1,94	1,09	3,79	0,63
Sve krave	LDL-K-direktni	2,07	2,04	1,17	3,53	0,51
(N=52)	LDL-K-Friedwald	2,33	2,28	1,09	4,35	0,76

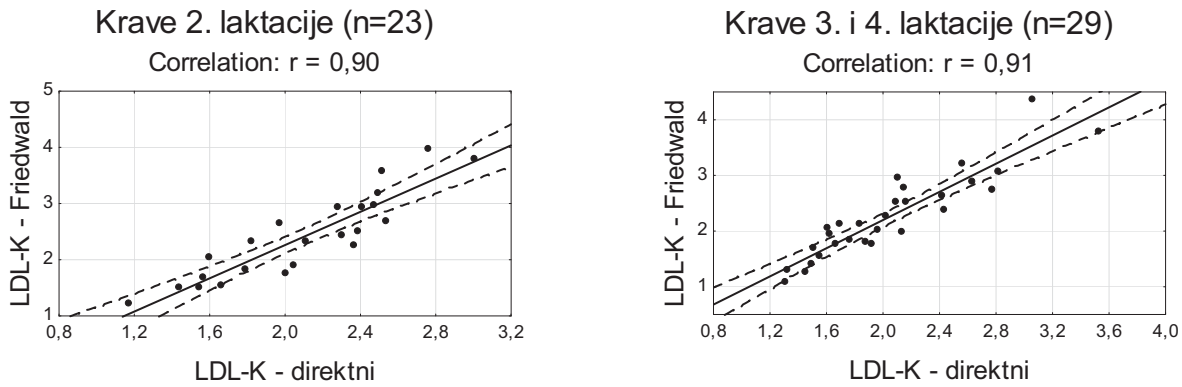
a)



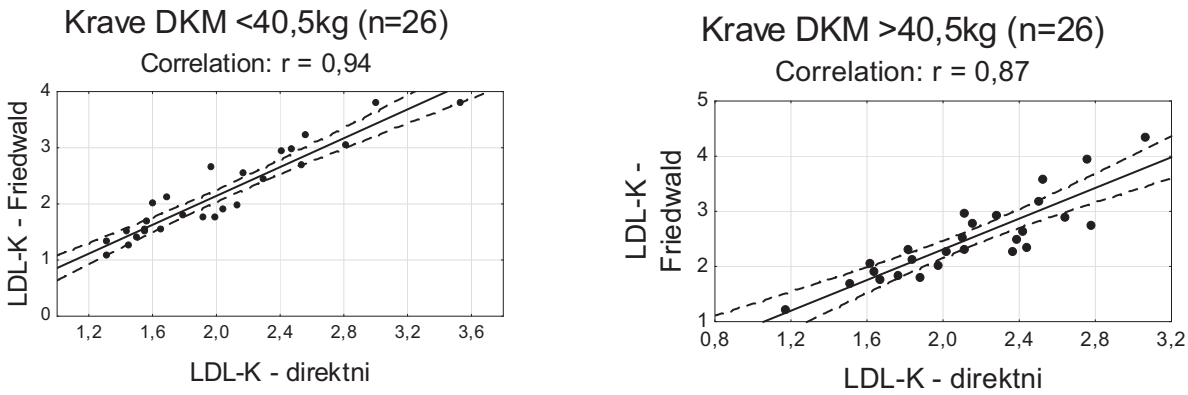




b)



c)



Grafikon 1. Pokazatelji povezanosti direktno i računski određenog kolesterola lipoproteina niske gustoće u plazmi krava Holstein pasmine s obzirom na a) farmu, b) broj laktacije i c) dnevnu proizvodnju mlijeka (DKM)

Zaključak

U našem istraživanju utvrdili smo značajnu i jaku povezanost direktne (putem komercijalnog kita) i računske (putem Friedwaldove jednadžbe) metode određivanja LDL-K. Manju preciznost Friedwaldova jednadžba pokazala je kod krava s DKM iznad 40,5 kg. S obzirom na dobivene rezultate možemo zaključiti da je Friedwaldova jednadžba dovoljno precizna za računsko određivanje LDL-K kod mliječnih krava Holstein pasmine.

Literatura

Ahmadi S. A., Boroumand M. A., Gohari-Moghaddam K., Tajik P., Dibaj S. M. (2008). The impact of low serum triglyceride on LDL-cholesterol estimation. Archives of Iranian Medicine. 11: 318–321.

Contois J. H., Warnick G. R., Sniderman A. D. (2011). Reliability of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B measurement. Journal of Clinical Lipidology. 5: 264–272.

Friedewald W. T., Levy R. I., Fredrickson D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 18: 499–502.

Kessler E. C., Gross J. J., Bruckmaier R. M., Albrecht C. (2014). Cholesterol metabolism, transport, and hepatic regulation in dairy cows during transition and early lactation. Journal of Dairy Science. 97: 5481-5490.

Martins J., Olorunju S. A., Murray L. M., Pillay T. S. (2015). Comparison of equations for the calculation of LDL-cholesterol in hospitalized patients. Clinica Chimica Acta. 444: 137-42. doi: 10.1016/j.cca.2015.01.037.

Miki Y. (1999). A homogeneous assay for the selective measurement of LDL-cholesterol in serum. Enzymatic selective protection method. Clinical Laboratory. 45: 398-401.

Miller W. G., Myers G. L., Sakurabayashi I., Bachmann L. M., Caudill S. P., Dziekonski A. (2010). Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures. Clinical Chemistry. 56: 977–86.

Nauck M., Warnick G. R., Rifai N. (2002). Methods for Measurement of LDL-Cholesterol: A Critical Assessment of Direct Measurement by Homogeneous Assays versus Calculation, Clinical Chemistry. 48: 236–254.

Van den Top A. M., Van Tol A., Jansen H., Geelen M. J., Beynen A. C. (2005). Fatty liver in dairy cows' *post-partum* is associated with decreased concentration of plasma triacylglycerols and decreased activity of lipoprotein lipase in adipocytes. Journal of Dairy Reasearch. 72: 129-37.

Comparison of the computational and direct method of low-density lipoprotein cholesterol determination in plasma of Holstein cows

Abstract

The aim of this study was to compare the results of a direct method for the determination of low-density lipoprotein cholesterol and computational determination using the Friedwald equation in Holstein cows. The correlation between the results of direct and calculation methods on both farms A and B exceeds 0.97. The correlation between the methods differs in cows whose daily milk production is below 40.5 kg (0.94) compared to those with daily milk production greater than 40.5 kg (0.87). Given the established correlations, we can conclude that the Friedwald equation is sufficiently accurate for the computational determination of LDL-K in Holstein dairy cows.

Key words: dairy cows, cholesterol, lipoproteins, Friedwald method

STRUČNI RAD

Utjecaj temperature vrhnja na trajanje tučenja i povećanje volumena šlaga

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Sažetak
Cilj istraživanja bio je utvrditi utjecaj temperature vrhnja za tučenje na trajanje i povećanje volumena tučenog vrhnja odnosno šlaga. Za potrebe istraživanja prikupljeno je ukupno dvanaest uzoraka vrhnja za tučenje s udjelom mliječne masti 30 % odnosno 36 % od četiri različita proizvođača. Od svakog proizvođača po jedan uzorak vrhnja koristio se za tučenje na temperaturama 4, 8 i 12 °C. Rezultati istraživanja pokazali su da se s povećanjem temperature vrhnja skraćuje trajanje tučenja ali i smanjuje volumen formiranog šlaga. Najveći *overrun* uz najkraće trajanje tučenja postignut je pri temperaturi od 8 °C.

Ključne riječi: temperatura, vrhnje za tučenje, trajanje tučenja, volumen šlaga

Uvod

Vrhnje za tučenje mliječni je proizvod bogat mliječnom masti, izuzetno popularan u izradi mnogobrojnih vrsta slastica. Prema Codex Standard (2018.) vrhnje za tučenje se definira kao tekuće vrhnje, rekonstituirano vrhnje i/ili rekombinirano vrhnje namijenjeno za tučenje, a da je pritom proizvedeno na način da potrošaču olakša proces tučenja. Za izradu takvog proizvoda preporuka je da udjel mliječne masti u vrhnju iznosi između 30 i 40 % (Hoffman, 2016.). Naime, zbog višeg udjela mliječne masti namirnice poput vrhnja za tučenje sposobne su tijekom tučenja formirati pjenu (Lim i sur., 2008.). Na formiranje i stabilnost pjene (šlaga) u najvećoj mjeri utječu svojstva sirovine odnosno mlijeka (starost mlijeka, sastav, podrijetlo i ostalo). Također, utjecaj imaju i tehnološki postupci proizvodnje (toplinska obrada, homogenizacija, način formiranja pjene, pH vrijednost i temperatura pri kojoj se vrhnje tuče) te sastojci poput stabilizatora i emulgatora (Ho i sur., 2020.). Tučenje vrhnja odvija se u tri faze: (I) inkorporacija mjehurića zraka uz istovremenu adsorpciju proteina na njihovu površinu, (II) adsorpcija i nakupljanje masnih globula na njihovu površinu, i (III) formiranje mreže agregata masnih globula (Hotrum i sur., 2005.). Protein koji u najvećoj mjeri okružuje mjehuriće zraka je površinski aktivan β-kazein koji se na nižim temperaturama nalazi u ne micelarnom obliku, a koji stabilizira šlag (Noda i Shiinoki, 1986.; Brooker i sur., 1986.; Anderson i sur, 1987.; Smiddy i sur., 2009.). Nadalje, niže temperature tučenja preporučuju i proizvođači pa se tučenje u kućanstvu najčešće provodi pri temperaturi vrhnja od oko 5 °C pri čemu je očekivano povećanje volumena (*overrun*) od primjerice 120 % moguće postići već nakon 1 min tučenja (Han i sur., 2018.). Ipak, Ihara i sur. (2010.) na temelju rezultata istraživanja smatraju da, za dobivanje šlaga dobre teksture uz postizanje željenog *overruna*, temperatura vrhnja za tučenje treba biti u rasponu od 7,5 do 12,5 °C.
Stoga je cilj ovog rada utvrditi utjecaj temperature od 4, 8 i 12 °C na trajanje tučenja i povećanje volumena šlaga.

Materijali i metode

Za potrebe istraživanja prikupljeno je ukupno 12 uzoraka vrhnja za tučenje od 4 različita proizvođača s udjelom mliječne masti 30 % odnosno 36 %. Uzorci vrhnja za tučenje sa 36 % mliječne masti proizvođača 1 i 2 bili su sterilizirani i homogenizirani. Uzorci vrhnja za tučenje sa 30 % mliječne masti proizvođača 3 i 4 bili su samo pasterizirani. Prije tučenja, uzorci vrhnja pohranjeni su u hladnjaku na 4 °C, a po potrebi zagrijani na 8 odnosno 12 °C. Od svakog proizvođača po jedan uzorak vrhnja se tukao na temperaturama od 4, 8 i 12 °C ručnim mikserom marke Bosch, snage 400 W.

Tučenje vrhnja provedeno je do trenutka postizanja željene čvrstoće, provjerom okretanja posude prema dolje uz izostanak pomicanja šlaga. Vrijeme potrebno za dobivanje čvrstog šlaga mjereno je štopericom. Povećanje volumena šlaga odnosno *overrun* izračunat je prema formuli (Fellows, 2009.):

$$VV\ (\%) = \frac{Vf - Vi}{Vi} \text{ x } 100$$

Vf = volumen vrhnja nakon tučenja
Vi = volumen vrhnja prije tučenja

Klasična statistička obrada podataka nije primijenjena s obzirom na različite tehnološke postupke u proizvodnji (sterilizacija, pasterizacija, homogenizacija) i različit udjel mliječne masti stoga je dat prikaz vrijednosti za svaku skupinu vrhnja za tučenje.

Rezultati i rasprava

Uobičajeno, vrhnje za tučenje na svojoj prodajnoj ambalaži ima navedenu preporučenu temperaturu hlađenja odnosno tučenja. Pritom se raspon temperature hlađenja vrhnja razlikuje između proizvođača te iznosi od 2 do 8 °C. Primjerice, Ihara i sur. (2018.) navode da je za tučenje vrhnja pogodna temperatura od 12,5 °C. Općenito, specifičnost proizvodnje tučenog vrhnja inkorporacija je mjehurića zraka u vrhnje pri čemu dolazi do povećanja njegovog volumena. Povećanje volumena ili *overrrun* se definira kao količina inkorporiranog zraka tijekom procesa tučenja vrhnja pri čemu dolazi do povećanja volumena tučenog vrhnja odnosno šlaga. U proizvodnji tučenog vrhnja *overrun* najčešće iznosi 100 – 120 % (Fellows, 2009.). Prema istraživanju Han i sur. (2018.) povećanje volumena od 120 % tučenjem steriliziranog vrhnja sa 36 % mliječne masti može se postići već za 1 min no što je trajanje tučenja duže dolazi do smanjenja *overruna*. Bruhn i Bruhn, (1988.) u svojim istraživanjima pak navode da tučenje steriliziranog vrhnja traje 40 % duže u odnosu na pasterizirano vrhnje. Toj tvrdnji idu u prilog i rezultati ovog istraživanja (Tablica 1). Za dobivanje čvrstog šlaga, najveći *overrun* uz najkraće trajanje tučenja vrhnja na temperaturi od 4, 8 i 12 °C utvrđeno je za pasterizirano vrhnje s udjelom mliječne masti od 30 % proizvođača 4. Zanimljivo, tučenje pasteriziranog vrhnja s 30 % mliječne masti proizvođača 3 rezultiralo je najmanjim *overrunom* te je trajalo najduže za sve temperaturne režime. Mogući razlog produženom trajanju tučenja mogu biti kvaliteta mlijeka odnosno vrhnja, tehnološki procesi u proizvodnji vrhnja za tučenje te „svježina“ vrhnja (Bruhn i Bruhn, 1988.). Naime, u proizvodnji vrhnja za tučenje izuzetno je važno da vrhnje bude prerađeno u što kraćem periodu.

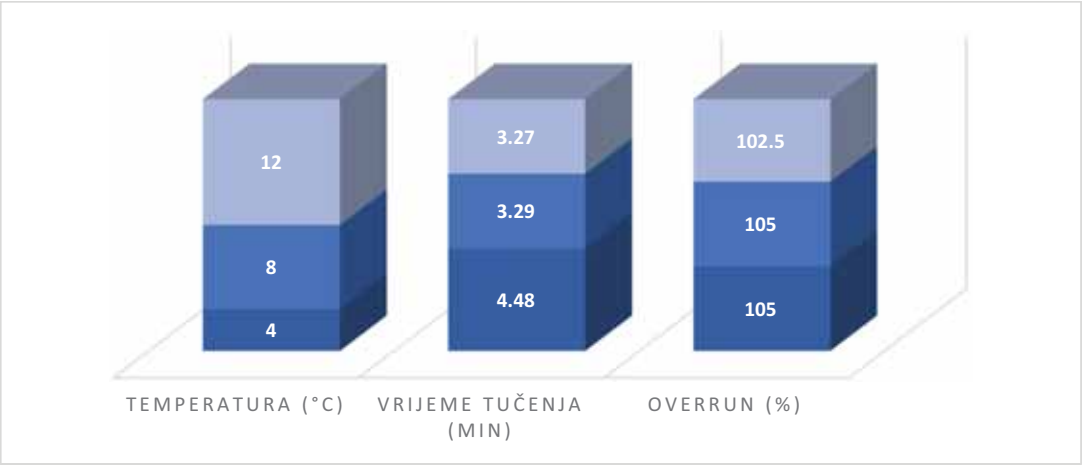
Tučenje steriliziranog vrhnja s udjelom mliječne masti od 36 % proizvođača 1 i 2 na svim je temperaturama trajalo između 2,14 i 3,51 min osim za tučenje vrhnja proizvođača 1 koje je trajalo najduže, čak 6,13 min pri 4 °C. Pritom se volumen šlaga povećavao s trajanjem tučenja neovisno o temperaturi vrhnja, što je u skladu s istraživanjem Ihara i sur. (2019.). Općenito, trajanje tučenja steriliziranog vrhnja je duže jer se danas u tehnologiji vrhnja za tučenje uobičajeno koristi postupak homogenizacije uz primjenu niskog tlaka (1 - 4,5 MPa)

radi sprečavanja izdvajanja vrhnja na površinu i stvaranja taloga tijekom pohrane (Dolenčić Špehar, 2018.).

Tablica 1. Utjecaj temperature vrhnja na trajanje tučenja i povećanje volumena šlaga

Uzorak	Udio mliječne masti (%)	Temperatura vrhnja (˚C)	Trajanje tučenja (min)	Volumen – prije tučenja (mL)	Volumen – nakon tučenja (mL)	Overrun (%)
Proizvođač 1	36	4	6,13	500	1100	120
Proizvođač 2	36	4	3,12	500	1000	100
Proizvođač 3	30	4	7,53	400	600	50
Proizvođač 4	30	4	1,56	400	1000	150
Proizvođač 1	36	8	2,53	500	1000	100
Proizvođač 2	36	8	3,02	500	1100	120
Proizvođač 3	30	8	6,30	400	600	50
Proizvođač 4	30	8	1,33	400	1000	150
Proizvođač 1	36	12	3,51	500	1050	110
Proizvođač 2	36	12	2,14	500	1000	100
Proizvođač 3	30	12	6,12	400	700	75
Proizvođač 4	30	12	1,33	400	900	125

Prosječne vrijednosti trajanja tučenja vrhnja i povećanja volumena šlaga neovisno o udjelu mliječne masti i tehnološkim postupcima u proizvodnji vrhnja za tučenje prikazane su na slici 1. Na temelju dobivenih rezultata vidljivo je da temperatura ima utjecaj na trajanje tučenja odnosno da se s povećanjem temperature vrhnja skraćuje trajanje tučenja i smanjuje volumen šlaga. Za tučenje vrhnja na temperaturi od 4 °C, koju najčešće preporučuju proizvođači, potrebno je najviše vremena za formiranje čvrstog šlaga uz prosječni *overrun* od 105 % što predstavlja prihvatljivu vrijednost. Najveći *overrun* uz najkraće trajanje tučenja postignut je pri temperaturi od 8 °C.



Slika 1. Prosječno trajanje tučenja skupine uzoraka vrhnja i povećanje volumena šlaga ovisno o temperaturi vrhnja (4, 8 i 12 °C)

Zaključak

Vrhnje za tučenje najčešće se koristi u izradi mnogobrojnih vrsta slastica. Preporuke za njegovu izradu prije svega uključuju temperaturu hlađenja vrhnja odnosno izrade šlaga koju proizvođači uobičajeno navode na svojoj prodajnoj ambalaži.

Temeljem toga, ovim je istraživanjem utvrđeno da temperatura vrhnja ima utjecaj na trajanje tučenja i povećanje volumena šlaga dok je najveći *overrun* uz najkraće trajanje tučenja postignut pri temperaturi od 8 °C.

Literatura

Anderson M., Brooker B. E., Needs E. C. (1987). The role of proteins in the stabilization/destabilization of dairy foams. U: Food Emulsions and Foams (Dickinson, E., ur.). The Royal Society of Chemistry, Cambridge. 100-109.

Brooker B. E., Anderson M., Andrews A. T. (1986). The development of structure in whipped cream. Food Microstructure. 5: 277-285.

Bruhn C. M., Bruhn J. C. (1988). Observations on the Whipping Characteristics of Cream. Journal of Dairy Science. 71:857-862.

Codex Standard (2018). Standard for cream and prepared creams CXS 288-1976, izmijenjen 2018.

Dolenčić Špehar, I. (2018). Tehnološki problemi u proizvodnji vrhnja za tučenje. 43. hrvatski simpozij mljekarskih stručnjaka s međunarodnim sudjelovanjem (Volarić, V.ur.), 7.-10. 11. 2018., Lovran, Hrvatska mljekarska udruga, Zbornik sažetaka simpozija, 37-38.

Fellows P. (2000). Properties of foods and processing theory. U: Food Processing Technology, Principles and Practice, Second Edition, (P. Fellows, ur.), Woodhead Publishing Limited and CRC Press LLC, 9-59.

Han J., Zhou X., Cao J., Wang Y., Sun, Bokang Li, Y., Zhang L. (2018). Microstructural evolution of whipped cream in whipping process observed by confocal laser scanning microscopy. International Journal of Food properties. 21(1): 593-605.

Ho T.M., Bhandari B., Bansal N. (2020). Influence of Milk Fat on Foam Formation, Foam Stability and Functionality of Aerated Dairy Products. U: Dairy Fat Products and Functionality (Truong, T., Lopez, C., Bhandari, B., Prakash, S., ur.). Springer, Cham, 583-606.

Hoffman W., Buchheim W. (2006). Significance of Milk Fat in Cream Products. U: Advanced Dairy Chemistry Volume 2 Lipids (Fox, P. F., McSweeney, P. L. H., ur.). 3rd edition. Springer, New York. 365-375.

Hotrum N. E., Cohen Stuart M. A., van Vliet T., Avino S. F., van Aken, G.A. (2005). Elucidating the relationship between the spreading coefficient, surface-mediated partial coalescence and the whipping time of artificial cream. Colloids and Surfaces. A: Physicochemical and Engineering Aspects. 260: 71-78.

Ihara K., Habara K., Ozaki Y., Nakamura K., Ochi H., Saito H., Asaoka H., Uozumi M., Ichihashi N., Iwatsuki K. (2010). Influence of whipping temperature on the whipping properties and rheological characteristics of whipped cream. Journal Dairy Science. 93: 2887-2895.

Ihara K., Nishimura Y., Habara K., Abe F. (2019). Influences of Beating Speed on Whipping and Physical Properties of Whipped Cream. Food Science and Technology Research, 25 (3): 391-397.

Lim S.-Y., Swanson G., Clark S. (2008). High Hydrostatic Pressure Modification of Whey Protein Concentrate for Improved Functional Properties. Journal of Dairy Science, 91: 1299-1307.

Noda M., Shiinoki Y. (1986). Microstructure and rheological behavior of whipping cream. Journal of Texture Studies. 17(2): 189-204.

Smiddy M. A., Kelly A. L., Huppertz T. (2009). Cream and related products. U: Dairy Fats and Related Products (Tamime, A. Y., ur.), Chichester, UK: Wiley-Blackwell. 61-85.

Influence of cream temperature on whipping duration and increase in whipped cream volume

Abstract
The aim of the study was to determine the effect of whipping cream temperature on the duration and increase in the volume of whipped cream. For the purposes of the research, twelve samples of whipping cream were collected with a milk fat content of 30% and 36% from a total of four producers. One sample of cream from each manufacturer was used for beating at temperatures of 4.8, and 12 °C. The results of the research showed that increasing the temperature of the cream shortens the duration of the whipping, but also reduces the volume of whipped cream. The highest overrun with the shortest whipping duration was achieved at a temperature of 8 °C.

Key words: temperature, whipping cream, whipping duration, whipped cream volume

IZVORNI ZNANSTVENI RAD

Plasman proizvoda pasmine kokoši hrvatica

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Sažetak
Cilj istraživanja bio je utvrditi jesu li uzgajivači pasmine kokoš hrvatica spremni povećati svoju proizvodnju, uložiti u promociju i marketing dobivenih proizvoda te kroz oznaku izvornosti povećati tržišnu vrijednost proizvoda. Istraživanje je provedeno anketiranjem uzgajivača pasmine kokoš hrvatica na izložbi izvornih pasmina peradi u Varaždinu, 2017. godine. Prema rezultatima ankete, ispitanici većinu proizvoda pasmine kokoš hrvatica plasiraju na tržište po većoj cijeni od tržišne cijene iste vrste proizvoda ostalih pasmina. Radi boljeg plasmana proizvoda spremni su se udruživati ali još uvijek u većoj mjeri ne znaju ili nisu spremni snositi dodatne troškove za dobivanje oznake izvornosti.

Ključne riječi: anketa, uzgajivači, pasmina kokoš hrvatica, oznaka izvornosti

Uvod

Peradarska proizvodnja ima značajnu ulogu u prehrani stanovništva kroz opskrbu animalnim proteinima, mineralnim tvarima i vitaminima. Konzumacija mesa peradi zauzima vodeće mjesto u odnosu na potrošnju svih ostalih vrsta mesa kako u svijetu tako i u Hrvatskoj (Kralik i sur., 2013.). Povećanje potrošnje i proizvodnje mesa peradi u Hrvatskoj kao i u većini razvijenih zemalja ima pozitivan trend koji se prema Grgiću i sur. (2015.) pripisuje relativno niskim cijenama peradskih proizvoda, kratkoći proizvodnih ciklusa i nedostatku vjerskih ograničenja. Potrošači su sve više počeli voditi brigu o onom što jedu s obzirom na svoje zdravlje, zaštitu okoliša te dobrobit životinja. Postali su sve zahtjevniji u odabiru hrane u odnosu na način uzgoja i držanja životinja (Al-Ajeeli i sur., 2018.).
U posljednjih deset godina na razini Europske unije potrošnja mesa peradi, za razliku od drugih vrsta mesa (goveđe, svinjsko, ovčje, kozje) bilježi kontinuirani rast po stanovniku od 1,9 % godišnje te predstavlja značajan izvor u podmirivanju potreba za proteinima. Kontinuirani rast od 0,5 % godišnje po stanovniku bilježi se i u potrošnji konzumnih jaja (EU, 2021.). Jaje je namirnica koja na dnevnoj bazi u ljudskoj prehrani osigurava značajne količine hranjivih tvari uz nisku kalorijsku vrijednost i kao takva se još naziva "nutritivno gusta" namirnica (Walsh i sur., 2009.). Intenzivnom proizvodnjom konzumnih jaja u Republici Hrvatskoj opskrbljuje se 70 % tržišnih potreba a ostatak od 30 % potječe s malih proizvodnih sustava i seoskih domaćinstava (Crnčan i sur., 2018.).
Na manjim obiteljskim gospodarstvima odvija se uzgoj peradi za proizvodnju mesa i jaja slobodnim načinom držanja u kojem se nerijetko koriste čiste pasmine, koje su svojim fenotipskim i genotipskim obilježjima prilagođene podneblju u kojem se uzgajaju (Magdelain, 2011.). Držanje u slobodnom uzgoju jedan je od najzastupljenijih alternativnih načina držanja peradi koji je u najvećoj mjeri usklađen s dobrobiti i zdravljem životinja. Dobrobit predstavlja stanje u kojem se jedinka pokušava nositi sa svojim okolišem (Broom, 2001.). U Republici Hrvatskoj slobodnim načinom držanja uzgaja se i jedina izvorna pasmina kokoši – hrvatica, u skladu s odobrenim Uzgojnim programom za kokoš hrvaticu.
Komercijalizacija uzgoja peradi favorizira korištenje visoko proizvodnih hibrida što je posljedično dovelo do smanjenja veličine populacije izvornih pasmina. Nadalje, izvorne

pasmine peradi u većini zemalja su zanemarene i daleko manje pažnje je posvećeno njihovom genetskom očuvanju u usporedbi s drugim vrstama domaćih životinja (Abebe i sur., 2015.). U Hrvatskoj je kokoš hrvatica kao izvorna pasmina priznata 1998. godine (NN, 127/98) ali dugi niz godina niti jedna jedinka nije bila evidentirana u službenim registrima. Krajem osamdesetih godina prošlog stoljeća zahvaljujući entuzijazmu pojedinaca iz Međimurske i Virovitičko-podravske županije pokrenuta je revitalizacija uzgoja pasmine kokoš hrvatica odabirom i povećanjem broja rasplodnih životinja (Vostrel, 2005.). Kralik i sur. (2013.) navode da uzgoj autohtonih pasmina i proizvodnja funkcionalne hrane na seoskim gospodarstvima može značajno proširiti ponudu u okviru seoskoga turizma s vizualnoga, gastronomskoga i zdravstvenoga gledišta. Budući da je značaj kokoši hrvatice kao izvorne pasmine prepoznat u društvu i broj uzgajivača se povećava iz godine u godinu. Za potrebe ovog istraživanja provedena je anketa s ciljem utvrđivanja spremnosti uzgajivača pasmine kokoš hrvatica da povećaju svoju proizvodnju, ulože u promociju i marketing dobivenih proizvoda te kroz oznaku izvornosti povećaju tržišnu vrijednost proizvoda.

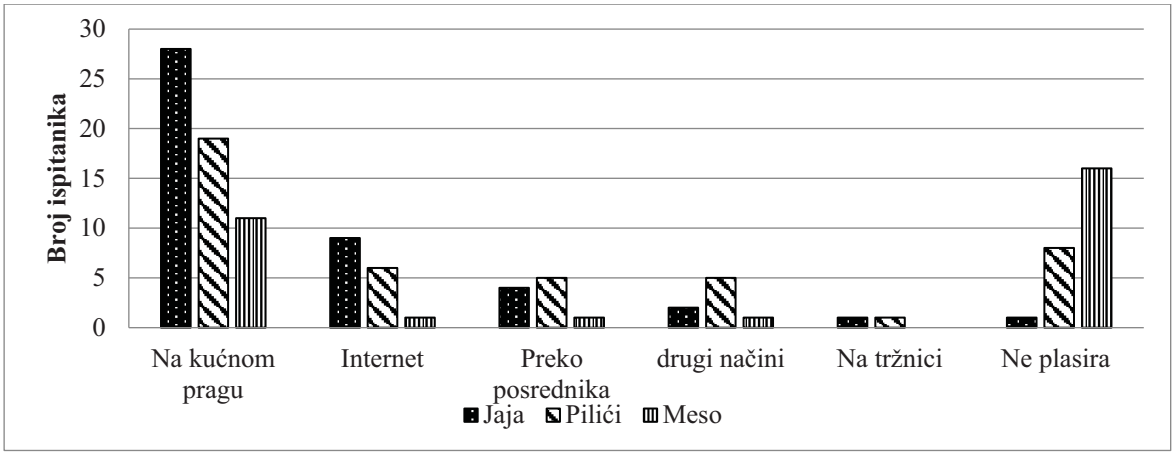
Materijal i metode

Podaci za rad su prikupljeni anketiranjem uzgajivača na državnoj izložbi izvornih pasmina peradi u studenom 2017. godine u Varaždinu. Na izložbi je svoje kolekcije izlagalo 55 uzgajivača, od kojih 38 pasminu kokoš hrvatica. Prikazano je ukupno 45 kolekcija pasmine kokoš hrvatica u 4 soja te 33 kolekcije pasmine zagorski puran. Prikupljanje podataka za istraživanje je unaprijed dogovoreno s organizatorom izložbe tako da su se anketni upitnici podijelili svim uzgajivačima. Anketni upitnik izrađen za potrebe ovog istraživanja sastojao se od 23 pitanja koja su raspoređena po područjima istraživanja i to: a) informacije o samom gospodarstvu (uzgajivačima), b) proizvodi i njihov plasman na tržište te c) promocija proizvoda s dodanom vrijednosti te spremnosti uzgajivača na ulaganja u promociju. Pitanja su bila koncipirana kroz otvoreni i zatvoreni tip te ljestvice kao posebne vrste zatvorenog tipa pitanja, a obrađena su korištenjem MS Excel programa.

Rezultati i rasprava

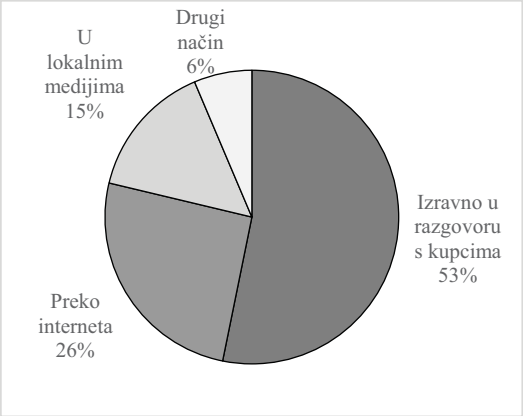
Od ukupno prisutnih 55 uzgajivača, anketni upitnik popunilo je 47 ispitanika u dobi od 22 do 83 godine, od kojih je više od pola (56,52 %) muškaraca. Većina ispitanika ima završenu srednju (44,44 %) ili višu/visoku školu (31,11 %) dok nešto manji udio ima završenu osnovnu školu (22,22 %). Samo jedan ispitanik nema nikakvu formalnu naobrazbu. Njih 68,88 % su članovi udruga ili zadruga. Od svih anketiranih ispitanika, pasminu kokoš hrvatica uzgaja 38 (80,85 %) uzgajivača, od kojih 18 uzgaja i pasminu zagorski puran. U Republici Hrvatskoj evidentiran je 2017. godine 231 uzgajivač pasmine kokoš hrvatica (HPA, 2018.), a anketni upitnik je popunilo 16,45 % uzgajivača ove pasmine, te su u nastavku prikazani rezultati samo tih ispitanika. Većina ispitanika (81,57 %) smatra da jaja kokoši hrvatice imaju poseban okus kao i boju (76,31 %), a samo manji dio (2,70 %) smatra da jaja imaju čvršću ljusku odnosno da su prosječno manja (2,70 %) od jaja ostalih nesilica konzumnih jaja. Većina ispitanika (91,66 %) smatra da meso pasmine kokoš hrvatica ima poseban okus. 44,11 % ispitanika smatra da je i boja mesa specifična, a 97,14 % smatra da osim okusa i boje meso nema drugih posebnosti. Pasmina kokoš hrvatica se prema odredbama Uzgojnog programa drži na otvorenom, na zatravnjenim ispustima za razliku od peradi držane u konvencionalnoj proizvodnji. Budući da se kod takvog načina uzgoja jedinke slobodno kreću kod njih je udio prsa i bataka veći, niža razina trbušne masti i bolje senzorne karakteristike mesa (Castellini i sur., 2012.), kako su naveli i ispitanici u svojim odgovorima. Od 38 ispitanika koji uzgajaju pasminu kokoš hrvatica, jaja na tržište ne plasira jedan ispitanik a njih 37,14 % na tržište plasira više od polovice svoje proizvodnje. Meso na tržište ne plasira 65,62 % ispitanika, a od onih koji plasiraju (34,37 %) njih 18,18 % na tržište

plasira više od polovice svoje proizvodnje. Jednodnevne piliće na tržište plasira 58,33 % ispitanika od kojih 33,33 % na tržište plasira više od polovice svoje proizvodnje. Na tržište ispitanici plasiraju proizvode kokoši hrvatice najviše na kućnom pragu, preko interneta, preko posrednika, mali dio na tržnicama, te na ostale načine plasiranja kako je prikazano u Grafikonu 1.

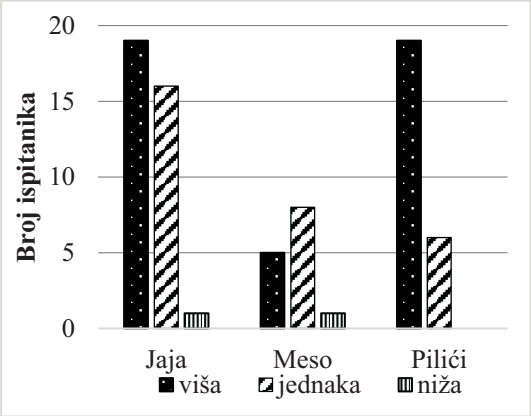


Grafikon 1. Načini plasiranja proizvoda pasmine kokoš hrvatica na tržište (jaja, mesa i jednodnevnih pilića)

Ovakvi kanali prodaje posljedica su ograničene proizvodnje po gospodarstvu budući da su gospodarstva s malim brojem jedinki (HPA, 2018.) i nemaju dovoljnu količinu proizvoda za drugačije oblike plasmana. Dio uzgajivača koristi mogućnosti promocije kroz dostupne kanale informiranja potrošača te više od 60 % uzgajivača promovira svoje proizvode. Budući da se radi o malim proizvodnim količinama i često izravnoj prodaji, većina ispitanika promovira svoje proizvode (Grafikon 2.) u izravnom kontaktu s potrošačima (75,75 %).



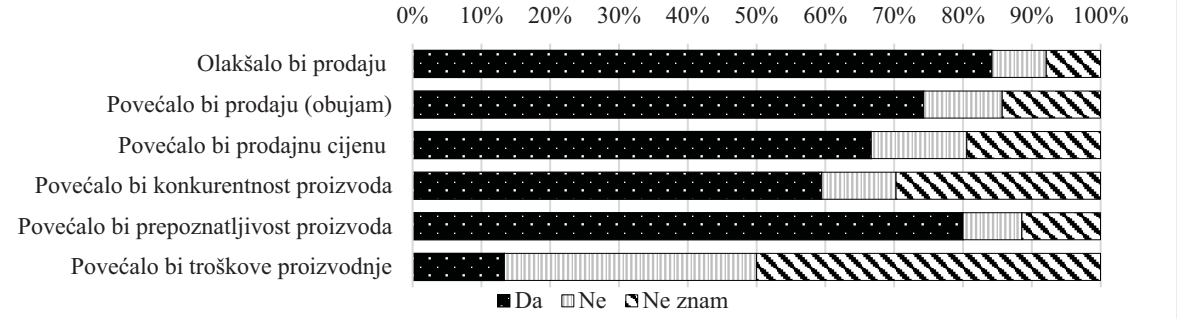
Grafikon 2. Načini promocije proizvoda kokoši hrvatice (jaja, mesa i jednodnevnih pilića) ispitanika



Grafikon 3. Mišljenje ispitanika o cijenama proizvoda kokoši hrvatice (jaja, mesa i jednodnevnih pilića) na tržištu u odnosu na iste proizvode komercijalnih pasmina peradi

Osim toga, značajan udio proizvođača se oslanja i na relativno jeftinije internetsko oglašavanje (37,50 %) te na lokalne medije (22,58 %) dok su drugi načini promocije manje zastupljeni (9,67 %). Prema ispitanicima, prodajne cijene proizvoda pasmine kokoš hrvatica, postigle su na tržištu višu ili jednaku cijenu u usporedbi s prosječnim cijenama istih vrsta

proizvoda ostalih pasmina kako je prikazano u Grafikonu 3. Višu je cijenu za jaja postiglo 52,77 %, a za piliće 76,00 % ispitanika dok je jednaku cijenu za meso postiglo 57,14 % ispitanika, što ukazuje da su proizvodi pronašli svoje kupce i postali prepoznati na tržištu.
Ispitanici su pokazali da su spremni na udruživanje s ostalim poljoprivrednicima radi zajedničkog nastupa na tržištu s proizvodima pasmine kokoš hrvatica. Većina ispitanika (52,63 %) je sigurna dok bi se njih 34,21 % vjerojatno udruživalo, samo manji dio ispitanika (2,63 %) se vjerojatno ne bi udruživao, a 10,52 % ne zna.
Udruživanje bi pomoglo manjim proizvođačima da postanu vidljivi na tržištu budući da samostalno nemaju dostatne količine proizvoda. Proizvodnju bi povećalo 42,10 % ispitanika dok bi njih 55,26 % zadržalo postojeći obim proizvodnje.
Proizvodi kokoši hrvatice, kao autohtone pasmine, imaju sve preduvjete za zaštitu oznakom izvornosti. Upitani jesu li spremni snositi troškove ishođenja oznake izvornosti proizvoda, tek 39,47 % je iskazalo spremnost, njih 13,15 % nije spremno, a 47,36 % ne zna bi li snosili te troškove. Troškove kontrole nakon dobivanja oznake izvornosti spremno je snositi 55,26 % ispitanika, a 36,84 % ne zna. Kako bi se upotreba oznake izvornosti odrazila na poslovanje s proizvodima dobivenim od kokoši hrvatice prema mišljenju ispitanika vidljivo je na Grafikonu 4.



Grafikon 4. Mišljenje ispitanika o doprinosu koji se očekuje od oznake izvornosti proizvoda

Zaključak

Prema rezultatima ankete, uzgajivači smatraju da proizvodi kokoši hrvatice, jaja (81,57 %) i meso (91,66 %) imaju poseban okus. No, iako na tržištu postižu cijenu veću od tržišne cijene iste vrste proizvoda iz konvencionalnog uzgoja, nešto više od polovice ispitanika bi ipak (55,26 %) zadržali postojeći obim proizvodnje. Spremni su se udruživati (52,63 % sigurno, 34,21 % vjerojatno) radi boljeg plasmana proizvoda ali još uvijek u većoj mjeri ne znaju (47,36 %) ili nisu spremni (13,15 %) snositi dodatne troškove za dobivanje oznake izvornosti koja bi uvelike doprinijela prepoznatljivosti proizvoda od strane potrošača. Rezultati pokazuju spremnost ispitanika za udruživanje ali bi trebala postojati organizirana inicijativa prema uzgajivačima u čemu bi značajno pomogle edukacije o potencijalnim prednostima koje nose oznake izvornosti proizvoda.

Literatura

Abebe A.S., Mikko S., Johansson A.M. (2015). Genetic Diversity of Five Local Swedish Chicken Breeds Detected by Microsatellite Markers. PLoS ONE, 10(4): e0120580. https://doi.org/10.1371/journal.pone.0120580

Al-Ajeeli M. N., Miller R. K., Leyva H., Hashim M. M., Abdaljaleel R. A., Jameel Y., i Bailey C. A. (2018). Consumer acceptance of eggs from Hy-Line Brown layers fed soybean or soybean-free diets using cage or free-range rearing systems. Poultry science, 97(5), 1848-1851.

Castellini C. Mugnai C., Dal Bosco A. (2012). Effect of organic production system on broiler carcass and meat quality. Meat Science. 60: 219-225.
Crnčan A., Jelić S., Kranjac D., Kristić, J. (2018). Poultry production in the Republic of Croatia: current state and future expectations. World's poultry science journal, 74(3), 549-558.
Broom, D. M. (2001). Coping, stress and welfare. Coping with challenge: Welfare in animals including humans. 1-9.
EC (2021), EU agricultural outlook for markets, income and environment, 2021-2031. European Commission, DG Agriculture and Rural Development, Brussels.
Grgić I., Zrakić M., Hadelan L., Salputra G. (2015). Proizvodno-potrošna bilanca mesa peradi u Republici Hrvatskoj. Poljoprivreda. 21(1): 82-88
HPA (2018.), Godišnje izvješće o uzgoju ovaca, koza i malih životinja za 2017. godinu. Hrvatska poljoprivredna agencija, Križevci. Raspoloživo: https://hpa.mps.hr/publikacije-godisnja-izvjesca/
Kralik G., Janječić Z., Kralik Z., Škrtić Z. (2013). Stanje u peradarstvu i trendovi njegova razvoja. Poljoprivreda. 19(2), 49-58.
Magdelain P. (2011). 1 Egg and egg product production and consumption in Europe and the rest of the world. In *Woodhead Publishing Series in Food Science, Technology and Nutrition, Improving the Safety and Quality of Eggs and Egg Products.* Nys, Y., Bain, M., Van Immerseel, F. (eds.) Woodhead Publishing, Cambridge
Popis izvornih i zaštićenih pasmina i sojeva domaćih životinja te njihov potrebit broj (1998). Narodne novine 127/98.
Vostrel V. (2005). Standard peradi. Hrvatski savez udruga uzgajatelja malih životinja, Virovitica.
Walsh, M., Hearty, Á., Nugent, A. (2009). An Overview of the Nutritional Role of Eggs in the Diet. UCD Institute of Food & Health. 1-39, Dublin, Ireland

Placement of hrvatica chicken products on the market - a survey of breeders

Abstract
The research aimed to review whether breed hrvatica chicken breeders are ready to increase their production, invest in the promotion and marketing of the products and increase the market value of products through quality labels. The research was conducted by surveying hrvatica chicken breeders at the Exhibition of native poultry breeds in Varaždin, 2017. According to the results of the survey, the respondents place most of the products of hrvatica chicken on the market at a higher price than the price of the same type of products from other breeds. For better product marketing, they are ready to unite, but they are still largely unaware or unwilling to have the additional costs of obtaining the quality label.

Key words: questionnaire, breeders, breed hrvatica chicken, mark of origin

IZVORNI ZNANSTVENI RAD

Anserin-funkcionalni sastojak u mesu pilića

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Sažetak
Cilj rada bio je istražiti obogaćivanje pilećeg mesa anserinom pri uporabi smjesa s dodatcima ß-alanina, L-histidina i magnezijevog oksida (MgO). Istraživanje je provedeno na 120 Cobb 500 tovnih pilića koji su bili podijeljeni u 4 pokusne skupine. Do 21. dana pilići su hranjeni starter smjesom komercijalnog sastava, a od 22.-42. dana finišer smjesama i to: P1=kontrolna skupina bez dodatka amino kiselina i MgO; P2=0,5 % ß-alanina+0,24 % MgO; P3=0,25 % L-histidina+0,24 % MgO i P4=0,20 % ß-alanina+ 0,10 % L-histidina+0,24 % MgO. Obradom podataka utvrđeno je da hranidbeni tretmani kao i interakcija tretmana i vrste tkiva nisu utjecali na deponiranje anserina (P>0,05) u mišićno tkivo. Međutim, ustanovljena je statistički visoko značajna razlika u koncentracijama anserina između mišića prsa i zabataka (P<0,001). Iako razlika između hranidbenih tretmana u sadržaju anserina u mišićnom tkivu prsa nije bila statistički značajna, rezultati upućuju da se dodatkom amino kiselina ß-alanina i L-histidina u smjese za tovne piliće, može utjecati na sadržaj anserina u prsnom mišićnom tkivu.

Ključne riječi: pilići, mišići prsa, mišići zabataka, amino kiseline.

Uvod

Karnozin i anserin prisutni su u mišićima i moždanom tkivu mnogih kralježnjaka, a posebno visoke koncentracije zabilježene su u prsnom mišićju pilića (Crush, 1970.; Nishimura i sur., 1988.; Bonfanti i sur., 1999.; Aristoy i Toldra, 2004.). Istraživanja su pokazala da se dodavanjem aminokiselina ß-alanina i L-histidina u smjese, pileće meso može obogatiti karnozinom (Kralik i sur., 2014., i 2018.). Anserin se sintetizira uz karnozin također u mišićima pilića, ovisno o sastavu obroka (Kopeć i sur., 2013.). U metabolizmu peradi karnozin se sintetizira iz ß-alanina i L-histidina pomoću enzima karnozin sintaze. Anserin nastaje u mišićima pilića na dva načina: a) metiliranjem karnozina u anserin pomoću karnozin-N-metiltransferaze i b) direktnom sintezom iz ß-alanina i L-histidina uz djelovanje karnozin sintaze (Drozak i sur., 2010.; Boldyrev i sur., 2013.). Song i sur. (2014.) smatraju da meso obogaćeno karnozinom i anserinom povećava nutritivna i funkcionalna svojstva mesa te predstavlja benefit za ljudsko zdravlje. Navedeni autori ističu da karnozin i anserin uneseni hranom mogu kod ljudi prevenirati progresiju metaboličkog sindroma. Radi sveg navedenog, cilj ovog rada bio je istražiti mogućnost deponiranja anserina u mišićno tkivo pilića, dodavanjem u hranu magnezijevog oksida (MgO) i različitih udjela aminokiselina ß-alanina i/ili L-histidina.Također je cilj bio utvrditi utjecaj vrste mišićnog tkiva na sadržaj anserina.

Materijal i metode

Smještaj i hranidba pilića

Istraživanje je provedeno sa 120 komada Cobb 500 tovnih pilića. Pilići su bili podijeljeni u 4 skupine, svaka skupina po 30 pilića (P1, P2, P3 i P4 u tri ponavljanja po 10 pilića). Od prvog do 21 dana pilići su hranjeni starter smjesom koja je sadržavala 21 % sirovog proteina i 13,5 MJ/kg ME. Od 22.-42. dana pilići su hranjeni finišer smjesama s različitim dodatkom aminokiselina uz prisustvo MgO kao katalizatora. Skupina P1 bila je kontrolna, a ostale skupine hranjene su posebno dizajniranim smjesama: P2=0,5 % ß-alanina+0,24 MgO; P3= 0,25 % L-histidina+0,24 % MgO i P4=0,20 % ß-alanina+0,10 % L-histidina+0,24 % MgO (Tablica 1.) Hranjenje i napajanje pilića bilo je a*d libitum*. Sve skupine pilića uzgajane su u istom objektu prema tehnološkim normativima za Cobb 500 hibrid.

Tablica 1. Sastav finišer krmnih smjesa korištenih u tovu pilića od 22.-42. dana

Sastojak (%)	P1	P2	P3	P4
Kukuruz	60,78	60,04	60,29	60,24
Pšenično brašno	5,00	5,00	5,00	5,00
Sojina pogača	23,85	23,85	23,85	23,85
Kvasac	2,50	2,50	2,50	2,50
Metionin DL	0,12	0,12	0,12	0,12
Lizin 99 %	0,12	0,12	0,12	0,12
Suncokretovo ulje	3,70	3,70	3,70	3,70
Sol	0,25	0,25	0,25	0,25
Na-bikarbonat	0,20	0,20	0,20	0,20
Vapnenac	1,22	1,22	1,22	1,22
Monokalcijev fosfat	1,61	1,61	1,61	1,61
Organske kiseline	0,40	0,4	0,40	0,40
Premix	0,25	0,25	0,25	0,25
ß-alanin	-	0,5	-	0,20
L-histidin	-	-	0,25	0,10
MgO	-	0,24	0,24	0,24
Ukupno	100	100	100	100

P1=kontrolna skupina bez dodatka amino kiselina i MgO; P2= 0,5 % β-alanina+0,24 % MgO; P3=0,25 % L-histidin+0,24 % MgO; P4=0,20 % β-alanin+0,10 % L-histidin+0,24 % MgO.

Sastav premiksa po kg smjese: vit. A (E672) 13,000 IU; vit. D3 (E671) 3,000 IU; vit. E (DL-α tokoferol) 35 mg; vit. K3 2.5 mg; vit. B1 3 mg; vit. B2 6 mg; nikotinamid 40 mg; kalcijev pantotenat 12 mg; vit. B6 5 mg; vit. B12 0.02 mg; biotin 0.18 mg; kolin klorid 500 mg; folina kiselina 1 mg; jod (E2 kalijev jodid) 1 mg; željezo (E1) 50 mg; bakar (E4 bakrov sulfat-pentahidrat) 15 mg; mangan (E5, mangan sulfat, hid.) 80 mg; cink (E6 cink sulfat, hid.) 60 mg; kobalt 0,1 mg; selen (E8 natrijev selenit) 0,2 mg; antioksidans BHT (E321) 100 mg; fitaza Ronozyme P (E1614 I) 500 FYT; organske kiseline 3.000 mg.

Određivanje sadržaja anserina u mišićima prsa i zabataka

Analiza sadržaja anserina u mišićnom tkivu prsa obavljena je na ukupno 40 uzoraka (10 po svakoj skupini) i zabataka 24 uzorka (6 uzoraka po svakoj skupini). Priprema uzoraka za analizu obavljena je prema metodi Aristoy i Toldra (2004.), a sadržaj anserina određen je na HPLC uređaju korištenjem kolone Zorbax ODS, 5 um (250 x 4,6 mm), fluorescentni detektor (valna dužina eksitacije 310 nm, valna dužina emisije 375 nm), temperatura pećnice 52°C, brzina protoka mobilne faze 0,5 mL/min., mobilna faza: 0,3 M natrijev acetat (pH 5,5): metanol : acetonitril = 75:15:10, injektirani volumen uzorka 20 uL.

Statistička obrada podataka

Rezultati istraživanja analizirani su korištenjem statističkog programa TIBCO® Data Sciencis Workbench version 14.0.0.15 (©1984-2020 Tibco Softwere Inc.) Rezultati su prikazani tablično, i to kao srednja vrijednost ($\bar{x}$), standardna devijacija (sd), te minimalna i maksimalna vrijednost. Razlike među skupinama utvrđene su analizom varijance (ANOVA). Razlike između promatranih učinaka testirane su Fisherovim LSD testom, i označene su različitim slovima [a, b]P<0,05.

Rezultati i rasprava

U tablici 2 prikazan je sadržaj anserina u mišićnom tkivu prsa i zabataka. Sadržaj anserina u mišićima prsa kretao se od 1451,28 mg/kg (P3) do 1561,92 mg/kg (P4). U mesu zabataka najmanji sadržaj anserina zabilježen je u skupini P4 i iznosio je 556,92 mg/kg, dok je najveći sadržaj zabilježen kod P1 skupine i iznosio je 621,17 mg/kg. Također je vidljivo da je sadržaj anserina u mišićima zabataka pokusnih skupina niži od kontrolne skupine (P>0,05). Na sadržaj anserina u mišićnom tkivu prsa i zabataka utjecaj nije imao hranidbeni tretman, kao ni interakcija tretmana i vrste tkiva, međutim statistički značajna razlika je bila u sadržaju anserina dva promatrana tkiva. Utvrđeno je da mišići prsa sadrže značajno više anserina u odnosu na mišiće zabataka (P<0,001).

Tablica 2. Sadržaj anserina u mišićnom tkivu prsa i zabataka (mg/kg)

Hranidbeni tretmani	Vrsta tkiva	Broj varijanti	Sadržaj anserina ($\bar{x}$±sd)	min.-max
P1	Prsa	10	1492,43±242,71[a]	1127,26-1959,49
	Zabatak	6	621,17±153,70[b]	432,71-799,73
P2	Prsa	10	1564,33±172,41[a]	1222,22-1780,80
	Zabatak	6	607,98±113,89[b]	424,22-769,87
P3	Prsa	10	1451,28±204,71[a]	1140,55-1793,83
	Zabatak	6	610,97±127,95[b]	462,11-814,32
P4	Prsa	10	1561,58±115,27[a]	1356,22-1746,70
	Zabatak	6	556,92±70,41[b]	510,32-695,31
Utjecaji				
Hranidbeni tretman			0,848	
Vrsta tkiva			***<0,001***	
Interakcija			0,521	

P1=kontrolna skupina bez dodatka amino kiselina i MgO; P2= 0,5 % β-alanina+0,24 % MgO; P3=0,25 % L-histidine+0,24 % MgO; P4=0,20 % β-alanine+0,10 % L-histidine+0,24 % MgO; vrijednosti u stupcu označene slovima [a, b,] razlikuju se na razini P<0.001.

Sadržaji anserina i karnozina su pod utjecajem tipa mišićnih vlakana. Mišićna vlakna razvrstavaju se u tri skupine: IIB vlakna-bijela glikolitička, oksidativna crvena mišićna vlakna koji prevladavaju kod tipa I i II A, kao i intermedijalna mišićna vlakna (Boldyrev i sur., 2013.). Naše istraživanje pokazuje veći sadržaj anserina u bijelim mišićnim vlaknima prsa za razliku od mišićnog tkiva zabataka. Bijela mišićna vlakna koja sadrže energetski bogate fosfatne estere značajne za anaerobne procese, bogatija su anserinom od crvenih mišićnih vlakana. Dodavanje 0,5 % ß-alanina kao i kombinacija 0,20 % ß-alanina + 0,10 % ßL-histidina te 0,24 % MgO u smjese za piliće, rezultiralo je 7,9 % odnosno 7,7 % povećanim odlaganjem anserina u odnosu na kontrolnu skupinu u mišićima prsa. Haug i sur. (2008.) su ustanovili da dodatak 3 g histidina po kg smjese povećava sadržaj karnozina za 64 %, a anserina za 10 % u pokusnoj u odnosu na kontrolnu skupinu. U istraživanju Kai i

sur. (2014.) navode da se uz dodavanje više razine histidina u smjese za tovne piliće značajno povećava sadržaj anserina u prsnom mišićnom tkivu, što nije sukladno našem istraživanju.

Zaključak

U radu se istražuje utjecaj dodatka aminokiselina L-histidina, ß-alanina i magnezijevog oksida u hranu pilića na deponiranje anserina u mišiće prsa i zabataka. Rezultati istraživanja pokazali su da hranidbeni tretmani nemaju utjecaj na deponiranje anserina u mišićno tkivo pilića. Međutim, utvrđena je statistički značajna razlika (P<0,001) u sadržaju anserina između mišića prsa i zabataka. U mišićnom tkivu prsa u skupinama P1, P2, P3 i P4 utvrđeno je 2,40; 2,57; 2,38 i 2,80 puta više anserina u odnosu na mišićno tkivo zabataka. Rezultati ukazuju da je mišićno tkiva prsa dobar izvor anserina za konzumente.

Napomena

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Literatura

Aristoy M.C., Toldra F. (2004). Histidine dipeptides HPLC-based test for the detection of mammalian origin proteins in feeds for ruminants. Meat Science. 67(2): 211-217.

Boldyrev, A.A., Aldini G., Derave W. (2013). Physiology and pathophysiology of carnosine. Physiological Review. 93(4): 1003-1845.

Bonfanti L., Peretto P., De Marchis S., Fasolo A. (1999). Carnosine-related dipeptides in the mammalian brain. Progress in Neurobiology. 59(4): 333-353.

Crush K.G. (1970). Carnosine and related substances in animal tissues. Comparative Biochemistry and Physiology. 34(1): 3-30.

Drozak J., Veiga-da-Cunha M., Vertommen D., Stroobant V., Van Schaftingen E. (2010). Molecular identification of carnosine synthase as ATP-grasp domain-containing protein 1 (ATPGD1). Journal of Biological Chemistry. 285(13): 9346-9356.

Haug A., Rodbotten R., Mydland L.T., Christophersen O.A. (2008). Increased broiler muscle carnosine and anserine following histidine supplementation of commercial broiler feed concentrate. Acta Agriculturae Scandinavica Section A. 58(2): 71-77.

Kai S., Watanabe G., Kubota M., Kadowaki M., Fujimura S. (2014). Effect of dietary histidine on contents of carnosine and anserine in muscles of broilers. Animal Science Journal. 86(5): 541-546.

Kopeć W., Jamroz D., Wiliczkiewicz A., Biazik E., Hikawczuk T., Skiba T., Pudło A., Orda J. (2013). Antioxidation status and histidine dipeptides content in broiler blood and muscles depending on protein sources in feed. Journal of Animal Physiology and Animal Nutrition. 97(3): 586-598.

Kralik G., Sak-Bosnar M., Grčević M., Kralik, Z. (2018). Effect of amino acids on growth performance, carcass characteristics, meat quality, and carnosine concentration in broiler chickens. The Journal of Poultry Science. 55(4): 239-248.

Kralik G., Sak-Bosnar M., Kralik Z., Galović O. (2014). Effects of β-Alanine Dietary Supplementation on Concentration of Carnosine and Quality of Broiler Muscle Tissue. The Journal of Poultry Science. 51(2): 151-156.

Nishimura T., Rhue M.R., Okitani A., Kato H. (1988). Components contributing to the improvement of meat taste during storage. Agricultural and Biological Chemistry 52(9): 2323-2330.

Song B.C., Joo N.S., Aldini G., Yeum K.J. (2014). Biological functions of histidine-dipeptides and metabolic syndrome. Nutrition Research and Practice. 8(1): 3-10.
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Anserine-functional ingredient in chicken meat

Abstract
The aim of this study was to investigate the enrichment of chicken meat with anserine using mixtures with the addition of ß-alanine, L-histidine, and magnesium oxide (MgO). The study was conducted on 120 Cobb 500 chickens, which were divided into 4 experimental groups. Until the 21st day, the chickens were fed with a starter mixture of commercial composition, and from the 22nd to the 42nd day with finisher mixtures as follows: P1=control group without the addition of amino acids and MgO; P2=0.5% ß-alanine+0.24% MgO; P3=0.25% L-histidine+0.24% MgO and P4=0.20% ß-alanine+0.10% L-histidine+0.24% MgO. Data processing revealed that feeding treatments, as well as the interaction of treatment and tissue type, did not affect anserine deposition ($P > 0.05$) in muscle tissue. However, a highly significant difference in anserine concentrations was found between breast and thigh muscles ($P<0.001$). Although the difference in anserine content in breast muscle tissue between feeding treatments was not statistically significant, the results suggest that the addition of amino acids ß-alanine and L-histidine to mixtures for chickens may affect anserine content in breast muscle tissue.

Key words: chickens, breast muscles, thigh muscles, amino acids

IZVORNI ZNANSTVENI RAD

Senzorna ocjena šunki turopoljskih svinja hranjenih žirom

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Sažetak
Cilj rada bio je istražiti senzorna svojstva suhih šunki (n=26) turopoljskih svinja hranjenih s ili bez dodatka žira hrasta. Šunke su prerađene u kontroliranim uvjetima i uzorkovane s 15 mjeseci u području *m.biceps femoris* i okolnog tkiva. Senzornu ocjenu proveo je deveteročlani panel kvantitativnom deskriptivnom analizom 19 senzornih deskriptora na nestrukturiranoj skali intenziteta s dva krajnja ekstrema. U obradi podataka, za svaki je deskriptor korišten prosječni rezultat svih panelista i repeticija uzorka uz analizu varijance ponovljenih mjerenja. Svojstava presjeka (boja i intenzitet boje mišićnog tkiva), mirisa (tipičnost mirisa) i okusa (slatko) mišićnog dijela, ocijenjena su višom ocjenom kod običnih šunki. Pozitivan utjecaj prihrane svinja žirom nije utvrđen.

Ključne riječi: turopoljska svinja, otvoreni uzgoj, šunka, žir, senzorna analiza

Uvod

Turopoljska svinja je ugrožena hrvatska autohtona pasmina koja se tradicionalno uzgaja u otvorenim proizvodnim sustavima povezanim s lokalnim hrastovim šumama i močvarnim livadama u Turopolju i okolnim područjima u središnjoj Hrvatskoj. Nedavna istraživanja s hrvatskim potrošačima pokazala su da u marketinškim strategijama za turopoljsku pasminu treba koristiti informacije o tradicionalnom sustavu proizvodnje i hranjenju žirom budući da takva reklama može pojačati motive potrošača za konzumaciju i kupnju proizvoda od turopoljske svinje, poput fermentiranih kobasica (Cerjak i sur., 2017.) ili suhe šunke (Kallas i sur., 2019.; Vitale i sur., 2020.), što je kritičan preduvjet za održivije upravljanje ovom pasminom u budućnosti. Stoga je za samoodrživi uzgoj turopoljskih svinja nedavno predložena nova strategija očuvanja temeljena na tradicionalnom proizvodnom sustavu i niši mesnih prerađevina s dodanom vrijednošću (Karolyi i sur., 2019.).
Kao jedan od mogućih premijskih proizvoda od turopoljske svinje, koji preradom najviše dobivaju na vrijednosti, ističe se salamurena i sušena turopoljska šunka. Međutim, za sada je dostupno vrlo malo ili nimalo podataka o intrinzičnim i senzornim svojstvima kvalitete suhomesnatih proizvoda od turopoljske svinje, uključujući i utjecaj lokalno dostupnih izvora hrane kao što je žir. Stoga je cilj ovog rada bio putem senzornog panela ispitati organoleptička svojstva suhih šunki od turopoljskih svinja iz otvorenog uzgoja koje su bile hranjene s ili bez dodatka žira.

Materijal i metode

Za istraživanje su korišteni uzorci suhih šunki (n=26) proizvedenih od nazimica i kastrata autohtone turopoljske pasmine uzgojenih u gateru pokušališta Agronomskog fakulteta iz Zagreba u Šiljakovačkoj Dubravi, hranjenih s ili bez dodatka žira hrasta lužnjaka (*Quercus robur L.*) tijekom završnog tova (1,5 mjesec prije klanja). Prosječna dob i završna masa tovljenika prije klanja iznosila je 18,15±1,4 mjeseci i 94,8±11,5 kg. Klanje i klaonička

obrada tovljenika obje skupine obavljeni su prema standardnoj proceduri u odobrenom objektu (Klaonica 32 d.o.o., Velika Mlaka), a rasijecanje polovica i prerada šunki u odobrenom mesno-prerađivačkom objektu u okolici Zagreba (IGO-MAT d.o.o., Otruševec) prema standardnoj tehnologiji u kontroliranim uvjetima. Uzorkovanje i senzorna analiza obavljeni su kada su šunke bile stare oko 15 mjeseci u senzornom laboratoriju Kmetijskog inštituta Slovenije u Ljubljani. Distribucija uzoraka šunki prema spolu i hranidbenoj skupini svinja bila je jednaka. Za senzornu ocjenu korišteni su naresci šunke (oko 1 mm debljine) izuzeti s kaudalne strane šunke u području *m.biceps femoris* (BF), *m.semitendinosus*, *m.semimembranosus* i pripadajućeg masnog tkiva. Naresci su posluženi na bijelom plastičnom tanjuru pri sobnoj temperaturi, uz čašu vode, kriške jabuke, mrkve i kruha za neutralizaciju okusa između kušanja uzoraka. Senzorna ocjena provedena je kvantitativnom deskriptivnom analizom korištenjem panela sastavljenog od devet obučenih članova, različite starosti (od 40 do 53 godine) i spola sa Kmetijskog inštituta Slovenije i Agronomskog fakulteta u Zagrebu. Prije početka prvog ocjenjivanja proveden je uvodni trening tijekom kojega je članovima panela prezentirano više komercijalnih šunki/pršuta različitog podrijetla i kakvoće (npr. stupanj osušenosti, slanost, mekoća, dimljenje i sl.) kako bi ih se što je više moguće upoznalo s varijacijama u osjetilnim svojstvima proizvoda ovoga tipa. Pojedinačni senzorni deskriptori definirani su na temelju onih opisanih u literaturi za istovjetne proizvode (Pugliese i sur., 2015.; Škrlep i sur., 2016.). Ukratko, panelisti su ocijenili 19 senzornih deskriptora: pet za svojstva izgleda cjelokupnog nareska na presjeku (mramoriranost, boja mišićnog tkiva, njezina ujednačenost i intenzitet, te boja potkožne masti), dva za svojstva mirisa cjelokupnog nareska (tipičnost mirisa i intenzitet dima), tri za svojstva okusa potkožne masti (užegli, slatki i strani okus), pet za svojstava okusa mišićnog dijela u području BF (slanost, kiselost, slatkoća, gorčina i strani okus), te četiri za svojstva teksture mišićnog dijela u području BF (mekoća, topivost, sočnost i pastoznost). Svaki od senzornih deskriptora je ocijenjen na 9 cm nestrukturiranoj ljestvici intenziteta s dva krajnja ekstrema (lijevi „ne-uočljivo“ i desni „vrlo intenzivno“). Ukupno je provedeno pet ocjenjivačkih sesija, po dvije dnevno u razmaku od 8 h sa po pet uzoraka, te zadnji dan jedna sa šest uzoraka. Pri statističkoj obradi, za svaki senzorni deskriptor korišten je prosječni rezultat svih panelista i repeticija uzorka uz primjenu analize varijance ponovljenih mjerenja. Dobiveni rezultati su obrađeni primjenom statističkog paketa SAS v 9.4. (SAS 2012.), korištenjem procedure PROC MIXED za utvrđivanje razlika između senzorne ocjene šunki turopoljskih svinja hranjenih s i bez dodatka žira.

Rezultati i rasprava

U tablici 1. prikazana je usporedba senzorne ocjene šunki turopoljskih svinja hranjenih sa i bez dodatka žira, dok je grafički prikaz rezultata senzorne ocjene prikazan u Grafikonu 1.

Iz prikazanih je rezultata vidljivo da je statistički značajna razlika ($P<0,05$) u senzornoj ocjeni šunki utvrđena kod svojstava izgled presjeka (boja mišićnog tkiva, intenzitet boje), miris (tipičnost mirisa) i okus mišićnog dijela (slatko), koja su redom bila bolje ocijenjena u skupini šunki bez dodatka žira u hranidbi svinja.

Poznato je da hranidba svinja žirom i pašom može povećati sadržaj intramuskularne masti i utjecati na boju na presjeku šunki, kao što je utvrđeno kod pršuta od iberijskih svinja (Carrapiso i sur., 2003.). Premda u provedenom istraživanju nisu utvrđene razlike u ocjeni mramoriranosti i boje potkožne masti, kao ni u ocjeni svojstva ujednačenosti boje – atributu koji je više vezan uz uspjeh procesa salamurenja, a manje uz hranidbu, boja mišićnog tkiva i intenzitet boje u prosjeku su bili značajno više ocijenjeni u skupini šunki „bez žira“. Navedeno može ukazivati da je izgled presjeka dobio višu senzornu ocjenu kod šunki od svinja koje su hranjene bez žira.

Kod svojstava mirisa, svojstvo tipičnosti mirisa bilo je ocijenjeno značajno više u skupini šunki od svinja koje su hranjene bez žira, što ukazuje da su u toj skupini svojstva mirisa šunki bila svojstvenija i intenzivnije izražena.

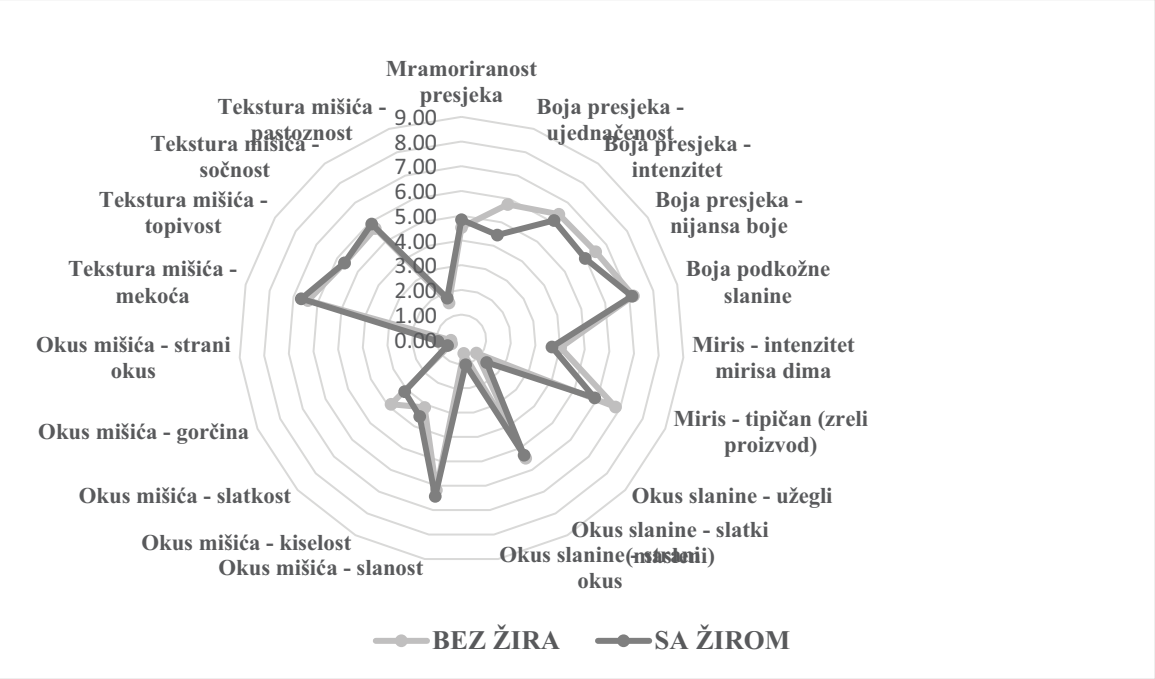
Tablica 1. Senzorna ocjena šunki turopoljskih svinja hranjenih s i bez dodatka žira

Svojstvo	Bez žira	Sa žirom	P-vrijednost
Izgled presjeka			
Mramoriranost	4,52 ± 0,379	4,84 ± 0,379	0,555
Boja mišićnog tkiva	5,77 ± 0,228	4,46 ± 0,227	**0,0004**
Ujednačenost boje	6,42 ± 0,227	6,09 ± 0,227	0,320
Intenzitet boje	6,48 ± 0,158	5,98 ± 0,157	**0,035**
Boja potkožne masti	7,17 ± 0,258	7,11 ± 0,258	0,866
Miris			
Intenzitet dima	4,02 ± 0,321	3,67 ± 0,321	0,449
Tipičnost mirisa	6,80 ± 0,262	5,88 ± 0,261	**0,019**
Okus potkožne masti			
Užeglo	0,82 ± 0,249	1,38 ± 0,249	0,122
Slatko	5,46 ± 0,217	5,32 ± 0,216	0,646
Strani okusi	0,57 ± 0,231	1,04 ± 0,231	0,163
Okus mišićnog dijela			
Slano	6,18 ± 0,267	6,43 ± 0,266	0,512
Kiselo	3.14 ± 0,165	3,54 ± 0,164	0,098
Slatko	3,87 ± 0,178	3,12 ± 0,177	**0,007**
Gorko	0,44 ± 0,133	0,61 ± 0,133	0,390
Strani okusi	0,43 ± 0,257	0,95 ± 0,257	0,163
Tekstura mišićnog dijela			
Mekoća	6,39 ± 0,229	6,68 ± 0,229	0,338
Topivost	5,64 ± 0,235	5,65 ± 0,234	0,983
Sočnost	5,68 ± 0,205	5,91 ± 0,204	0,429
Pastoznost	1,56 ± 0,243	1,76 ± 0,242	0,574

Prikazane su srednje vrijednosti procijenjene metodom najmanjih kvadrata ± standardna greška

Glede svojstava okusa masnog i mišićnog dijela, niti u jednoj skupini nije utvrđen značajniji intenzitet stranih ili gorkih okusa koji se mogu povezati s greškama koje nastaju tijekom tehnološkog procesa prerade i sazrijevanja šunki (Hernández-Ramos i sur., 2020.). Također, između skupina nisu utvrđene značajnije razlike u intenzitetu ostalih analiziranih senzornih deskriptora okusa, izuzev svojstva slatkoće mišićnog dijela, koje je u prosjeku bilo ocjenjeno značajno više u skupini šunki „bez žira“. Navedeni rezultat je u suprotnosti s poznatim utjecajem žira na karakterističnu aromu i okus suhomesnatih proizvoda od lokalnih pasmina svinja uzgajanih u ekstenzivnim uvjetima, kakvi se susreću u tradicionalnim silvopastoralnim sustavima u južnoj Europi (Pugliese i Sirtori 2012.). Navedeno se, makar djelomično, može objasniti s razlikama u vrsti, masno-kiselinskom sastavu i količini konzumiranog žira i ostalih lokalno dostupnih krmiva, a u obzir svakako treba uzeti i ostale čimbenike poput genetike ili procesnih parametara tijekom prerade. Osim toga, korištenje žira u hranidbi svinja, koje je u prošlosti bilo uobičajeno na ovim prostorima, danas je vrlo rijetko, pa u obzir treba uzeti i moguću nenaviknutost ocjenjivača na okus proizvoda žirom hranjenih svinja.

Kod svojstva teksture mišićnog dijela (mekoća, topivost, sočnost, pastoznost) razlike između analiziranih skupina nisu bile značajne.



Grafikon 1. Senzorna ocjena šunki turopoljskih svinja hranjenih sa i bez dodatka žira

Zaključci

Za marketinški uspjeh premijskih proizvoda od turopoljske svinja, kakav je turopoljska suha šunka, važno je uspostaviti vezu između tradicionalnog sustava proizvodnje i lokalno dostupnih hranidbenih resursa s kvalitetom proizvoda. U tom kontekstu, ovaj je rad imao za cilj istražiti utjecaj hranidbe žirom na senzorna svojstva suhih šunki turopoljskih svinja iz tradicionalnog uzgoja na otvorenom. Značajna razlika u senzornoj ocjeni šunki utvrđena kod svojstava izgled presjeka (boja mišićnog tkiva, intenzitet boje), miris (tipičnost mirisa) i okus mišićnog dijela (slatko), koja su redom bila ocijenjena više u skupini običnih šunki te nije utvrđen pozitivan utjecaj hranidbe svinja žirom. Ipak, istraživanja ove vrste treba nastaviti, budući da intrinzična ili organoleptička svojstva proizvoda povezana uz prirodne uvjete uzgoja i hranidbu životinja, kada postoje, mogu predstavljati vrlo moćan alat za diferencijaciju u marketingu proizvoda lokalnih pasmina i pridonijeti da se ugrožene pasmine očuvaju na održiviji način.

Napomena

Opisana proizvodnja svinja i prerada butova, kao i senzorna ocjena šunki provedeni su u sklopu projekta TREASURE financiranog iz programa Europske unije za istraživanja i inovacije Obzor 2020 (br. ugovora 634476). Rad je izvod iz diplomskog rada Valentine Jakopović, mag.ing. Proizvodnje i prerade mesa pod naslovom „Senzorna ocjena šunki turopoljskih svinja hranjenih žirom“.

Literatura

Carrapiso A.I., Bonilla F., García C. (2003). Effect of crossbreeding and rearing system on sensory characteristics of Iberian ham. Meat Science. 65: 623-629.

Cerjak M., Petrčić M., Karolyi D. (2017). Effect of information about animal feeding on consumer acceptability of sausages from Turopolje pig breed. Agriculturae Conspectus Scientificus. 87(2): 151-154.

Hernández-Ramos P., Vivar-Quintana A.M., Revilla I., González-Martín M.I., Hernández-Jiménez M., Martínez-Martín I. (2020). Prediction of Sensory Parameters of Cured Ham:

A Study of the Viability of the Use of NIR Spectroscopy and Artificial Neural Networks. Sensors. 20: 5624.

Kallas Z., Varela E., Čandek-Potokar M., Pugliese C., Cerjak M., Tomažin U., Karolyi D., Aquilani C., Vitale M., Gil J.M. (2019). Can Innovations in Traditional Pork Products Help thriving EU Untapped Pig Breeds? A Non-Hypothetical Discrete Choice Experiment with hedonic evaluation. Meat Science. 154: 75-85.

Karolyi D., Luković Z., Salajpal K., Škorput D., Vnučec I., Mahnet Ž., Klišanić V., Batorek-Lukač N. (2019.). Turopolje Pig (Turopoljska svinja). Objavljeno u *European Local Pig Breeds - Diversity and Performance. A study of project TREASURE*, Čandek-Potokar M., Linan R. M. N. (eds), 267-277. London, UK: IntechOpen.

Pugliese C., Sirtori F. (2012). Quality of meat and meat products produced from southern European pig breeds. Meat Science. 90: 511-518.

Pugliese C., Sirtori F., Škrlep M., Piasentier E., Calamai L., Franci O., Čandek-Potokar M. (2015). The effect of ripening time on the chemical, textural, volatile and sensorial traits of Bicep femoris and Semimembranosus muscles of the Slovenian dry-cured ham Kraski prsut. Meat Science. 100: 58-68.

SAS (2012). SAS Version 9.3. SAS Institute Inc., Cary, NC, USA

Škrlep M., Čandek-Potokar M., Lukač N.B., Povše M.P., Pugliese C., Labussiere E., Flores, M. (2016). Comparison of entire male and immunocastrated pigs for dry-cured ham production under two salting regimes. Meat Science. 111: 27-37.

Vitale M., Kallas Z., Rivera-Toapanta E., Karolyi D., Cerjak M., Lebret B., Lenoir H., Pugliese C., Aquilani C., Čandek-Potokar M., Gil M., Oliver M. À. (2020). Consumers' expectations and liking of traditional and innovative pork products from European autochthonous pig breeds. Meat Science. 168: 108179.

Sensory evaluation of hams from Turopolje pigs fed acorn

Abstract

The aim of this study was to investigate the sensory characteristics of dry-cured hams (n=26) obtained from Turopolje pigs raised in the outdoor and fed with or without acorns before slaughter. The hams were processed under the controlled conditions and sampled after 15 months in the area of the biceps muscle and associated fatty tissue. Sensory evaluation was performed by quantitative descriptive analysis by a panel of 9 trained members who rated a total of 19 sensory descriptors on a 9 cm unstructured intensity scale with two end extremes. For data processing, the average score of all panellists and sample repetitions was used for each descriptor using repeated measures analysis of variance. A statistically significant difference was found in the cross section appearance (lean colour and colour intensity), muscle part odour (typicality) and taste (sweetness), all of which were rated higher in hams from pigs not fed acorn. The conclusion is that, contrary to expectations, the positive effect of feeding pigs with acorns on the sensory properties of ham from Turopolje pig could not be demonstrated.

Key words: Turopolje pig, outdoor farming, dry-cured ham, acorn, sensory analysis

ORIGINAL SCIENTIFIC PAPER

Partition of genetic trend for milk yield by gender and flock in Pag sheep

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Abstract
Breeding programs for dairy sheep seek to improve a set of economically and/or functionally important traits. Their success is measured by genetic trends, and their partitioning allows the contributions of different selection pathways to be quantified. This study aimed to estimate the genetic trend for daily milk yield in Pag sheep and to partition it by gender and flock. The estimated genetic gain was ~1 kg per production year (120 days). Females contributed to selection gain much more than males, while the within-flock contribution varied over the years, making it difficult to rank their contribution in the overall gain. The results imply that rams should be selected more carefully, preferably based on estimated breeding values.

Key words: Pag sheep, genetic trend, partition, gender, flock

Introduction

Pag sheep is a Croatian autochthonous breed with great traditional, cultural, ecological, and economical importance. The high purchase prices of ovine milk (~1.7 €/L) and ovine cheese (~30 €/kg) on the island of Pag encourage breeders to raise this breed and also to produce more milk per ewe. Some breeders made significant improvements in dairy sheep management (housing, nutrition, machine milking), and further improvements could be obtained by intermating genetically superior animals. Genealogical and performance recording in this population started about two decades ago. BLUP genetic evaluation has been conducted for several dairy traits in order to support breeders in their selection decision. The evaluation relies on the pedigree based test-day repeatability animal genetic model (Špehar et al., 2020), but a substantial part of the population has been genotyped (50K SNP chip) in order to establish single-step genomic evaluation (Legarra et al., 2009). The breeders are advised to select based on EBVs, but they are not obliged to do so. Trait-orientated selection usually provides genetic gain for trait(s) of interest. In order to check the success of selection, genetic trends need to be examined. They have been traditionally obtained by averaging estimated breeding values (EBVs) by birth year of animals (Blair and Pollak, 1984) or by regressing EBVs on birth year (Bernardes et al., 2018). However, evaluating single parts of the selection scheme is more complicated due to different selection intensities from different sources. One approach in tackling this issue is to calculate selection differentials for the different paths (Van Tassell and Van Vleck, 1990), which is not a trivial task (overlapping pedigrees). The more sophisticated method, proposed by Garcia-Cortes et al. (2008), relies on a direct transformation of EBVs by pedigree. Decomposition of EBVs with this methodology has already been carried out in cattle (Gorjanc et al., 2011; Gorjanc et al., 2012; Špehar et al., 2011), pigs (Škorput et al., 2015), and sheep (Špehar et al., 2021). Success of past selection work in this population has never been examined on such level, so we aimed to determine genetic trend for milk yield and decompose EBVs by gender and flock to examined their different contribution in the overall genetic trend for daily milk yield.

Material and methods

Genetic evaluation

Test day-records (n=113,075 collected on 9,629 ewes) for daily milk yield (DMY), information on systematic effects, (age, days in milk, parity, flock, litter size, season), and genealogical information (n=10,594 animals) were provided by the Ministry of Agriculture. Test-day records were obtained in accordance with ICAR guidelines (ICAR, 2018). Covariance components (estimated with REML) and BVs (predicted with BLUP) were estimated using the same multi-trait repeatability test–day genetic animal model (Špehar et al., 2020). Parity, litter size, season of lambing, and flock were fitted as class, while days in milk (DIM) and age at lambing (AL) as continues fixed predictors. The Ali–Schaeffer lactation curve (Ali and Schaeffer, 1987) nested within parity and litter size was used to model DIM. Age at lambing was modelled as a linear regression nested within parity. Flock–test–day, permanent environmental effect within lactations, and direct additive genetic effect were fitted as random effects. The matrix notation of the model was: $\mathrm{y} = \mathrm{Xb} + \mathrm{W_c c} + \mathrm{W_p p} + \mathrm{W_a a} + \mathrm{e}$ where: y was a vector of the phenotypes and, X and W were incidence matrices for the fixed and random effects, respectively. $\mathrm{W_c}$, $\mathrm{W_p}$, $\mathrm{W_a}$ were incidence matrices; and c, p, and a were vectors of unknown random effects, for the flock–test–day, permanent environmental, and additive genetic effects, respectively.

Decomposition of genetic trends

Genetic trends were obtained as the average of $\hat{a}$ (BVs) by year of birth for animals born from 2010 to 2018, and the $\hat{a}$ was partitioned by the gender and flock in R (R Core Team, 2020) with the package AlphaPart 0.8.1. (Gorjanc et al., 2021). This was done by following the method of Garcia-Cortes et al. (2008) with the formula $\hat{a} = TP_1T^{-1}\hat{a} + TP_2T^{-1}\hat{a} + \cdots + TP_kT^{-1}\hat{a}$. T traces gene flow from one generation to another, i.e., it describes the expected genetic contribution of genes identical by descent between each individual and all its ancestors, while P_i matrix is the 'path' matrix for the $i-th$ origin that selects a partition of animals' BV attributed to the selection work performed by the $i-th$ origin. Only a subset of the population (21 flocks) was included in this part of the study.

Results and discussion

The overall genetic trend for DMY in the Pag sheep breed and its decomposition by gender is presented in Figure 1. The overall genetic trend during the studied period was positive. By regressing BVs on birth year, the annual DMY was estimated to be 0.0087 kg. This result arose from the BVs obtained by the fixed regression repeatability test day animal model, which means that this estimate reflects annual genetic progress for average DMY. Multiplying this estimate by 120 days of lactation, cumulative lactation gain per year was estimated to be ~1 kg. Decomposition of the trend by gender revealed that females were the main source of genetic gain in this population. This result was quite unexpected at first, because raw means of EBVs by year and gender suggested the opposite conclusion. However, after detailed inspection of the data, applied procedures, and feedback from the field, it was determined that a positive response to selection could be attributable to the female selection path. The results obtained suggest that rams should be selected more carefully, preferably based on BVs. A similar pattern, i.e., a higher contribution of females to the overall genetic trend has been obtained by Abdollahi-Arpanahi et al. (2021) in the population of a selected maternal pig line. Their explanation for such a result was that selection decisions in a maternal pig line were placed more on the females because 40% of the traits under selection were measured only in females and because the selected females had more progenies than the sires. The former reason can be applied to some extent to our population, where all traits were measured on females and selection of replacement ewes is

based on female ancestral performance. This is rarely the case in selection of rams. Decomposition of genetic trends with this methodology has been insufficiently applied on real data and the patterns obtained on simulated data (Garcia-Cortes et al., 2008; Obšteter et al., 2021) can hardly be compared with our results due to the large discrepancy in data structure and applied selection schemes.

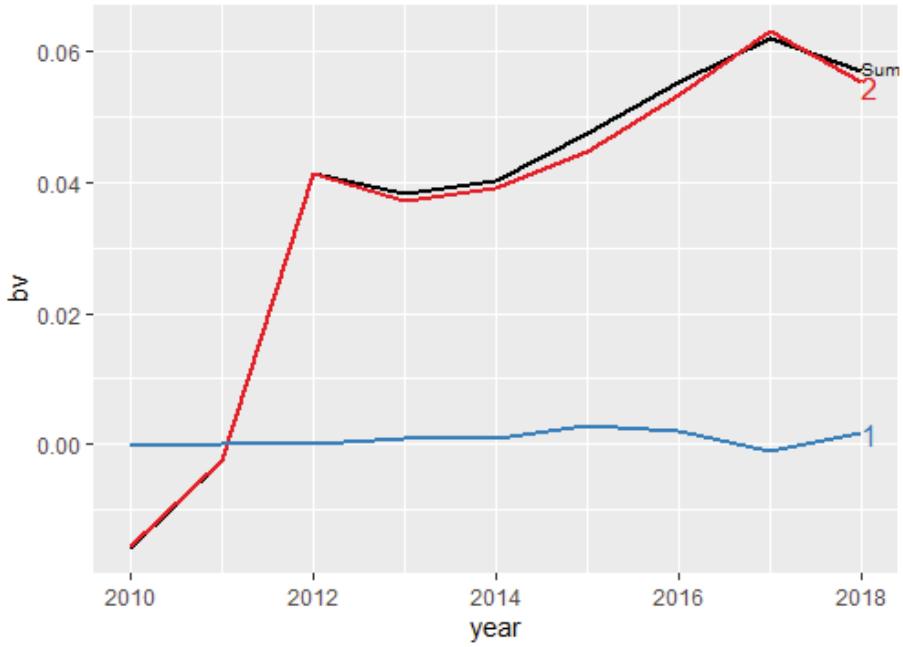

Figure 1. The estimated genetic trend (black) and contribution of sires (blue) and dams (red) in selection on daily milk yield in the Pag sheep breed

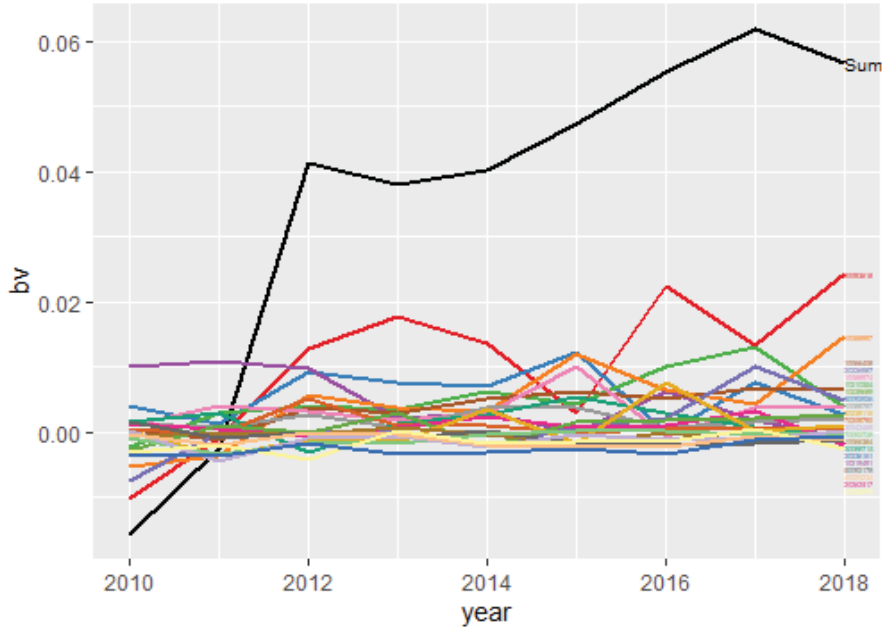

Figure 2. Flocks' contributions to the overall genetic trend for daily milk yield in the Pag sheep breed

The decomposition of genetic trend by flock (Figure 2) was less conclusive, however it revealed to some extent which flocks contributed most to the overall genetic trend. According to the estimated partitions, it seems that their relative importance to the overall

genetic gain was inconsistent between years, suggesting non-systematic selection with changeable success. Genetic progress of the population under consideration usually comes from different selection pathways that are exposed to different selection pressures, making this task very challenging. When attempting to study the impact of genetic effects on any complex (quantitative) trait, it is necessary to eliminate, or to account for and control, other potential sources of phenotypic variation. Studies conducted on livestock field data therefore heavily rely on the appropriate statistical model used to disentangle genetic from environmental effects. In this study, all available information (as explained in M&M) was used to estimate EBVs as accurately as possible (true breeding value is never known), and a purposely developed method and software was used to decompose them by gender and flock. Despite some shortcomings of the data (low genetic connectedness between flocks and insufficient pedigree information for some animals), we think that the estimates are a credible reflection of the true genetic change and the main contributors of genetic gain in this population. In addition to answering some important questions about past selection work in this population, we hereby demonstrated the "power" of decomposition of genetic trends on real data. Implementation of such procedures is necessary in practice in order to reveal benefits and pitfalls of applied selection schemes and to take actions to guide future selection activities in right direction.

Conclusion

The decomposition of genetic trends is an important "tool" to monitor past breeding activities in a population, ant it should be regularly applied in advanced breeding programs. Results of this study revealed that females were the main contributors of selection gain in this population and that flocks included in the national genetic evaluation system had non-uniform contribution. The results should serve to improve selection practices in this population, but also to demonstrate the importance and power of comprehensive genetic trend analysis in livestock populations under selection.

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Literature

Abdollahi-Arpanahi R., Lourenco D., Legarra A., Misztal I. (2021). Dissecting genetic trends to understand breeding practices in livestock: a maternal pig line example. Genetic Selection Evoltion. 53(89): https://doi.org/10.1186/s12711-021-00683-6

Ali T.E., Schaeffer L.R. (1987). Accounting for covariances among test day milk yields in dairy cows. Canadian Journal of Animal Science. 67: 637–644. Bernardes P.A., Grossi D.A., Savegnago R.P., Buzanskas M.E., Urbinati I., Bezerra L.A., Lôbo R.B., Munari D.P. (2015). Estimates of genetic parameters and genetic trends for reproductive traits and weaning weight in Tabapuã cattle. Journal of Animal Science. 93(11): 5175–5185.

Blair H. T., Pollak E.J. (1984): Estimation of genetic trend in a selected population with and without the use of a control population. Journal of Animal Science. 58(4): 878–886.

García-Cortés L.A., Martinez-Ávila J.C., Toro M.A. (2008). Partition of the genetic trend to validate multiple selection decisions. Animal. 2(6): 821–824.

Gorjanc G., Hely F., Amer P. (2012). Partitioning international genetic trends by origin in Holstein bulls. ICAR 38th Annual Meeting, 28 May - 1 June 2012; Cork, Ireland.

Gorjanc G., Obšteter J., Oliveira T.P. (2021) AlphaPart: Partition/Decomposition of Breeding Values by Paths of Information. https://CRAN.R project.org/package=AlphaPart

Gorjanc G., Potočnik K., García-Cortés L. A., Jakobsen J, Dürr J. (2011). Partitioning of international genetic trends by origin in Brown Swiss bulls. In *Proceedings of the 2011 Interbull meeting,* Jorjani H. (ed.), 81–86. Stavanger, Norway.

ICAR (2018). Section 16. Guidelines for Performance Recording in Dairy Sheep and Dairy Goats. Utrecht, The Netherlands: International Committee for Animal Recording. Available from: https://www.icar.org/index.php/icar-recording-guidelines/11.

Legarra A., Aguilar I., Misztal I. (2009). A relationship matrix including full pedigree and genomic information. Journal of Dairy Science. 92(9): 4656–4663.

Obšteter J., Holl J., Hickey J.M., Gorjanc G. (2021). AlphaPart—R implementation of the method for partitioning genetic trends. Genetics Selection Evolution. 53(30): 1–11.

R Core Team. (2020). R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing. Vienna, Austria. Available from: https://www.R-project.org

Škorput D., Gorjanc G., Kasap A., Luković Z. (2015). Partition of genetic trends by origin in Landrace and Large-White pigs. Animal. 9(10): 1605–1609.

Špehar M., Ivkić Z., Bulić V., Barać Z., Gorjanc G. (2011). Partitioning of Genetic Trends by Origin in Croatian Simmental Cattle. Agriculturae Conspectus Scientificus. 76(4): 301–304.

Špehar M., Mulc D., Barać Z., Mioč B., Kasap A. (2020). Estimation of genetic parameters for dairy traits in Pag sheep with single and multi-trait test–day models. Small Ruminant Research. 183: 106029.

Špehar M., Ramljak J., Kasap A (2021). Partitioning of genetic trends by flock in Istrian sheep breed. In: Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science, 228, Davos, Switzerland.

Van Tassell C. P., Van Vleck L. D. (1990). Estimates of genetic selection differentials and generation intervals for four paths of selection. Journal of Dairy Science. 74(3): 1078–1086.

Particija genetskog trenda prema spolu i stadu za dnevnu količinu mlijeka Paške ovce

Sažetak

Uzgojni programi u mliječnom ovčarstvu nastoje unaprijediti skup ekonomski i/ili funkcionalno važnih osobina ovaca. Uspjeh njihove provedbe se iskazuje genetskim trendovima, čija dekompozicija omogućuje kvantifikaciju doprinosa pojedinih selekcijskih puteva. Cilj rada bio je procijeniti genetski trend za dnevnu količinu mlijeka Paške ovce i napraviti njegovu dekompoziciju temeljem spola i stada. Trend je bio pozitivan, sa stopom promjene od 0,0087 kg/dan/godina (~1 kg po laktaciji). Ovce su imale veći doprinos selekcijskom napretku od ovnova, dok je doprinos pojedinih stada varirao tokom godina i time otežao rangiranje doprinosa pojedinih stada. Rezultati upućuju na potrebu veće pažnje kod odabira ovnova koji bi se trebao temeljiti prvenstveno na procijenjenim uzgojnim vrijednostima.

Ključne riječi: Paška ovca, genetski trend, dekompozicija, spol, stado

STRUČNI RAD

Mogućnosti korištenja stajskog gnoja kao sirovine u proizvodnji bioplina

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Sažetak
Ovisno o količini te načinu skladištenja i korištenja, stajski gnoj može doprinijeti onečišćenju zraka, vode, tla te emisiji stakleničkih plinova. Anaerobna digestija stajskog gnoja tijekom proizvodnje bioplina smanjuje rizik od navedenih onečišćenja. Proizvedeni bioplin može biti iskorišteni za proizvodnju biometana, dok se nusproizvod proizvodnje bioplina, digestat, može koristi se kao gnojivo ili stelja. Stoga je cilj ovog rada prikazati svojstva stajskog gnoja te njegove prednosti i nedostatke kao sirovine u proizvodnji bioplina.

Ključne riječi: stajski gnoj, bioplin, metan, anaerobna digestija, očuvanje okoliša

Uvod

Stočarstvo je sastavni dio poljoprivredne proizvodnje i gospodarstva neke zemlje. No, uzgoj domaćih životinja pored primarnih proizvoda (mlijeko/meso/vuna/vlakno/jaja) rezultira i neizbježnim nusproizvodom - stajskim gnojem. Na razini Europske unije ne postoji jedinstvena zakonska definicija stajskog gnoja. U Regulativi EC/1069/2009 navodi se da je stajski gnoj životinjski nusproizvod, odnosno svaki ekskrement i/ili urin farmskih životinja, izuzev riba, sa ili bez stelje. Direktiva 91/676/EEC, poznatija kao Nitratna direktiva, definira stajski gnoj domaćih životinja kao otpadni produkt koje izlučuju domaće životinje ili mješavinu stelje i otpadnih produkata koje izlučuju domaće životinje (Köninger i sur., 2021.). U Republici Hrvatskoj pojam „stajski gnoj“ definiran je u II. Akcijskom programu zaštite voda od onečišćenja uzrokovanog nitratima poljoprivrednog podrijetla (NN 60/2017.). Prema navedenom Programu stajski gnoj čine kruti stajski gnoj, gnojovka, gnojnica i stajski gnoj kao organski gnoj u prerađenom obliku. Pojam *krutog stajskog gnoja* odnosi se na smjesu stelje, krutih i tekućih životinjskih izlučevina različitog stupnja biološke razgrađenosti, stabilnosti i zrelosti. *Gnojovka* je polutekuće stajsko gnojivo, smjesa krutih i tekućih životinjskih izlučevina, tj. stajski gnoj uglavnom bez stelje, dok je gnojnica tekući stajski gnoj, najčešće smjesa tekućih životinjskih izlučevina i otpadnih voda (obično nastaje kao tekući ostatak izlučevina koje stelja ne uspije upiti). Prema sadržaju vode stajski gnoj se dijeli na topli i hladni. Zbog velikog sadržaja vode, goveđi i svinjski gnoj su hladni, dok je stajski gnoj konja, magaraca, ovaca, koza, mazgi i mula topli (Brdarić i sur., 2009.).
U Europi (EU-27+Velika Britanija) godišnje se proizvode oko 1400 milijuna tona stajskog gnoja, pri čemu šest zemalja (Njemačka, Španjolska, Francuska, Italija, Poljska, Velika Britanija) proizvodi oko 68 % ukupne količine. Oko 75 % ukupne količine stajskog gnoja u EU potječe od goveda, a svinjogojstvo i peradarstvo doprinose svaki sa po 12 % ukupne količine proizvedenog stajskog gnoja (Köninger i sur., 2021.). Također, 60-70 % stajskog gnoja u EU-27 nastaje na farmama, dok 30-40 % nastaje pri pašnom držanju, poglavito goveda, ovaca i koza. Više od 50 % stajskog gnoja je u obliku gnojnice i/ili gnojovke, dok manje od 50 % je u formi krutog stajskog gnoja i sadrži stelju (Oenema i sur., 2007.).
Ukoliko se stajski gnoj pravilno ne skladišti i ne koristi na propisani način, može doprinijeti oneščišćenju površinskih i podzemnih voda, kao i zraka. Stoga je cilj ovoga rada prikazati sastav i svojstva stajskog gnoja te mogućnosti njegova korištenja kao sirovine u proizvodnji bioplina.

Sastav i svojstva stajskog gnoja

Većina proizvedenog stajskog gnoja koristi se kao gnojivo na poljoprivrednim površinama stoga što popravlja fizikalna i kemijska svojstva tla (zahvaljujući visokom puferskom kapacitetu), osigurava esencijalna mineralna hranjiva (N:P:K), povećava zalihe ugljika u tlu, stabilizira sadržaj organske tvari te omjer ugljika i dušika u tlu (Köninger i sur., 2021.). Dostupnost dušika kao primarnog hranjiva iz stajskog gnoja ovisi o formi u kojoj se nalazi. U krutom stajskom gnoju (>20 % suhe tvari) dušik se nalazi vezan za organsku tvar te je njegova mineralizacija spor proces koji traje 5-10 godina (Webb i sur., 2013.). Dušik iz gnojovke (4-20 % suhe tvari) i gnojnice (<4 % suhe tvari) podrijetlom od preživača sadrži dušik uglavnom u obliku amonijaka koji biljke mogu odmah koristiti, osobito u tlu siromašnom dušikom (Webb et al., 2013.). Međutim, problemi primjene stajskog gnojiva nastaju u slučajevima kad se zbog ograničenih i/ili nedovoljnih skladišnih kapaciteta stajski gnoj izlaže na poljoprivrednim površinama u svježem ili nedovoljno prerađenom stanju. Prema ranijim procjenama, oko 90 % hranjiva iz stajskog gnoja u EU završi na poljoprivrednim površinama u neprerađenom ili neznatno prerađenom obliku (Foged i sur., 2011.). U tim slučajevima hranjiva (dušik, fosfor i kalij) se nakupljaju u tlu u količinama koje biljke ne mogu iskoristiti.

Skladištenje, dozrijevanje te primjena stajskog gnoja na poljoprivrednim površinama rezultira i gubitkom dijela hranjiva uslijed hlapljenja. Procjenjuje se da u EU27 + UK stajski gnoj doprinosi emisiji metana (CH_4) sa 18,6 % i emisiji dušikovog(I)oksida (N_2O) sa 11,3 % (Eurostat, 2018.). Kako bi se spriječilo i kontroliralo onečišćenje voda nitratima iz poljoprivrednih izvora, na razini Europske unije propisano je da u tijeku jedne kalendarske godine poljoprivredno gospodarstvo može gnojiti poljoprivredne površine stajskim gnojem do granične vrijednosti primjene od 170 kg dušika/ha. Ujedno, skladištenje stajskog gnoja mora se provoditi na uređenim gnojištima, kao što su: platoi za kruti stajski gnoj, gnojišne jame, lagune, jame za gnojnicu, ili drugi spremnici. Svojom veličinom spremnici moraju omogućiti prikupljanje stajskog gnoja tijekom šestomjesečnog razdoblja (NN 60/2017.).

Količina i sastav proizvedenog stajskog gnoja određene su brojnim čimbenicima, kao što su: vrsta domaćih životinja, kategorija, hranidba i količina vode koju životinje popiju, proizvodni sustav, vrsta stelje i uvjeti skladištenja (Rayne i Aula, 2020.). Stoga se dostupni literaturni podaci o sastavu stajskog gnoja pojedinih vrsta domaćih životinja često razlikuju. Stajski gnoj prosječno sadrži 0,20-0,60 % dušika (N_2), 0,04-0,30 % fosfora (P_2O_5), 0,10-0,80 % kalija (K_2O), 0,07-1,00 % kalcija (CaO) i 0,03-0,06 % magnezija (MgO) (Brdarić i sur., 2009.). U II. Akcijskom programu zaštite voda od onečišćenja uzrokovanog nitratima poljoprivrednog podrijetla navodi se da stajski gnoj goveda, ovaca i koza proizveden tijekom jedne godine sadrži 70 kg N/uvjetnom grlu (UG), stajski gnoj konja 60 kg N/UG, svinja 80 kg N/UG te peradi 85 kg N/UG. Salajpal i sur. (2014.) navode da mliječne krave godišnje proizvedu 22,6-23,4 t tekućeg stajskog gnoja, junad 8 t tekućeg stajskog gnoja, kobile i pastusi 10,9 t krutog stajskog gnoja, ovce 0,75-0,88 t krutog stajskog gnoja, krmače s prasadi 5,1 t tekućeg stajskog gnoja, svinje u tovu 1,2 t tekućeg stajskog gnoja, kokoši nesilice 0,053 t te brojleri 0,011 t krutog stajskog gnoja. Brdarić i sur. (2009) navode da jedno UG goveda dnevno izluči oko 46 L ekskrementa (uključujući urin), dok jedno UG svinja izluči oko 50 L ekskremenata.

Proces proizvodnje bioplina

Sukladno odredbama Direktive 2009/28/EZ o poticanju uporabe energije iz obnovljivih izvora, članice EU su do 2020. godine trebale ostvariti 20 % udjela obnovljive energije u ukupnoj potrošnji energije. Ujedno, u EU je prihvaćen cilj da se u razdoblju od 2020. do 2030. godine ostvari smanjenje emisije stakleničkih plinova za 40 % u odnosu na razinu iz 1900.-ih (Scarlet i sur., 2018.). Istodobno u većini zapadnoeuropskih zemalja postoji

problem iskorištenja svih količina stajskog gnoja kao gnojiva na poljoprivrednim površinama (ograničenje od 170 kg N/ha). Stoga, kako bi se spriječio negativan utjecaj na okoliš u vidu potencijalnog onečišćenja zraka, tla i voda te oslobađanja metana i ugljičnog dioksida tijekom skladištenja, primjenjuju se različiti tehnološki procesi kao što su spaljivanje, piroliza, rasplinjavanje. No, ovi procesi što zbog visokih ulaganja i/ili neučinkovitosti nisu značajnije zastupljeni u tretiranju stajskog gnoja. Anaerobnom digestijom (AD) stajskog gnoja kao biološkim tretmanom ostvaruje se bolji balans između ulaganja i učinkovitosti te rezultira korisnim (nus) proizvodima (bioplin, digestat) (Moeller i sur., 2004.; Al Seadi i sur., 2008.; Bošnjak i sur., 2018.). Bioplin nastaje tijekom biokemijskog procesa u kojem se složeni organski spojevi razgrađuju djelovanjem različitih vrsta bakterija u anaerobnim uvjetima. U većini slučajeva, u proizvodnji bioplina se koristi homogena mješavina dva ili više različita supstrata te se taj postupak naziva kodigestija. Proces AD može se odvijati pri različitim temperaturnim uvjetima: psifrofilnim (<25 ºC), mezofilnim (25-45 ºC) i termofilnim (45-70 ºC). Ipak, većina suvremenih bioplinskih postrojenja koristi termofilne uvjete tijekom AD zbog prednosti u odnosu na druge: učinkovito uništenje patogena, veća stopa rasta metanogenih bakterija, kraće trajanje digestije, poboljšana razgradnja i iskoristivost hranjivih tvari iz supstrata, bolja mogućnost razdvajanja tekuće i krute frakcije supstrata. Potencijalni nedostatci su: veća potrošnja energije tijekom zagrijavanja do viših temperatura, nastanak veće količine amonijaka koji inhibira proces AD te teže održavanje optimalnih uvjeta AD (Al Seadi i sur., 2008.). Proces AD se odvija u digestoru ili fermentatoru, zračno nepropusnom spremniku koji je toplinski izoliran i grijan. Ovisno o ulazu i izlazu sirovine iz fermentatora postoje obročni (sirovina se puni u jednom obroku, prolazi proces fermentacije i po završetku se u potpunosti prazni) i kontinuirani (kontinuirano punjenje sirovinom i stalna proizvodnja bioplina). Kontinuirani fermentatori ovisno o izvedbi mogu biti vertikalni i horizontalni. Prosječno vrijeme zadržavanja supstrata u digestoru je između 20 i 40 dana (ovisni o sirovini i temperaturi)

Sirovine za proizvodnju bioplina razvrstavaju se najčešće ovisno o sadržaju suhe tvari, prinosu metana i udjelu hlapljivih tvari u ukupnoj suhoj tvari. U stajskom gnoju se osnovne komponente suhe tvari (ugljikohidrati, proteini i masti) razgrađuju tijekom četiri glavne faze anerobne digestije: hidroliza, acidogeneza, acetogeneza i metanogeneza. Bioplin sadrži 55-80 % metana, 20-40 % ugljikovog dioksida, u tragovima sumporovodika i ostale primjese (Truong i Abatzoglou, 2005.). Prednosti korištenja stajskog gnoja u proizvodnji bioplina su niska cijena, laka dostupnost, prirodna prisutnost anaerobnih bakterija te visoki sadržaj vode koja je otapalo za druge tvari (Al Seadi i sur., 2008.). Prilikom proizvodnje bioplina nastaje nusproizvod digestat koji ima smanjen udio hlapljivog dušika, ali poboljšava kvalitetu tla i prinose. U odnosu na stajski gnoj digestat sadrži veći udio amonijaka koji biljke lakše i brže koriste čime se sprječava procjeđivanje i dospijevanje dušika u vode (Díaz-Vázquez i sur., 2020.). Također, proizvodnjom bioplina smanjuje se pojava neugodnih mirisa i insekata koji inače nastaju tijekom skladištenja stajskog gnoja.

U stajskog gnoju goveda, svinja i peradi uglavnom se nalazi oko 80 % hlapljivih tvari u ukupnoj suhoj tvari te se prinos metana kreće od 0,20 do 0,60 m^3/kg hlapljivih tvari. Prinos metana po toni suhe tvari za stajski gnoj je 200-250 m^3 te je gotovo upola manji u odnosu na biljne sirovine (npr. ostatke iz prerade šećera, proizvodnje piva i alkoholnih pića, energetski usjevi). Stoga je kodigestija stajskog gnoja i biljnih komponenti uobičajena u povećanju učinkovitosti proizvodnje bioplina (Al Seadi i sur., 2008.). Dodatak zelene biljne mase povećava proizvodnju bioplina za 18-40 %, dok kodigestija sa sjetvenim ostatcima sa većim udjelom suhe tvari (npr. slama) povećava prinose bioplina 10-80 % (Al Seadi i sur., 2008.). Naime, stajski gnoj nastaje kao rezultat probave biljne hrane u probavnom sustavu domaćih životinja te je stoga njegov potencijal za proizvodnju metana manji u odnosu na druge supstrate, odnosno biljne komponente. Također, stajski gnoj sadrži i veliki udio teže

probavljivih komponenti jer su one lakše probavljive već razgrađene u probavnom sustavu životinje (Liebetrau i sur., 2021.).
Glavni čimbenici učinkovitosti procesa AD su potencijal stajskog gnoja za proizvodnju bioplina, sadržaj vode u stajskom gnoju, nepoželjne i inhibirajuće komponente u stajskom gnoju, veličina stada odnosno količina proizvedenog stajskog gnoja (Liebetrau i sur., 2021.). Pri tome, sve ove komponente su međusobno ovisne. Visoki udio vode u stajskom gnoju (<10 % suhe tvari; npr. svinjska gnojovka) zahtjeva veći utrošak energije za postizanje i održavanje optimalne temperature digestije te povećava troškove prijevoza i potrebu za korištenjem dodatnih supstrata koji će povećati prinos metana. S druge strane, gnoj peradi ima veliki postotak suhe tvari što olakšava manipulaciju i prijevoz na veće udaljenosti te visoki postotak dušika. No, peradski stajski gnoj sadrži veće količine inertnih materijala (pijesak, kamenčići i sl.) koji mogu abrazivno djelovati na pokretne dijelove digestora i uzrokovati mehaničke probleme, a ujedno se talože na dno digestora, smanjujući njegov efektivni volumen. Osim toga, visok udio dušika predstavlja problem i izazov u održavanju optimalnih uvjeta u digestoru budući da rezultira nastankom većih količina amonijaka koji inhibira rast anaerobnih mikroorganizama.
Stajski gnoj goveda sadrži povoljniji udio dušika u odnosu na peradski ili svinjski stajski gnoj te se lako prevodi u metan. Brdarić i sur. (2009.) navode da bioplin iz goveđe gnojovke u RH sadržava prosječno 4,35 % dušika (N_2), 68,83 % metana i 26,81 % ugljikovog dioksida. Isti autori navode da se anaerobnom fermentacijom u trajanju od 40 dana pri mezofilnim uvjetima iz 1 kg goveđe gnojovke proizvede 31 L bioplina, a iz 1 kg svinjske gnojovke 14,83 L bioplina. Isplativost i opravdanost ulaganja u izgradnju bioplinskih postrojenja koja će koristi stajski gnoj kao sirovinu treba zasnivati i na veličini farmi, odnosno broju domaćih životinja na pojedinom području. Tako stado od 50 mliječnih krava (što je manje-više prosjek veličine stada u većini razvijenih zemalja) nije dovoljno za proizvodnu dostatnih količina stajskog gnoja za ekonomski isplativu proizvodnju bioplina (Liebetrau i sur., 2021.). Stoga, s obzirom na broj domaćih životinja i veličinu farmi u RH, preporučuje se izgradnja mikro bioplinskih postrojenja (10-50 kW) (Energetika-net, 2018.).

Zaključak

Stajski gnoj je zbog niske cijene, dostupnost, prirodno prisutnih anaerobnih bakterija te visokog sadržaj vode poželjna sirovina u proizvodnji bioplina. Ipak, kako bi se ostvarili veći prinosi bioplina poželjno je stajski gnoj koristiti u kodigestiji sa biljnim sirovinama. Stajski gnoj goveda ima bolji potencijal proizvodnje bioplina u odnosu na peradski ili svinjski.

Literatura

Al Seadi T., Rutz D., Prassl H., Köttner M., Finsterwalder T., Volk S., Janssen R. (2008). Biogas handbook. Esbjerg, Denmark: University of Southern Denmark.

Bošnjak R., Prpić Z., Kelava Ugarković N., Konjačić M., Vnučec I. (2018). Possibilities for mitigating the environmental footprint of dairy ruminants. Mljekarstvo. 68(3): 153-168.

Brdarić, D., Kralik, D., Kukić, S., Spajić, R., Tunjić G. (2009). Konverzija organskog gnoja u bioplin. Poljoprivreda. 15(2): 3-7.

Direktiva 2009/28/EZ Directive 91/676/EEC of the Council of the European Communities of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources.

Energetika-net (2018.). Raspoloživo: http://www.energetika-net.com

Eurostat (2018). Agri-Environemntal Indicatore Greenhouse Gas Emissions. Raspoloživo: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Archive:Agri-environmental_indicator_-_greenhouse_gas_emissions&oldid=374989

Foged, H., Flotats, X., Blasi, A.B., Palatsi, J., Magri, A., Schelde, K.M. (2011). Inventory of manure processing activities in Europe. Technical Report No. I concerning 'Manure Processing Activities in Europe'to the European Commission, Directorate-General Environment.

Köninger J., Lugato E., Panagos P., Kochupillai M., Orgiazzi A., Briones M.J.I. (2021). Manure management and soil biodiversity: Towards more sustainable food systems in the EU. Agricultural Systems. (194): 103251.

Liebetrau J., O'Shea R., Wellisch M., Lyng K.A., Bochmann G., McCabe B.K., Harris P.W., Lukehurst C., Kornatz P., Murphy J.D. (2021). Potential and utilization of manure to generate biogas in seven countries. Objavljeno u: IEA Bioenergy Task, Murphy J.D. (Ed.), 37, 2021: 6.

Moeller H.B. , Sommer S.G. , Ahring B.K. (2004). Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy. 26: 485-495.

NN 60/2017. II. Akcijski program zaštite voda od onečišćenja uzrokovanog nitratima poljoprivrednog podrijetla. Zagreb: Ministarstvo poljoprivrede.

Oenema O., Oudendag D., Velthof G.L. (2007). Nutrient losses from manure management in the European Union. Livestock Science 112(3): 261-272. Raspoloživo: https://doi.org/10.1016/j.livsci.2007.09.007

Rayne N., Aula L. (2020). Livestock manure and the impacts on soil health: a review. Soil Systems. 4: 64. Raspoloživo: https://doi.org/10.3390/soilsystems4040064

Regulation 1069/2009 of the European Parliament and of the Council of 21 October 2009 laying down health rules as regards animal by-products and derived products not intended for human consumption.

Sajalpal K., Vnučec I., Konjačić M. (2014). Analiza opterećenja površinskih i podzemnih voda iz stočarske proizvodnje. Objavljeno u: *Utjecaj poljoprivrede na onečišćenje površinskih i podzemnih voda u Republici Hrvatskoj*. Zagreb: Sagra.

Scarlat N., Fahl F., Dallemand J.F., Monforti F., Motola V. (2018). A spatial analysis of biogas potential from manure in Europe. Renewable and Sustainable Energy Reviews. 94: 915-930. Raspoloživo: https://doi.org/10.1016/j.rser.2018.06.035.

Truong L.V.A, Abatzoglou N. (2005). A H_2S reactive adsorption process for the purification of biogas prior to its use as a bioenergy vector. Biomass Bioenergy. 29(2): 142–151.

Webb J., Sørensen P. , Velthof G., Amon B., Pinto M., Rodhe L., Salomon E., Hutchings N., Burczyk P., Reid J. (2013). An assessment of the variation of manure nitrogen efficiency throughout Europe and an appraisal of means to increase manure-N efficiency. Advanteges in Agronomy. 119: 371-442.

Possibilities of using manure as a raw material in biogas production

Abstract

Depending on the yield, storage and application, manure can contribute to pollution of air, water, soil and greenhouse gas emissions. Anaerobic digestion of manure during biogas production reduces the risk of these contaminants. The produced biogas can be used for biomethane production, while the by-product of biogas production, digestate, can be used as fertilizer or litter. Therefore, the aim of this paper is to present the properties of manure and its advantages and disadvantages as raw materials in biogas production.

Key words: livestock manure, biogas, methane, anaerobic digestion, environment protection

ORIGINAL SCIENTIFIC PAPER

Serum biochemical parameters of Alpine goat kids during suckling and post-weaning period

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Abstract
The aim of the present study was to determine the difference between the biochemical serum parameters of goat kids during suckling and post-weaning period. The study was carried out with 10 French Alpine goat kids aged 32 (±3) days in the suckling period and 87 (±3) days in the post-weaning period. When the goat kids were weaned, the concentrations of total proteins (61.7 vs. 54.3 g/l; P=0.002), globulins (31.2 vs. 25.4 g/l; P=0.017) and the activities of AST (107.3 vs. 70.3 U/l; P=0.003) and GGT (47.5 vs. 36.9 U/l; P=0.038) in serum increased compared to the values during the suckling period. These changes are a consequence of the normal growth and organ maturation of goat kids during transition from non-functional ruminant during suckling to functional one in post-weaning period.

Key words: goat kids, biochemical parameters, serum, weaning, growth

Introduction

Of the total number of goats in the Republic of Croatia (86 258; FAOSTAT, 2022) the French Alpine was the most numerous breed with 4 193 goats in the herdbook goat breed structure (HAPIH, 2021). The farming goal of this breed is milk, while male and female goat kids which are not intended for reproduction are further used for meat production (Mioč et al., 2011). Goat kids are very sensitive, susceptible to diseases and insufficient growth and development, due to poor immunity and poorly developed organs and biological mechanisms in their early life during the suckling period (Antunović et al., 2008). During this period, the kids are very sensitive to changes in diet, especially to adjustment to diet containing voluminous feed and cereals with a gradual reduction of milk protein (Antunović et al., 2015). It is well known that weaning period represents a stressful condition for animals. Therefore, weaning must be gradual to ensure optimal rumen development. Raising healthy animals is very important, because health status can affect goat kids production at a young age and in adulthood. Problems include diarrhea and lower daily weight gain, as well as respiratory diseases, that can lead to high levels of mortality (Diao et al., 2019). Determination of biochemical parameters is a clinical tool that helps in diagnosis of various diseases. It can also be used to evaluate animals´ metabolic profile by mainly assesing the nutritional management and reproductive performance (Souza et al., 2020). It is known from other studies that age of ruminants plays an important role in the concentration of many blood parameters (Antunović et al., 2017). However, for goat kids, a better understanding is required, especcially for the differences between suckling and post-weaning period. Threfore, the aim of the present study was to determine the differences in biochemical parameters of goat kids between the suckling and post-weaning period.

Material and methods

The study was conducted within the legal regulations according to the Animal Protection Act of Croatia (NN 133/06, NN 37/13 and NN 125/13) and within the European Union

Directive 2010/63/UE regarding animal protection as approved by the Bioethics Committee for Research on Animals of the Faculty of Agrobiotechnical Sciences, Osijek.
The research was conducted with 10 goat kids (50%♂: 50%♀) of the French Alpine breed on the family farm in Marjančaci. All ten goat kids were monitored at the age of 32 (± 3) and 87 (± 3) days in the period of suckling and post-weaning, respectively. Goat kids were kept in a semi-intensive farming system, together with goats, until the age of two months. Until the age of 32 ± 3 days, all goat kids were kept together with the goats and were suckling *ad libitum*, with milk (3.22% of fat, 2.72% of protein, 4.28% of lactose) being the only source of food. After this, the kids were weaned from their mothers, by gradually reducing milk feeding and by including feed mixture (87.6% od dry matter, 16.2% of crude proteins; 5.6 % of crude fat; 4.1% of crude fibre, 13.2 MJ/kg of metabolizable energy) and hay (clover-grass mixture). The goat kids were kept separated from their mothers, during the night, and after the morning milkings kids were returned to the pens where they stayed with the goats until they were two months old when they were completely weaned. Goat kids had a free access to fresh drinking water, feed mixtures and hay *ad libitum*.
At the beginning and at the end of the study period, when kids were 32 (suckling, pre-weaning) and 87 (post-weaning) days of age, blood samples were taken. Blood samples were collected from the jugular vein into sterile vacuum tubes (Venoject®; Leuven, Belgium) in the morning. Within 4 h after sampling, blood samples were centrifuged (10 min) at 1609.92×*g* when serum was obtained. Serum was separated and transferred to 2 ml safe-lock tubes (Eppendorf, Hamburg, Germany). These samples were frozen at −20°C until analysis. Following biochemical parameters were determined in serum: glucose, total proteins, urea, albumin, cholesterol, high density lipoprotein (HDL-cholesterol), low density lipoprotein (LDL-cholesterol), triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl-transferase (GGT). The concentration of globulins was obtained by subtracting the albumin from the total protein. Beckman Coulter AU400 (Beckman Coulter Inc., Brea, CA, USA) automatic analyser was used to determine biochemical parameters by using Total Protein Reagent (Beckman Coulter Inc., Brea, CA, USA).
The results were analysed with MEANS procedure and expressed as arithmetic mean, standard deviation, minimal and maximal value, as well as standard error of mean and coefficient of variation. The ANOVA was used to determined significant difference in biochemical parameters between suckling and post-weaning period of goat kids. All results were analysed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

Table 1. presents a descriptive statistics of biochemical parameters in serum of goat kids. The concentration of glucose, urea, albumin, cholesterol, LDL, HDL, tryglicerides and ALT in goat kids did not differ between suckling and post-weaning period (Table 2). It is evident that the most of serum biochemical parameters were within the reference values, whereas the highest coefficient of variation was determined LDL-cholesterol concentrations and ALT activity.
The concentration of glucose did not differ during the growth of goat kids. Concentration of glucose in serum is ussually not influenced very much by the feeding, because of homeostatic mechanism which keeps its concentration constant, as reported by Sundrum (2015). Since protein content in milk was 2.72%, and the sampling was done during the peak of lactation, the total proteins in goat kids´ serum was lower during the suckling at the age of 32 days compared to age of 87 days when goat kids consumed concentrate feed mixture rich in proteins. As reported by Mthi et al. (2021) an adequate supply of protein is a fundamental factor for proper growth of goats. Adequate proportion of crude proteins and

energy provided by concentrate feed mixture (16.2% of crude protein; 13.2 MJ/kg of metabolizable energy) explains the higher (P<0.01) serum total proteins in goat kids in post-weaning period. However, protein concentration in serum was slightly bellow reference values, which was expected, since reference values were estimated for adult goats, and not younger categories. Osman et al. (2020) determined similar concentration of total proteins in weaned goat kids, which was 61 ± 4.1 g/l. According to Souza et al. (2020) lower values for total proteins and globulins at 30 days represent the end of the degradation process of immunoglobulins passively received via colostrum and by the initial phase of active production of immunoglobulins by the animal itself. In the present study globulin concentration was higher (P=0.017) in goat kids post-weaning compared to suckling period. After few months, the increase of globuline concentration in serum mainly come from the increase of gamma globulin concentrations related to adaptation on environment conditions as at this age animal already has matured immune system, which was determined in lambs by Santos et al. (2017). The albumin is mostly influenced by dietary intake, and is ussually increasing in lambs until 90 days, as these animals are already ruminating and are capable to use nitrogen from the diet (Souza et al., 2020). The albumin concentration in serum of goat kids during suckling period were at lower limit of the reference values, while in post-weaning period was within the reference values, although without significant differences.

Table 1. Descriptive statistics of serum biochemical parameters of growing goat kids

Parameters	Mean	Sd	Min	Max	CV
Glucose, mmol/l	3.83	0.63	3.00	5.05	16.3
Total protein, g/l	57.8	5.43	48.7	68.6	9.40
Urea, mmol/l	4.40	1.11	2.30	6.86	25.2
Albumin, g/l	29.7	2.02	25.9	33.6	6.82
Globuline, g/l	28.1	5.23	18.4	39.2	18.6
Cholesterol, mmol/l	2.45	0.84	1.68	4.62	34.2
LDL-cholesterol, mmol/l	0.85	0.51	0.32	2.25	60.4
HDL-cholesterol, mmol/l	1.42	0.33	1.11	2.26	23.3
Triglycerides, mmol/l	0.40	0.13	0.25	0.70	32.3
AST, U/l	86.5	27.6	53.2	155.8	32.0
ALT, U/l	16.6	7.13	10.6	36.9	43.0
GGT, U/l	42.2	10.4	28.7	60.6	24.7

Sd-standard deviation; CV-coefficient of variation; LDL-low density lipoprotein; HDL-high density lipoprotein; AST-aspartate aminotransferase; ALT-alanine aminotransferase; GGT-γ-glutamyl transferase.

The cholesterol and tryglicerides concentration was presented by high coefficient of variation, which is normal since weaning is a period in which milk is progressively replaced by feed mixtures and forage. Besides, as goat kids start to consume dry feed, the digestive system begin to develop (Diao et al., 2019). As a consequence of rumen development, elementary changes in metabolic pathways occur in the liver (Baldwin et al., 2004), which has a higher ability to metabolize lipids compared to early age of life. This was also confirmed in the present study by higher acitivities of examined liver enzymes, such as AST (P=0.003) and GGT (P=0.038) in goat kids post-weaning compared to suckling period. As goat kids are growing, the AST and GGT activities are increasing in post-weaning period due to greater mass of muscle and metabolic activities as explained by Cruz et al. (2017). The increase in GGT activity in goat kids post-weaning can be related to functional adaptation of liver during growth.

Table 2. Serum biochemical parameters of goat kids during suckling and post-weaning period

Parameters	Goat kids		SEM	P-value	Reference values[1]
	Suckling	Post-weaning			
Glucose, mmol/l	3.54	4.04	0.167	0.146	2.4-4.0
Total protein, g/l	54.3	61.7	1.32	0.002	62-79
Urea, mmol/l	4.21	4.61	0.269	0.475	4.0-8.6
Albumin, g/l	29.0	30.5	0.49	0.126	29-43
Globuline, g/l	25.4	31.2	1.27	0.017	35-57[2]
Cholesterol, mmol/l	2.44	2.47	0.204	0.955	1.0-3.0
LDL-cholesterol, mmol/l	0.81	0.89	0.124	0.752	1.37±0.36[3]
HDL-cholesterol, mmol/l	1.45	1.40	0.081	0.794	1.27±0.19[3]
Triglycerides, mmol/l	0.42	0.39	0.032	0.662	0.33±0.07[3]
AST, U/l	70.3	107.3	6.72	0.003	46-161[4]
ALT, U/l	13.1	19.6	2.00	0.099	14-32[5]
GGT, U/l	36.9	47.5	2.53	0.038	34-65[4]

[1]Jackson and Cockcroft (2002); [2]Kaneko 2008; [3]Antunović et al. (2017)-determined in goat kids aged 50 days; [4]Smith et al. (2002); [5]Tschuor et al. (2008); LDL-low density lipoprotein; HDL-high density lipoprotein; AST-aspartate aminotransferase; ALT-alanine aminotransferase; GGT-γ-glutamyl transferase.

Conclusions

The results of the present study indicate changes during animals growth, especially between suckling and post-weaning period in goat kids aged 32 and 87 days, respectively. After weaning, the concentrations of total proteins, globulins, as well as activities of liver enzymes such as AST and GGT increased compared with the values during the suckling period. These changes are a consequence of normal growth and organ maturation of goat kids, as well as adaptation to environmental conditions during growth and transition from non-functional ruminant during suckling period to functional ruminant in post-weaning period.

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References

Antunović Z., Šperanda M., Senčić Đ., Domaćinović M., Novoselec J. (2008). Efficiency of probiotic preparation „Probios 2b“ in goat kids feeding. Krmiva. 50(2): 73-78.

Antunović Z., Novoselec J., Klir Ž. (2015). Body growth of goat kids in organic farming. Macedonian Journal of Animal Science. 5(2): 59-62.

Antunović Z., Šperanda M., Novoselec J., Đidara M., Mioč B., Klir Ž., Samac D. (2017). Blood metabolic profile and acid-base balance of dairy goats and their kids during lactation. Veterinarski Arhiv. 87(1): 43-55.

Baldwin R. L., McLeod K. R., Klotz J. L., Heitmann R. N. (2004). Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. Journal of Dairy Science. 87:(E. Suppl.): E55–E65.

Croatian Agency for Agriculture and Food, HAPIH (2021). Sheep, goats and small animals breeding. Annual report. 68, Osijek, Croatia: Croatian Agency for Agriculture and Food.

Cruz R. E. S., Rocha F. M., Sena C. V. B., Noleto P. G., Guimarães E. C., Galo J. A., Mundim A. V. (2017). Effects of age and sex on blood biochemistry of Dorper lambs. Semina: Ciências Agrárias. 38: 3085-3094.

Diao Q., Zhang R., Fu T. (2019). Review of strategies to promote rumen development in calves. Animals. 9, 490.

FAOSTAT (2022). Available from: https://www.fao.org/faostat/en/#data

Jackson P. G. G., Cockcroft P. D. (2002). Clinical examination of farm animals. 302-305, USA, New Yersey: Wiley-Blackwell.

Kaneko J. J., Harvey J. W., Bruss M. L. (2008). Clinical biochemistry of domestic animals. 144-916, USA, San Diego: Academic Press.

Mioč B., Sušić V., Antunović Z., Prpić Z., Vnučec I., Kasap A. (2011). Study on birth weight and pre-weaning growth of Croatian multicolored goat kids. Veterinarski Arhiv. 81(3): 339-347.

Mthi S., Nyangiwe N., Gwaze F. R., Yawa M., Tyasi T. L., Tokozwayo S., Thubela T., Jansen M. S., Goni S., Khetani T., Qokweni L., Washaya S., Guza B., Magwaza M., Mbangi B. B., Ndobeni T. (2021). Exploratory study on relationship among body weight, body condition score and some blood biochemical parameters of non-descriptive goats in Mzimvubu local municipality: A case of Santombe Village. Open Journal of Animal Sciences. 11: 646-657.

Osman O. A., Elkhair N. M., Abdoun K. A. (2020). Effects of dietary supplementation with different concentration of molasses on growth performance, blood metabolites and rumen fermentation indices of Nubian goats. BMC Veterinary Research. 16: 411.

Santos R. P., Lima Macedo G. J., Pedro da Silva S., Fernandes de Sousa L., Barbosa Andrade M. E. (2017). Inclusion of propylene glycol in the diet of sheep and its effect on their lambs' protein and mineral metabolites. Acta Scientiarum Animal Sciences. 39(3): 297-302.

Souza D. F., Reijers T. S. S. S., Gilaverte S., Cruz T. A., Hentz F., Castilhos B. Q., Dittrich R. L., Monteiro A. L. G. (2020). Dynamics of biochemical parameters in lambs during the first four months of life. Revista Brasileira de Zootecnia. 49: e20190167.

Smith B. P. (2002). Large animal internal medicine. London, United Kingdom: Copyright Mosby, Inc.

Sundrum A. (2015). Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals. 5(4): 978–1020.

Tschuor A. C., Riond B., Braun U., Lutz H. (2008). Hämatologische und klinisch-chemische Referenzwerte für adulte Ziegen und Schafe. Schweiz Arch Tierheilk. 150(6): 287-295.

Biokemijski pokazatelji jaradi u serumu tijekom razdoblja sisanja i nakon odbića

Sažetak

Cilj ovog istraživanja bio je utvrditi razlike u koncentracijama biokemijskih pokazatelja jaradi tijekom razdoblja sisanja i nakon odbića. Istraživanje je provedeno na 10 jaradi pasmine francuska alpina u prosječnoj dobi od 32 (± 3) dana tijekom razdoblja sisanja i dobi od 87 (± 3) dana, odnosno nakon odbića. Koncentracije ukupnih proteina (61,7 : 54,3 g/l; P=0,002), globulina (31,2 : 25,4 g/l; P=0,017), kao i aktivnosti AST (107,3 : 70,30 U/l; P=0,003) i GGT (47,5 : 36,9 U/l; P=0,038) u serumu povećane su u jaradi nakon odbića u odnosu na sisajuće razdoblje. Navedene promjene u serumu posljedica su normalnog rasta i razvoja organa jaradi tijekom prijelaza iz sisajućeg razdoblja u razdoblje nakon odbića, odnosno tijekom rasta i razvoja u funkcionalnog preživača.

Ključne riječi: jarad, biokemijski pokazatelji, serum, odbiće, rast

IZVORNI ZNANSTVENI RAD

Fizikalne i senzorske promjene svježeg svinjskog karea tijekom zrenja

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Sažetak
Cilj rada bio je utvrditi utjecaj trajanja zrenja (7 i 14 dana) na fizikalne i senzorske karakteristike svinjskog karea. Istraživanjem je utvrđeno značajno povećanje pH vrijednosti svinjskog karea nakon 7 i 14 dana mokrog zrenja u odnosu na početnu vrijednost. Pri kraćem zrenju utvrđeno je povećanje vrijednosti svih pokazatelja boje u odnosu na vrijednost s početka zrenja. Kalo zrenja bilo je ujednačeno bez obzira na trajanje, ali je nakon odmrzavanja najveće kalo utvrđeno kod skupine s najdužim zrenjem. Najveće kalo toplinske obrade utvrđeno je kod skupine bez zrenja, a najmanje kod skupine s najdužim zrenjem. Ustanovljeno je da su promjene teksture i okusa najvažnije senzorske promjene uzrokovane zrenjem.

Ključne riječi: svinjski kare, pH, boja, kalo, senzorska analiza

Uvod

Zrenje mesa uključuje razlaganje mišićne proteinske strukture dominantno pod djelovanjem endogenih proteaza. Pritom se najviše razlažu citoskeletni proteini poput titina, nebulina ili desmina, što dovodi do omekšavanja strukture mesa u procesima nakon rigor mortisa (Nowak, 2011.; Devine, 2014.). Unatoč poznatom učinku zrenja mesa na mekoću, vrlo često se u industriji i preradi mesa zanemaruje utjecaj zrenja svinjskog mesa na okus kroz stvaranje peptida i aminokiselina (Lee i sur., 2016.; Ngapo i sur., 2012.). Kao potpora tome brojna su istraživana koja pokazuju da svinjsko meso na zrenju od 6 do 10 dana ima bolje senzorske karakteristike od mesa koje zrije jedan ili dva dana (Channon i sur., 2004.; Juárez i sur., 2011.; Lee i sur., 2016.). Primjena zrenja jako je raširena kod junećeg mesa, dok se kod svinjskog mesa gotovo i ne provodi, a sam se proces zrenja odvija uglavnom tijekom distribucije mesa do krajnjeg potrošača. S druge strane, sve su izraženije pritužbe potrošača na kvalitetu i okus svinjskog mesa (Ngapo i sur., 2012.) što predstavlja izazov za suvremenu mesnu industriju. Stoga je cilj ovog istraživanja bio utvrditi utjecaj trajanja zrenja od 7 i 14 dana na fizikalne i senzorske karakteristike svinjskog karea.

Materijal i metode

U istraživanju je korišteno meso muških kastriranih tovljenika (N=5) pasmine njemački landras tjelesne mase oko 200 kg i starosti 12 mjeseci uzgojenih na gospodarstvu Mioković kod Siska. Klanje svinja provedeno je prema *Uputi za postupanje prilikom klanja svinja za potrošnju u vlastitom kućanstvu* (Ministarstvo poljoprivrede, 2014.). Nakon klanja provedeno je toplo rasijecanje trupova, a kare odnosno dugi leđni mišić (*lat. musculus longissimus dorsi*) je iskošten i ohlađen na 4 °C tijekom 18 sati. Nakon hlađenja pripremljeni su uzorci karea mase oko 1 kg, a potom nasumično razvrstani po deset uzoraka u tri skupine; Z0 – kontrolna skupina bez zrenja, Z7 – pokusna skupina sa 7 dana zrenja i Z14 – pokusna

skupina s 14 dana zrenja. Uzorci su 24 sata nakon klanja pojedinačno izvagani na digitalnoj vagi točnosti ± 1 g, izmjerena je pH vrijednost pomoću prijenosnog pH metra IQ150 (Texas Instruments, SAD) opremljenog ubodnom sondom BlueLine 21 (Shott Instruments, Njemačka) i boja mesa pomoću kolorimetra Chroma Meter CR 410 (Konica Minolta, Japan) s 50 mm dijametarskim područjem mjerenja i spektrom boja L*, a*, b* (CIE, 1976) uz standardnu iluminaciju za meso D-65 s vremenom stabilizacije boje (blooming time) od 30 min. Uzorci svih skupina potom su vakumirani i zamrznuti na -20 °C (uzorci skupine Z0) odnosno ostavljeni u hladnjaku na temperaturu + 3 °C (uzorci skupina Z7 i Z14). Nakon zrenja od 7 (skupina Z7) i 14 dana (skupina Z14) uzorci su ponovo izvagani radi izračuna kala zrenja te je izmjerena pH vrijednost i boja. Uzorci Z7 i Z14 potom su vakumirani i zamrznuti na temperaturu -20 °C do senzorske analize.

Senzorska analiza provedena je nakon 3 mjeseca čuvanja uzoraka u zamrzivaču. Uzorci su najprije odmrznuti u hladnjaku na 4 °C tijekom 24 sata, potom su izvagani radi izračuna kala odmrzavanja. Zatim su uzorci stavljeni u HDPE vrećice (polietilen visoke gustoće) i toplinski obrađeni na temperaturi od 90 °C do postizanja 80 °C u centru proizvoda. Nakon hlađenja do 60 °C na sobnoj temperaturi uzorci su izvagani radi izračuna kala toplinske obrade te su pripremljeni za serviranje u obliku kockice veličine 1 x 1 x 1 cm. Uzroci su do serviranja čuvani u zatvorenoj posudi na temperaturi 60 °C. U senzornoj analizi korišten je triangl test pomoću 22 prethodno educirana ocjenitelja iz redova studenata i djelatnika Agronomskog fakulteta s ujednačenim odnosom spolova (55 % muški : 45 % ženski) u individualnim boksovima (Lawless i Heymann, 2010.). Pri provedbi triangl testa ocjeniteljima su u jednom nizu predstavljena tri uzorka označena troznamenkastom šifrom pri čemu su morali odabrati uzorak koji smatraju različitim od preostala dva i odrediti u čemu percipiraju razliku (mirisu, okusu, teksturi ili ostalome). Ukupno su bila prezentirana 4 niza, od čega su dva niza bila sastavljena iz uzoraka Z0-Z7 i dva niza iz uzoraka Z0-Z14. Redoslijed nizova u triangl testu kao i redoslijed uzoraka unutar niza bio je slučajno određen. Ocjenitelji su zamoljeni da nakon uzimanja svakog pojedinačnog niza konzumiraju kruh i vodu radi neutralizacije usta i odmora osjetila.

Dobiveni podaci obrađeni su pomoću statističkog programa SAS Studio University Edition 3.71 (SAS Institute, 2018.). U analizi pH vrijednosti, kala i boje korištena je procedura GLM uz primjenu Tukey-Kramer post-hoc testa za utvrđivanje značajnosti razlika između skupina ($P<0,05$). Podaci dobiveni triangl testom obrađeni su izračunom statističke značajnosti bazirane na broju točnih odgovora i usporedbom sa statističkim tablicama (Lawless i Heymann, 2010.).

Rezultati i rasprava

U tablici 1 prikazane su vrijednosti pH i boje (L*, a* i b*) uzoraka karea prije zrenja (skupina Z0) i nakon zrenja od 7 dana (skupina Z7) odnosno 14 dana (skupina Z14). pH vrijednost kontrolne skupine Z0 iznosila je 5,40, dok se nakon 7 dana zrenja pH vrijednost povećala na 5,53, a gotovo identična vrijednost utvrđena je i nakon 14 dana zrenja (pH = 5,54). U obje pokusne skupine sa zrenjem povećanje pH vrijednosti bilo je statistički značajno ($P<0,05$) u odnosu na skupinu bez zrenja. Dobiveni rezultati u skladu su s navodima istraživanjima Juáreza i sur. (2011.) koji su utvrdili značajno povećanje ($P<0,05$) pH vrijednosti tijekom 7 i 14 dana zrenja, ali u manjem iznosu; 0,06 i 0,05 pH jedinica. Slično tome, Lee i sur. (2016.) utvrdili su značajno povećanje ($P<0,05$) pH vrijednosti pri zrenju svinjskih trupova u trajanju od 40 dana s 5,54 na 5,66, odnosno za 0,12 pH jedinica. Isti autori zaključuju da bi povećanje pH vrijednosti moglo biti uzrokovano povećanjem količine bazičnih aminokiselina oslobođenih tijekom degradacije proteina djelovanjem degradacijskih enzima mesa. Za razliku od prikazanog, Ngapo i sur. (2012.) nisu utvrdili značajne razlike u pH vrijednosti tijekom čuvanja svinjskog karea u trajanju 12 dana s dvodnevnim intervalima mjerenja.

Boja mesa iskazana kao L* (svjetlina mesa), a* (stupanj crvenila mesa) i b* (stupanj žutila mesa) statistički se značajno mijenjala tijekom zrenja. Utvrđeno je da je u skupini Z7 došlo do značajnog povećanja (P<0,05) L* vrijednosti, dok je kod skupine Z14 povećanje bilo manje i nije bilo statistički značajno. Stupanj crvenila mesa a* statistički se značajno (P<0,05) povećao tijekom zrenja kod skupine Z7 i Z14. Stupanj žutila mesa b* značajno se povećao (P<0,05) kod skupine Z7 od početka do kraja zrenja, dok je kod skupine Z14 čak došlo do smanjenja, ali bez značajnog utjecaja. Iz tih rezultata je vidljivo da je pri zrenju od 7 dana utvrđeno značajno povećanje vrijednosti svih pokazatelja boje u odnosu na vrijednosti uzoraka skupine koja nije bila na zrenju. Međutim, nakon 14 dana zrenja ustanovljeno je značajno povećanje samo pokazatelja boje a* u odnosu na početne vrijednosti skupine Z0. Dobiveni rezultati djelomično su u skladu s prethodnim istraživanjima Juáreza i sur. (2011.) koji su tijekom zrenja od 7 i 14 dana utvrdili statistički značajno povećanje L* vrijednosti. Nasuprot tome, istraživanjem Ngapo i sur. (2012.) nisu utvrđene značajne promjene kod svih pokazatelja boje (L*, a* i b*) tijekom zrenja od 12 dana uz uzorkovanje svakih dva dana.

Tablica 1. pH vrijednosti i pokazatelji boje uzoraka karea prije i nakon zrenja

Svojstvo	Skupina[1]		
	Z0	Z7	Z14
pH	5,40±0,010 [b]	5,53±0,006 [a]	5,54±0,009 [a]
Boja – L*	51,41±0,995 [b]	56,31±0,814 [a]	53,10±0,122 [b]
Boja – a*	19,04±0,343 [b]	20,20±0,118 [a]	20,16±0,358 [a]
Boja – b*	6,95±0,639 [b]	8,96±0,342 [a]	6,93±0,254 [b]

[1] Z0: skupina bez zrenja; Z7: skupina sa zrenjem u trajanju 7 dana; Z14: skupina sa zrenjem u trajanju 14 dana; [a, b] : vrijednosti označene različitim slovima unutar reda statistički značajno se razlikuju (P<0,05)

U tablici 2 prikazani su iznosi kala zrenja, odmrzavanja, toplinske obrade i sveukupnog kala. Kalo zrenja kod skupine Z0 nije mjereno jer ti uzorci nisu stavljeni na zrenje. Između skupina Z7 i Z14 kalo zrenja nije se značajno razlikovao. Predmetnim je istraživanjem utvrđeno statistički značajno veće (P<0,05) kalo odmrzavanja kod skupine Z14 u odnosu na skupine Z7 i Z0. Izraženo relativno, skupina Z14 imala je za 46,4 % veći gubitak na masi od skupine Z7, odnosno za 100,2 % veći gubitak u odnosu na skupinu Z0. Znanstvene studija o kakvoći zamrznutog mesa svinja (Hrvatska agencija za hranu, 2014.) navodi da kalo odmrzavanja nakon tri mjeseca čuvanja iznosi 8,67 %, što je vrlo slično vrijednosti koja je utvrđena kod skupine Z0 (8,45 %).

Tablica 2. Vrijednosti kala (%) nakon zrenja, odmrzavanja i toplinske obrade

Svojstvo	Skupina[1]		
	Z0	Z7	Z14
Kalo zrenja, %	-	6,55±0,006	6,58±0,006
Kalo odmrzavanja, %	8,45±0,006[b]	11,56±0,006[b]	16,92±0,006[a]
Kalo toplinske obrade, %	51,20±0,010[a]	34,52±0,010[b]	26,63±0,010[c]
Sveukupno kalo, %	64,00±0,010[a]	61,37±0,010[ab]	54,07±0,010[b]

[1] Z0: skupina bez zrenja; Z7: skupina sa zrenjem u trajanju 7 dana; Z14: skupina sa zrenjem u trajanju 14 dana; [a, b, c]: vrijednosti označene različitim slovima unutar reda statistički značajno se razlikuju (P<0,05)

Nakon toplinske obrade najveće kalo utvrđeno je kod skupine Z0 (51,20 %), zatim kod skupine Z7 (34,52 %), dok je najmanje kalo toplinske obrade utvrđeno kod skupine Z14 (26,63 %). Do sličnih zaključaka došli su Lee i sur. (2016.) koji navode da pri 40-dnevnom suhom zrenju svinjskih polovica dolazi do značajnog smanjenja kala kuhanja (za 21,9 %).

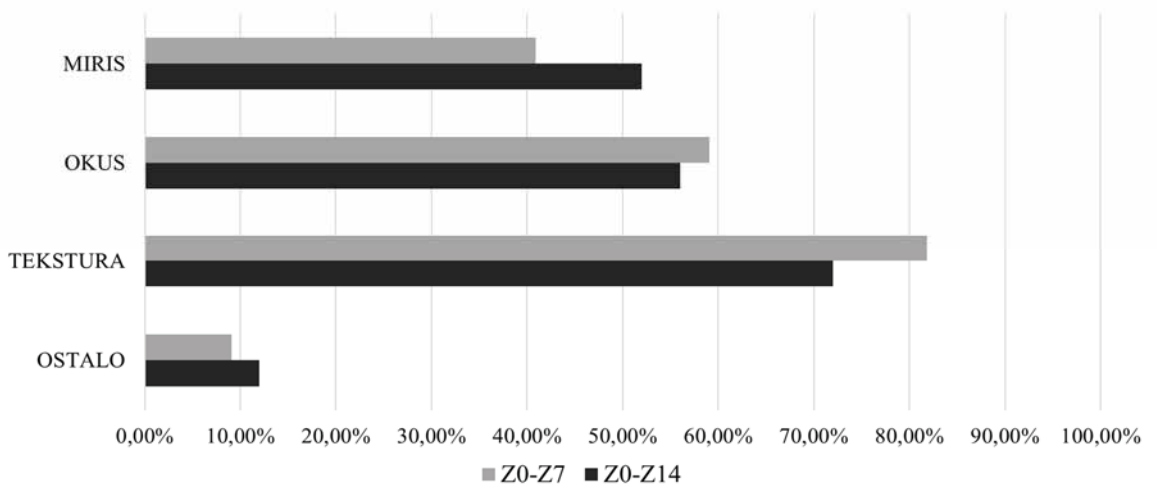
Juárez i sur. (2011.) su pak utvrdili manje, ali statistički značajne razlike u kalu kuhanja svinjskog karea (25,02 % kod kontrolne skupine, 23,28 % kod 7 dana zrenja te 21,43 % kod 14 dana zrenja). Male, ali značajne razlike u kalu kuhanja uzoraka pri 12-dnevnom zrenju svinjskog mesa navode Ngapo i sur. (2012.) u iznosu od 19,9 % (kontrolna skupina) do 17,5 % (12 dana zrenja). Ukoliko bi dobivene vrijednosti izrazili relativno, tada je kalo toplinske obrade skupine Z7 bio za 29,60 %, a skupine Z0 za 92,3 % veći od skupine Z14.
Sveukupno kalo skupine Z7 nije se statistički razlikovalo od skupine Z0 i Z14. No, sveukupno kalo skupine Z14 bilo je statistički značajno manje u odnosu na skupinu Z0 za 9,93 % ili relativno izraženo za 18,4 %. Iz ovih rezultata je vidljivo da je između skupina s različitom dužinom zrenja najveće gubitke na masi pri odmrzavanju imala skupina koja je bila najduže u zrenju (Z14), dok je najmanje kalo odmrzavanja bilo kod skupine koja uopće nije bila na zrenju (Z0). Nasuprot tome, nakon toplinske obrade najveći je gubitak na masi utvrđen upravo kod skupine bez zrenja (Z0), a najmanji kod skupine koja je bila najduže na zrenju (Z14). Navedeno se može objasniti manjim sadržajem vode kod skupine Z14 zbog većeg gubitka vode u ranijim fazama (nakon zrenja i odmrzavanja) što potvrđuju rezultati Lee i sur. (2016.). Uz navedeno, predmetnim je istraživanjem utvrđena značajno veća pH vrijednost uzoraka nakon zrenja pri čemu se pH odmiče od izoelektrične točke miofibrilarnih proteina možebitno uslijed povećanog ukupnog naboja na proteinima i posljedično povećane sposobnosti vezanja vode (Huff-Lonergan i Lonergan, 2005.). Dodatno, Kristensen i Purslow (2001.) napominju da zbog degradacije citoskeletnih proteina tijekom zrenja dolazi do manjeg skraćivanja miofibrila uzrokovano rigorom, što omogućava izvanstaničnoj vodi ulazak u mišićnu stanicu i posljedično povećanje sposobnosti vezanja vode.
U tablici 3. prikazani su rezultati triangl testa uzoraka svinjskog karea različitog trajanja zrenja. U testiranju parova je 50-56,8 % ocjenitelja uočilo razliku između skupina. S obzirom na broj ponavljanja prema statističkim tablicama (Lawless i Heymann, 2010.) utvrđeno je da su ocjenitelji uočili statistički značajne razlike u senzorskim svojstvima u parovima skupina Z0-Z7 (P=0,024) i Z0-Z14 (P=0,02). Također, točnost odgovora bila je veća kod parova skupina Z0-Z14, odnosno ocjenitelji su kod tog para jasnije percipirali razliku.

Tablica 3. Rezultati triangl testa svinjskog karea različitog trajanja zrenja

Parovi uzoraka[1]	Broj točnih odgovora	Udio točnih odgovora, %	P-vrijednost
Z0-Z7	22 od 44	50,0	0,024
Z0-Z14	25 od 44	56,8	0,002

[1] Z0: skupina bez zrenja; Z7: skupina sa zrenjem u trajanju 7 dana; Z14: skupina sa zrenjem u trajanju 14 dana

Na grafikonu 1. prikazane su učestalosti označavanja svojstava prema kojima su ocjenitelji percipirali razliku između uzoraka. Vidljivo je da se preko 70 % uočenih razlika odnosilo na teksturu, s time da je to svojstvo u 82 % slučajeva bilo označeno kod para Z0-Z7, dok je kod para Z0-Z14 bilo označeno u 72 % slučajeva. Okus je bio označen kao različit u 59 % kod para Z0-Z7, a kod para Z0-Z14 u 56 % slučajeva. Međutim, svojstvo mirisa bilo je češće označeno kao različito kod para Z0-Z14 (52 % slučajeva) nego kod para Z0-Z7 (41 % slučajeva). Iz navedenog bi mogli zaključiti da je promjena teksture tijekom zrenja glavna senzorska manifestacija. Promjena okusa druga je važna manifestacija, ali se njezin značaj pri dužem trajanju zrenja izjednačava s promjenom mirisa.



Grafikon 1. Učestalost označavanja svojstava u kojima je percipirana razlika kada su odgovori u triangl testu bili točni
Z7: skupina sa zrenjem u trajanju 7 dana; Z14: skupina sa zrenjem u trajanju 14 dana

Lee i sur. (2016.) navode da je zrenje svinjskog karea dovelo do značajno manjih vrijednosti instrumentalno određene tvrdoće, kohezivnosti, otpora pri žvakanju te sili presijecanja iz čega su autori zaključili da su uzorci nakon zrenja generalno mekaniji. Do sličnih rezultata došli su i Juárez i sur. (2011.) koji su pri zrenju svinjskog karea u trajanju 7 i 14 dana utvrdili povećanje mekoće i sočnosti, s time da je intenzitet okusa bio značajno povećan nakon 7, ali ne i nakon 14 dana u odnosu na uzorke bez zrenja. Dobiveni rezultati o smanjenju razlike u okusu nakon 7 dana zrenja sukladni su rezultatima istraživanja Juárez i sur. (2011.). Naime, poboljšanje mekoće povezuje se sa strukturnom degradacijom miofibrila uzrokovano degradacijom miofibrilarnih i citoskeletnih proteina tijekom zrenja (Nowak, 2011.).

Zaključak

Temeljem dobivenih rezultata možemo istaknuti da se pH vrijednost svinjskog karea značajno povećava u prvom tjednu zrenja i zadržava u drugom tjednu bez izraženijeg naknadnog povećanja. Iako je kalo zrenja bilo ujednačeno, najveće kalo odmrzavanja utvrđeno je kod skupine s trajanjem zrenja od 14 dana, dok je kod skupine bez zrenja bilo najmanje. Za razliku od tog, najveće kalo toplinske obrade utvrđeno je kod skupine bez zrenja, a najmanje kod skupine s najdužim zrenjem. Senzorskom analizom ustanovljene su statistički značajne razlike između skupina bez i sa zrenjem. Pritom vrijedi istaknuti da je promjena teksture tijekom zrenja glavna senzorska manifestacija. Promjena okusa je druga važna manifestacija, ali se njezin značaj pri dužem trajanju zrenja izjednačava s promjenom mirisa. Slijedom dobivenih rezultata može se zaključiti da je preporučljivo provoditi zrenje svinjskog karea u minimalnom trajanju 7 dana zbog značajnog utjecaja na senzorska svojstva.

Napomena

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Literatura

Channon H.A., Kerr M.G., Walker P.J. (2004). Effect of Duroc content, sex and ageing period on meat and eating quality attributes of pork loin. Meat Science. 66: 881-888.

Devine C.E. (2014). Conversion of muscle to meat - Aging. Objavljeno u Encyclopedia of Meat Sciences (Second Edition), Dikeman M., Devine, C. (ed.), 329-338. Academic Press, Nizozemska.

Huff-Lonergan E. i Lonergan S.M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science. 71: 194-204.

Hrvatska agencija za hranu. (2014). Kakvoća zamrznutog mesa svinja - Znanstvena studija o kakvoći zamrznutog mesa svinja. Raspoloživo: https://www.hah.hr/pregled-upisnika/?preuzmi_misljenje=40

Juárez M., Caine W. R., Dugan M.E.R., Hidiroglou N., Larsen I.L., Uttaro B., Aalhus J.L. (2011). Effects of dry-ageing on pork quality characteristics in different genotypes. Meat Science. 88: 117-121.

Kristensen L. i Purslow P.P. (2001). The effect of ageing on the water-holding capacity of pork: Role of cytoskeletal proteins. Meat Science. 58: 17-23.

Lawless H. T., Heymann H. (2010). Sensory Evaluation of Food Principles and Practices. Second edition. Springer. USA.

Lee C.W., Lee J.R., Kim M.K., Jo C., Lee K.H., You I., Jung S. (2016). Quality Improvement of Pork Loin by Dry Aging. Korean Journal for Food Science of Animal Resources. 36 (3): 369-376.

Ministarstvo poljoprivrede (2014). Uputa za postupanje prilikom klanja svinja za potrošnju u vlastitom kućanstvu. Uprava za veterinarstvo i sigurnost hrane. Raspoloživo: http://www.veterinarstvo.hr/UserDocsImages/dobrobitZivotinja/DZklanje/Uputa%20za%20postupanje%20prilikom%20klanja%20svinja%20za%20potro%C5%A1nju%20u%20vlastitom%20ku%C4%87anstvu%20studeni%202014.pdf

Ngapo T.M., Riendeau L., Laberge C., Fortin J. (2012). Marbling and ageing - Part 1. Sensory quality of pork. Food Research International. 49: 396-405.

Nowak D. (2011). Enzymes in tenderization of meat - The system of calpains and other systems - A review. Polish Journal of Food and Nutrition Sciences. 61(4): 231-237.

SAS Institute (2018). SAS Studio University Edition, release: 3.71.

Physical and sensory changes of fresh pork loin during ageing

Abstract

The aim of this study was to determine the effect of 7- and 14-day ageing on the physical and sensory characteristics of pork loin. It was found that the pH of pork loin increased significantly during the first week of wet ageing and was maintained during the second week. With shorter ageing, a significant increase in the values of all colour indicators was observed compared to the initial values. Weight loss during ageing was consistent regardless of the duration, but after thawing, the greatest weight loss was observed in the group with the longest ageing. The highest weight loss during heat treatment was observed in the group without ageing, and the lowest in the group with the longest ageing. Changes in texture and flavour were found to be the most important sensory changes affected by ageing.

Key words: pork loin, pH, weight loss, colour, sensory analysis

STRUČNI RAD

Lanci kvalitete u proizvodnji i trgovini svinjskim mesom

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Sažetak
Kakvoća svinjskog mesa te posljedično i kvaliteta proizvoda ovise o brojnim čimbenicima, kao i njihovoj međusobnoj interakciji. Ovo je u svinjogojskoj proizvodnji rezultiralo razvojem specijalnih lanaca kvalitete koji se koriste u proizvodnji i trgovini svinjskim mesom, definirani temeljem posebnih karakteristika proizvoda. Obzirom na različite proizvodne sustave i pasmine svinja temeljem javnih ili privatnih standarda kakvoće moguće je razviti lance proizvodnje tradicionalnih proizvoda koji bi bili implementirani i u sustavima proizvodnje svinja u Republici Hrvatskoj.

Ključne riječi: kakvoća mesa, sustavi uzgoja, lanac, svinjsko meso

Uvod

Posljednja desetljeća prošlog i početak tekućeg stoljeća obilježile su različite krize povezane s proizvodnjom hrane animalnog podrijetla kao što su BSE (bovina spongiformna encefalopatija), dioksinska otrovanja, klasična svinjska kuga, ptičja i svinjska gripa te skorašnja afrička svinjska kuga. I današnju pošast u obliku Covid-19 pandemije mnogi znanstvenici smatraju zoonozom, iako postoje i druge, jednako moguće teorije (Skitarelić i sur., 2020.). Isto tako, svijet se trenutno suočava s novim izazovima koje nameće stočarska proizvodnja koji se u prvom redu odnosi na zaštitu okoliša, zdravstvene probleme koji se povezuju s prekomjernim unosom animalnih proizvoda te dobrobiti životinja. U tom smislu došlo je do velikih promjena u percepciji kakvoće proizvoda svinjogojstva, od svinjskih trupova i polovica preko svinjskog mesa i masti do različitih mesnih prerađevina. Općenito, široka paleta proizvoda svinjogojstva povećava očekivanja potrošača u odnosu na kvalitetu pojedinih proizvoda, ali i održivost sustava proizvodnje svinja, humanost u tretmanu životinja i poboljšanje zdravlja ljudi. Sve to čini kakvoću svinjskog mesa vrlo kompleksnim, višedimenzionalnim pitanjem. Prema Međunarodnoj organizaciji za standardizaciju, kvaliteta proizvoda definirana je skupom atributa koji mogu zadovoljiti navedene ili implicitne potrebe krajnjeg korisnika (Lebret i Čandek Potokar, 2022.). U takvoj se situaciji može očekivati povećan interes za takozvane atribute vjerodostojnosti koje potrošači, uz klasična svojstva organoleptičke kakvoće poput izgleda i okusa, uzimaju u obzir pri kupnji. To su oni atributi koje potrošači ne mogu procijeniti, ni prije ni nakon kupnje, ali ih je potrebno na neki način priopćiti (Fernqvist i Ekelund, 2014.), primjerice različitim deklaracijama ili drugim oznakama kojima se omogućava razlikovanje na tržištu. Takav pristup omogućava potrošačima uzimanje u obzir svih aspekata procesa proizvodnje svinja pri odabiru proizvoda. To je od posebne važnosti jer se od njih očekuje spremnost plaćanja više cijene za svinjsko meso koje je proizvedeno uz brigu o etičkim pretpostavkama kao što su dobrobit životinja, karakteristikama vezanim uz zdravstvenu funkcionalnost ili uz ekološku prihvatljivost proizvodnih sustava.

Ti novi kriteriji kojima današnji potrošači vrednuju poljoprivredne proizvode obuhvaćaju socijalne, okolišne i ekonomske aspekte u konceptu koji se naziva PPP prema skraćenici koja potječe od engleskih riječi za ljude, planet i profit (People, Planet, Profit). U tom kontekstu najvažniju ulogu igraju zdravlje i sigurnost (Ljudi), učinak na okoliš (Planet) u određenom ekonomskom okviru (Profit). Na tim temeljima počiva „Europski zeleni sporazum“, plan za održivu ekonomiju u kojoj posebnu ulogu ima proizvodnja hrane uz primjenu strategije „Od polja do stola“. U tim dokumentima se navodi potreba da europska hrana ostane sigurna, hranjiva i visoke kakvoće, ali ujedno s minimalnim utjecajem na prirodu. Ključ za uspješnu tranziciju poljoprivrede u tom smjeru predstavljaju farmeri. Isto tako, strategija „Od polja do stola“ ima za cilj pomoći u borbi protiv falsificiranja hrane prevencijom, detekcijom i borbom protiv lažiranja kroz koordinaciju s zemljama članicama Europske Unije i zemljama izvan Europske Unije. Zbog svega toga, od dionika u proizvodnji svinjskog mesa očekuje se posvećivanje još veće pozornosti na kontrolu kakvoće i sigurnosti što dovodi do diferencijacije svinjogojske proizvodnje uz sve češće promjene ekonomskih i tehnoloških orijentacija prema tematskim pitanjima kao što su sigurnost hrane, zdravstvena ispravnost hrane, dobrobit životinja i učinci na okoliš.

Konvencionalni proizvodni sustavi u svinjogojstvu

Potrebe za svinjskim mesom koje su u konstantnom porastu postavile su visoke zahtjeve za unaprjeđenjem svinjogojske proizvodnje. U skladu s tim, mnoge uzgajivačke tvrtke usmjerile su svoje selekcijske programe prema unaprjeđenju dnevnih prirasta, konverzije hrane i povećanju mesnatosti svinjskih polovica. Da bi se ti uzgojni ciljevi ostvarili razvijena je visoka tehnologija hranidbe i smještaja svinja u zatvorenim industrijskim objektima. U tim intenzivnim sustavima, biološka sigurnost i upravljanje zdravljem glavni su prioriteti pa se u njima može uočiti visok zdravstveni status, ograđene barijere i ograničen pristup, a većina svinja smještena je u objektima s potpuno ili djelomično rešetkastim podovima i sustavima za rukovanje tekućim gnojem. Međutim, u ovim sustavima zanemarena je dobrobit životinja, a uočena su i neka pogoršanja u kvaliteti mesa te negativan utjecaj na okoliš, pa danas u Europskoj Uniji svjedočimo velikom porastu alternativnih, manje intenzivnih sustava proizvodnje. To uključuje tov na dubokoj stelji, zatvorene sustave s ispustima ili vanjske sustave proizvodnje za reproduktivne i tovne svinje na otvorenom. Tako se na primjer sustavi držanja svinja na dubokoj stelji često kombiniraju s jeftinijim građevinskim konstrukcijama kao što su neizolirane šupe u sjevernoj Europi ili obručne strukture (slične šatorima) u Velikoj Britaniji i Sjevernoj Americi.

Alternativni proizvodni sustavi

Zapravo, korištenje alternativnih proizvodnih sustava s podlogom od slame ili s pristupom vanjskom dvorištu te u ekstenzivnim uvjetima već se otprije koristi za proizvodnju vrhunskih proizvoda iz specifičnih kvalitativnih shema kao što su eko proizvodi ili proizvodi sa zemljopisnim indikacijom (zaštićena oznaka izvornosti, ZOI ili oznaka zemljopisnog podrijetla, OZP). U posljednje se vrijeme bilježi veliki porast proizvoda od svinjetine koji se prodaju sa službenim oznakama kvalitete ili zaštitnim znakovima, kao i broj svinja proizvedenih u alternativnim proizvodnim sustavima. Zamjetna je i sve veća raznolikost proizvoda od svinjetine i sustava proizvodnje svinja (IFIP, 2021.). Primjerice, europska ekološka proizvodnja svinja porasla je za 50 % od 2015. do 2018. i nastavlja stalno rasti, iako predstavlja samo mali udio u proizvodnji svinja - 0,9 % u 2018. (Agence Bio, 2020.). Dosadašnji pregledi izvještavaju o oko 80 proizvodnih sustava u svinjogojskim proizvodnjama Europske Unije. Od toga broja, 40 ih ne podrazumijeva nikakvu diferencijaciju; to su takozvani konvencionalni sustavi. Ostali se diferenciraju kao alternativni na temelju jedne ili više pretpostavki pa se time i razlikuju s obzirom na različite

kriterije kao što je prikazano u tablici 1. Iz prikazanih podataka očito je da većina sustava ima nekoliko osnova za diferencijaciju.

Tablica 1. Diferencijacija alternativnih sustava proizvodnje svinja s obzirom na isticanu tvrdnju o posebnosti (Boneau i Lebret, 2010.)

Osnova za diferencijaciju (najmanje jedna)	%
Dobrobit životinja	68
Poboljšana gurmanska vrijednost	70
Poboljšana nutritivna vrijednost	25
Smanjen utjecaj na okoliš	41
Organska proizvodnja	25
Lokalna pasmina	9

Tvrdnja o vrhunskoj kvaliteti može se temeljiti i na dokumentaciji koja se odnosi na hranidbu, dob ili težinu pri klanju i uvjetima uzgoja. Na primjer, u Francuskoj oznaka kvalitete „Label Rouge“ ima specifikaciju koja propisuje dob pri klanju višu od 182 dana. U ovom sustavu se propisuje i minimalna živa masa pri klanju, brzina rasta svinja kontrolira se pomoću hranidbe, a time se izravno utječe na kakvoću mesa. Sastav obroka pri tome je vrhunske važnosti i zato se u diferencijaciji alternativnih sustava često ističu različite strategije hranidbe svinja. Primjeri tih strategija su korištenje tzv. kompenzatornog rasta gdje svinje na početku tova hrane vrlo restriktivno, a u posljednjoj fazi tova omogućuje im se neograničen pristup hrani. Nadalje, neke strategije osnivaju se na varijacijama u nutritivnoj vrijednosti krme kroz omjer bjelančevina i energije ili kroz promjene u aminokiselinskom sastavu krme. Negdje se samo radi o produženom tovu ili povećanja težine pri klanju. Osnova za diferencijaciju može biti i pasmina. Na primjer, utvrđeno je da meso autohtonih pasmina posjeduje poželjnija tehnološka i organoleptička svojstva u usporedbi s konvencionalnim pasminama svinja. Vrhunska kakvoća svinjetine iz proizvodnih sustava koji koriste lokalne pasmine zapravo je rezultat kombinacije čimbenika koji uključuju genotip, uvjete držanja i hranidbe (Boneau i Lebret, 2010.). S druge strane, iako poboljšana gurmanska vrijednost nije osnova za diferencijaciju u organskoj proizvodnji, potrošači su često uvjereni da je meso organski proizvedenih životinja u tom pogledu kvalitetnije (Edwards, 2005.). Sve to predstavlja veliki potencijal za brendiranje proizvoda korištenjem neke oznake ili standarda kakvoće. Međutim, za ovo je potrebno uvođenja sustava sljedivosti kroz opskrbni lanac.

Standardi/norme kakvoće (sheme osiguranja kakvoće)

Standardi kakvoće općenito se mogu podijeliti na javne (obavezne) i privatne. Glede javnih standarda Europska Unija je razvila širok spektar zakonodavnih zahtjeva s obzirom na sigurnost hrane. Ti su zahtjevi opće smjernice koje moraju ispuniti zemlje EU. Primjerice, HACCP je usvojen u gotovo svim lancima opskrbe u Europi. Zemlje članice se mogu oslanjati isključivo na zakonodavstvo Europske Unije ili ga koristiti kao osnovu na kojoj se moraju graditi svoja vlastita pravila. Pored javnih propisa, privatna su tijela pokrenula inicijative za razvijanje standarda za osiguravanje kvalitete i sigurnosti u opskrbnim lancima. Ovi standardi sadrže sveobuhvatne norme u pogledu sigurnosti hrane, upravljanja proizvodima i postupcima te higijene osoblja i proizvodnog okruženja. Velike maloprodajne kompanije u Europi razvile su standarde koji obvezuju svoje dobavljače na stroga pravila upravljanja kvalitetom hrane. Na primjer, QS (Qualität und Sicherheit) u Njemačkoj ili IKB (Integrale Keten Beheersing) u Nizozemskoj. Slično, Global-GAP (GAP, od engl. Good

Agricultural Practice = dobra poljoprivredna praksa) osniva se na principima HACCP-a, a proizvođači se obavezuju prikazati predanost pitanjima kao što su smanjivanje štetnosti u odnosu na okoliš, efikasnoj uporabi prirodnih resursa, zdravlje i sigurnost uposlenih i sljedivost. Uz ekološku proizvodnju (oznaka EKO) službene oznake vrednuju i tipične regionalne proizvode (zaštićena oznaka izvornosti (ZOI), zaštićena oznaka zemljopisnog podrijetla (ZOZP) i zajamčeni tradicionalni specijalitet (ZTS). Ovi standardi naročito su zanimljivi u proizvodnji tradicionalnih proizvoda od svinjskog mesa u ruralnim područjima jer obuhvaćaju aktivnosti koje se odvijaju uzduž cijelog lanca, od proizvodnje svinja do izrade proizvoda. Zaštićena oznaka izvornosti zaštićena oznaka zemljopisnog podrijetla specificiraju faze proizvodnje koji se provode u određenom zemljopisnom području i doprinose tipičnosti proizvoda; za ZOI su to sve faze, a za ZOZP samo neke. U te standarde ubraja se još i zajamčeni tradicionalni specijalitet koji opisuje tradicionalni način proizvodnje ili recept.

Lanci kakvoće u prometu proizvoda od svinjskog mesa

Dosadašnja istraživanja jasno pokazuju da kakvoća svinjskog mesa ponajviše zavisi o pasmini, genotipu, primijenjenom režimu hranidbe, manipulaciji prije klanja i ostalim čimbenicima te njihovim interakcijama (Olsson i Pickova 2005.; Li i sur., 2013.). Na osnovi te činjenice u posljednje se vrijeme proizvodnja mesa i proizvoda od svinjskog mesa Europske Unije organizira u vidu lanaca koji se definiraju na osnovi neke posebne karakteristike, primjerice poboljšanje sastava trupa ili kakvoće svinjskog mesa. Pri tome se cijeli lanac opisuje određenom vrstom specifikacije iz koje je razvidno na kojoj se pretpostavci osniva posebna kakvoća proizvoda. Neki proizvodni lanci orijentirani su na proizvodnju visoko mesnatih polovica svinja, dok su neki usmjereni na proizvodnju svinjetine poboljšanog okusa, boje ili drugih svojstava kakvoće. Postoje i proizvodni lanci koji su osnovani s ciljem poboljšavanja nekih drugih dimenzija od društvene važnosti, kao na primjer dobrobiti životinja. Njihova autentičnost dokazuje se shemama diferencijacije koje imaju za cilj razlikovati proizvode kroz određene karakteristike, a korist za proizvođače očituje se kroz poboljšavanje svoje tržišne pozicije i kroz višu cijenu za svoje proizvode. Ove sheme podliježu certifikaciji i pri tome koriste različite oznake kako bi se razlikovali od konkurentskih proizvoda. Na primjer, francusko Ministarstvo poljoprivrede potvrđuje da već spomenuta oznaka „Label Rouge“ garantira da proizvod ima specifičan skup karakteristika koje čine pozitivnu kvalitativnu razliku od nekog sličnog proizvoda na tržištu. Visoka kakvoća mesa lokalnih pasmina svinja proizvedenih u specifičnim ekstenzivnim sustavima, kao što je silvo-pastoralni, vrlo je često isticana karakteristika u mnogim istraživanjima, a posebno se to odnosi na visok sadržaj intramuskularne masti (Pugliese i Sirtori, 2012.; Budimir i sur., 2014.). Slična percepcija kakvoće odnosi se i na proizvode od svinjskog mesa kao što su različite inačice pršuta ili kobasičarskih proizvoda, pogotovo onih koji se povezuju uz određenu regiju. U tim slučajevima mogu se primijeniti različite sheme i pripadajuća označivanja. Tako su de Roest i sur. (2014.) opisali tri različita lanca u proizvodnji talijanskih pršuta uz pripadajuća oznake: parmski pršut zaštićen oznakom izvornosti (ZOI) (Dentoni, 2013.; Prosciutto di Parma PDO Consortium, 2013.), tradicionalni pršut dobiven od svinja Cinta Senese, lokalne pasmine u Toskani, te generički (nezaštićeni) suhi pršut proizveden od butova uvoznih svinja.

Zaključak

Proizvodni lanci mogu se definirati na sličan način i u nas, ali to još nije postala uobičajena praksa prilikom isticanja svojstava kakvoće mesa ili proizvoda od mesa, nego se više računa na povjerenje potrošača u oznake kao što su Meso hrvatskih farmi i slično. Kao definirani kvalitativni lanci u Hrvatskoj, odnosno programi upravljanja kakvoćom hrane, u smislu

nadzora nad proizvodnim procesom od strane certificirajućeg tijela, mogu se navesti jedino zaštićene oznake izvornosti (ZOI) i zaštićenog zemljopisnog podrijetla (ZOZP) te oznaka hrvatski EKO proizvod. U proizvodnji tradicionalnih proizvoda od svinjskog mesa poželjno je koristiti sirovine podrijetlom iz definiranih proizvodnih lanaca i na taj način promovirati njihovu visoku kakvoću i posebnu vrijednost. Pri uvođenju takvih shema valja biti oprezan jer proliferacija shema certificiranja, raznolikost oznaka i preklapanja među njima te nedostatak dosljednosti mogu dovesti do kontra učinaka. Potrošači mogu postati jednostavno zbunjeni do te mjere da počnu ignorirati oznake. Oni također mogu sumnjati da neke informacije nedostaju ili su skrivene, uključujući točno podrijetlo. Osim toga, neke su sheme potencijalno obmanjujuće jer nose naziv zemlje ili regije, ali proizvodi ne potječu nužno u potpunosti iz određenog područja. Zbog toga je važno uspostaviti lance kakvoće sa pripadajućim sustavom sljedivosti kojim se jednostavno može pratiti proizvodnja od farme do potrošača, a današnji razvoj informatičkih tehnologija zasigurno može pružiti veliki izbor rješenja.

Literatura

Agence Bio. (2020). Organic farming and market in the European Union. Ed Agence Bio, 93 Montreuil France. Dostupno na: https://www.agencebio.org/2020/04/23/an-english-version-of-our-publication-on-organic-farming-and-market-is-now-available/

Boneau M., Lebret B. (2010). Production systems and influence on eating quality of pork. Meat science. 84(2): 293-300.

Budimir K., Margeta V., Kralik G., Margeta, P. (2013). Silvo-pastoralni način držanja crne slavonske svinje. Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme. 55(3): 151-157.

Dentoni D., Menozzi D., Capelli M.G. (2012). Group heterogeneity and cooperation on the geographical indication regulation: The case of the "Prosciutto di Parma" Consortium. Food Policy, 37(3): 207-216.

de Roest K., Pignedoli S., Belletti G., Menozzi D., Arfini F. (2014). Glamur project Italian case study: local and global cured ham chains. CRPA.

Edwards S. A. (2005). Product quality attributes associated with outdoor pig production. Livestock Production Science. 94: 5–14.

Fernqvist F., Ekelund L. (2014). Credence and the effect on consumer liking of food – A review. Food Quality and Preference. 32: 340–353.

IFIP (2021). Partenaire de vos innovations. Available from: https://ifip.asso.fr/

Lebret B., Čandek-Potokar M. (2022.): Review: Quality attributes of pork from farm to fork. Part I. Carcass and fresh meat. Animal, in press.

Olsson V., Pickova J. (2005). The influence of production systems on meat quality, with emphasis on pork. AMBIO: A Journal of the Human Environment. 34(4): 338-343.

Pugliese C., Sirtori F. (2012). Quality of meat and meat products produced from southern European pig breeds. Meat science. 90(3): 511-518.

Skitarelić N., Dželalija B., Skitarelić N. (2020). Covid-19 pandemija: kratki pregled dosadašnjih spoznaja – Medica Jadertina. 50(1): 5-8.

Quality chains in production and trade of pig meat

Abstract

Pig meat quality, and consequently the quality of the product, depends on many factors together with their interaction. In pig breeding, this resulted in the development of special quality chains used in the production and trade of pig meat and are defined based on special product characteristics. Due to the existence of different production systems and pig breeds in Croatia, it is possible to develop chains for traditional products that would be implemented in pig production using public or private standards.

Key words: meat quality, breeding systems, chains, pork

ORIGINAL SCIENTIFIC PAPER

Some blood biochemical parameters of ewes in gestation and lactation

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Abstract
The study aimed to assess metabolic demands during late gestation and lactation by variation of some biochemical blood parameters. The research was conducted on 10 Merinolandschaf late gestation and lactation ewe kept on the farm. Most of the investigated indicators were in the reference range, except for a slight deviation in the concentration of urea, triglycerides, albumin, and LDL which can be related to intensive metabolic process, energy demands of the fetal-placental unit in gestation, and early lactation ewes, as well as the ration rich in protein. A significantly increased concentration of glucose and triglyceride in lactating compared to highly pregnant ewes is expected given the early lactation and lactation curve of ewes.

Key words: biochemical parameters, ewes, gestation, lactation

Introduction

Gestation and lactation are high demanding physiological phases considered to modify animal metabolism, which must be supported by increased uptake of nutrients such as glucose. Throughout pregnancy, placental energy expenditure decreases, and by the end of gestation, most energy is shifted to the fetuses (Antunović et al., 2017; Pesantez-Pacheo et al., 2019). Lactation involves significant metabolic changes in the mammary and other tissues. The mammary secretory gland utilizes 80% of the blood circulating metabolites for milk synthesis. The main nutrient consumed is glucose (Bell, 1995; Piccione et al., 2009). Glucose is needed for the synthesis of lactose, the most important osmotic solute in milk (Bell and Bauman, 1997). Other blood biochemical parameters inclouding total protein triglycerides, urea, and free fatty acids which are important indicators of the metabolic activity in lactation (Antunović et al., 2004; Karapehlivan et al., 2007). In heavily pregnant ewes, the concentration of blood serum lipids is usually increased, especially total cholesterol, triglycerides, and lipoproteins. This is mainly due to the increased mobilization of fatty acids from adipose tissue, making available new nutrients for fetal growth (Picione et al., 2009). Peripartum diseases can be predicted by determining lipid profiles, respectively circulating blood triglycerides that are widely used for milk fat synthesis (Nazifi et al., 2002), while a decrease in blood protein concentration in late gestation was detected in ewes. Amino acids are used for protein synthesis in the fetal muscles (Antunović et al., 2002). Krajničakova et al. (2003) reported an increase in the serum total protein in lactating goats with the progress of the lactation due to the catabolism of protein for milk synthesis. Urea levels increased during pregnancy, riching the highest value at parturition (El-Sherif and Assad 2001). Pregnancy and lactation are competitive physiological phases, in which the fetus must compete for nutrients with the demands of lactation (Kamal et al., 2014). At the end that has effects both on maternal health and offsprings birth weight. These two phases are energetically the most demanding periods that can cause metabolic problems like liver damage, pregnancy toxemia, and ketosis thereby affecting production (Karagiannis et al.,

2014). This study aimed to assess metabolic demands during late gestation and lactation by variation of some biochemical blood parameters.

Material and methods

Ten clinically healthy Merinolandschaf ewes kept on a family farm (average age of 4 years in good condition) were selected from a large flock and used for the research. Selected ewes were in late gestation (90th days of pregnancy, single lamb) and continued with the same ewes after lambing in lactation. All ewes were housed in a barn and fed twice a day with a meal consisting of Alfalfa hay *ad libitum* (dry matter – 91.40%, crude proteins – 17.74%, crude fiber 30.90, crude fat 1%, crude ash 7.60), 300 g/day/animal feed mixture (dry matter –88%, crude proteins – 17.73%, crude fiber 6.40, crude fat 3%, crude ash 7%) and 150 g/day/animal of barley (dry matter –87.1%, crude proteins – 8.35%, crude fiber 3.80%, crude fat 1.30%, crude ash 2.87%). Blood sampling was carried out in heavily pregnant ewes (two weeks before parturition) and in lactating ewes (three weeks after parturition). Blood samples were taken in the morning before feeding from the jugular vein into a sterile vacuum tube Venoject® (Leuven, Belgium). Blood samples were centrifuged at 1609.92 g for 10 minutes to obtain the serum samples. Blood biochemical parameters (glucose, urea, cholesterol, triglyceride, total protein, albumin, globulin, creatinine, HDL – cholesterol, LDL – cholesterol, total bilirubin, were analyzed out on an automatic Olympus AU 400 (Olympus, Japan). The results were statistically evaluated using descriptive statistical procedures. The mean values of the obtained research results were calculated by the MEANS procedure in the computer program TIBCO Statistica® 14.0.0. Differences between mean values of analyzed parameters were checked by the t-test method between dependent variables at the significance level $P<0.05$ or less.

Results and discussion

In the present research, most of the investigated indicators were in the reference range, except for a slight deviation in the concentration of urea, triglycerides, albumin, and LDL (Table 1).

Table 1. Descriptive statistic blood biochemical indicators in ewes (n=20)

Indicator	$\bar{x}$	Sd	Min	Max	CV, %	Reference values[1]
Glucose, mmol/L	3.72	0.56	2.70	5.20	15.09	2.78 – 4.44
Urea, mmol/L	9.06	1.43	4.90	11.40	15.84	2.86 – 7.14
Cholesterol, mmol/L	1.69	0.33	1.00	2.50	19.36	1.35 – 1,97
Triglycerides, mmol/L	0.25	0.09	0.10	0.50	36.23	0.19 – 0.25[2]
Total protein, g/L	75.53	6.56	62.60	89.50	8.68	60 – 79
Albumin, g/L	30.90	5.91	12.10	35.30	19.13	24 – 30
Globulin, g/L	44.62	10.75	30.60	75.80	24.09	35 – 57
Creatinine, µmol/L	78.13	8.05	66.00	93.00	10.31	70 –105[3]
HDL, mmol/L	0.83	0.16	0.52	1.19	19.68	1.09-1.18[2]
LDL, mmol/L	0.74	0,24	0.16	1.13	32.12	0.36-0.40[2]
Total bilirubin, µmol/L	2.96	0.93	1.00	5.00	31.39	1.71 – 8.55

$\bar{x}$=mean value; Sd= standard deviation; CV=coeficient value; HDL-high density lipoprotein; LDL-low density lipoprotein; [1] Kaneko et al. (2008); [2]Antunović et al. (2011); [3]Jackson and Cockcroft, (2002)

Urea is the main metabolic product of nitrogenous substances in the body. Feeds rich in protein or increased protein metabolism due to temperature, trauma, or intensive metabolic stages may affect elevated urea concentration. An increase or decrease in the concentration

of urea in the blood may be due to increased or decreased water intake, as well as oliguria, or polyuria (Ramadan and Harapin, 1998). The higher urea concentration, which was above the reference value, indicates the fact that the ration was rich in protein. It is known that the concentration of urea in the blood can be taken as a reliable indicator of the protein content in the ration of ewes (Whitney et al., 2009). Albumin makes a large contribution to plasma colloid osmotic pressure (Piccione et al., 2013), also acts as a carrier protein for many insoluble organic substances and plays a role as a reserve energy source whenever there is an inadequate intake for some reason (Tothova et al., 2014). Slightly increased triglyceride in demanding reproduction phases appears to be related to the energy demands of the fetal-placental unit in pregnant ewes (Watson et al., 1993). An increase in the concentration of triglycerides in blood plasma is the result of their increased intestinal resorption and increased synthesis in the liver. The increased triglycerides and low-density lipoproteins above reference found in this study may reflect elevated transportation of accumulated triglycerides from the liver to peripheral tissue to improve hepatic lipidosis according to Oikawa et al. (2010).

Table 2. Biochemical indicators of ewes depending on reproductive status

Indicator	Reproduction stage		SEM	*P*-value
	Gestation	Lactation		
	Mean ± Sd	Mean ± Sd		
Glucose, mmol/L	$3.48^{a} \pm 0.52$	$3.99^{b} \pm 0.49$	0.56	0.023
Urea, mmol/L	9.55 ± 1.73	8.52 ± 0.78	1.43	0.084
Cholesterol, mmol/L	1.69 ± 0.38	1.68 ± 0.28	0.33	0.944
Triglycerides, mmol/L	$0.29^{A} \pm 0.08$	$0.20^{B} \pm 0.08$	0.02	0.010
Total protein, g/L	75.03 ± 5.35	76.07 ± 7.91	1.37	0.711
Albumin, g/L	31.51 ± 6.18	30.25 ± 5.83	1.23	0.620
Globulin, g/L	43.52 ± 9.39	45.83 ± 12.42	2.24	0.618
Creatinine, µmol/L	75.33 ± 6.29	81.18 ± 8.93	1.68	0.081
HDL, mmol/L	0.84 ± 0.17	0.82 ± 0.17	0.03	0.780
LDL, mmol/L	0.72 ± 0.25	0.77 ± 0.23	0.05	0.615
Total bilirubin, µmol/L	3.0 ± 0.85	2.91 ± 1.04	0.19	0.820

Mean=mean value; Sd= standard deviation; SEM=mean standard eror; HDL-high density lipoprotein; LDL-low density lipoprotein; ^{A,}P<0.01; ^{a,} P<0.05

A significantly higher concentration of glucose was determined in early lactating ewes compared to highly pregnant ewes (Table 2). Nutrition has the greatest impact on the concentration of glucose and urea in the blood of ewes. Restrictions in nutrition, especially during late pregnancy lead to a drop of glucose concentration in blood (Lekatz et al., 2011). In this research, glucose was within the reference range, indicating adequate nutrition. An increased (P>0.05) plasma glucose concentration in early lactating compared to highly pregnant goats was determined Allaoua and Mahdi, (2018) and previously Manat et al. (2016). The determined glucose concentration in highly pregnant and early lactation ewes was expected given the lactation curve. A significant decrease in triglyceride (Table 2) of serum during early lactation ewes shown in present research has also been reported by Piccione et al. (2009) and Gradinski-Vrbanac et al. (1986). Nazifi et al. (2002) determined the lowest concentrations of the triglycerides during the second to the third week of lactation. In lactation, insulin inefficiently stimulates lipogenesis, which is confirmed by the significant decrease in serum triglycerides concentration and total cholesterol in early lactation compared to late gestation (Watson et al., 1993) after lambing increased lipoprotein lipase activity induct the enzyme in mammary tissue to provide for milk fat synthesis.

Conclusion

Determined biochemical indicators in the blood of ewes were mostly in the reference range. Slightly higher concentrations of urea, triglycerides, albumin, and LDL can be related to intensive metabolic process, energy demands of the fetal-placental unit in gestation, and early lactation ewes, as well as the ration rich in protein. A significantly increased concentration of glucose and triglyceride in lactating compared to highly pregnant ewes is expected given the early lactation and lactation curve of ewes. Based on shown biochemical indicators, we can conclude that the nutrition of ewes in gestation and lactation is adequate.

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Literature

Allaoua S. A., Mahdi D. (2018). Plasma biochemical and minerals parameters in Arabia goats of a semi-arid region of North-Eastern Algeria during different stages of production. Veterinarski arhiv. 88(5): 643-660.

Antunović Z., Senčić Đ., Šperanda M., Liker B. (2002). Influence of the season and the reproductive status of ewes on blood parameters. Small Ruminant Research. 1: 39-44.

Antunović Z., Šperanda M., Steiner Z. (2004). The influence of age and the reproductive status to the blood indicators of the ewes. Archives Animal Breeding. 47(3): 265-273.

Antunović Z., Novoselec J., Šperanda M., Vegara M., Pavić V., Mioč B., Djidara M. (2011). Changes in biochemical and hematological parameters and metabolic hormones in Tsigai ewes blood in the first third of lactation. Archiv Tierzucht. 54(5): 535-545.

Antunović Z., Novoselec J., Šperanda M., Steiner Z., Ćavar S., Pavlović N., Lendić K.V., Mioč B., Paćinovski N., Klir Ž (2017). Monitoring of blood metabolic profile and milk quality of ewes during lactation in organic farming. Mljekarstvo. 67(4): 243 - 252.

Bell A. W. (1995). Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of animal science. 73(9): 2804-2819.

Bell A. W., Bauman D. E. (1997). Adaptations of glucose metabolism during pregnancy and lactation. Journal of mammary gland biology and neoplasia. 2(3): 265-278.

El-Sherif M. M. A., Assad F. (2001). Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. Small Ruminant Research. 40(3): 269-277.

Gradinski-Vrbanac B., Mitin V., Mikulec K., Karadjole I. (1986). Triglycerides and phospholipid values in sheep serum in the course of a year. Veterinary Archiv. 55: 29-31.

Jackson, P. G. G., Cockcroft, P. D. (2002). Clinical examination of farm animals. Oxford, UK: Blackwell Science. 217.

Kamal, M. M., Van Eetvelde, M., Depreester, E., Hostens, M., Vandaele, L., Opsomer, G. (2014). Age at calving in heifers and level of milk production during gestation in cows is associated with the birth size of Holstein calves. Journal of Dairy Science. 97(9): 5448-5458.

Kaneko J.J., Harvey J.W., Bruss M.L. (2008). Clinical Biochemistry of Domestic Animals. 6th ed. Elsevier/ Academic Press, Amsterdam, 932 p.

Karagiannis I., Panousis N., Kiossis E., Tsakmakidis I., Lafi S., Arsenos G., Brozos C. (2014). Associations of pre-lambing body condition score and serum β-hydroxybutyric acid and non-esterified fatty acids concentrations with periparturient health of Chios dairy ewes. Small ruminant research. 120(1): 164-173.

Karapehlivan M., Atakisi E., Atakisi O., Yucayurt R., Pancarci S. M. (2007). Blood biochemical parameters during the lactation and dry period in Tuj ewes. Small Ruminant Research. 73(1-3): 267-271.

Krajničakova M., Kovac G., Kostecky M., Valocky I., Maracek I., Sutiakova I., Lenhardt L. (2003). Selected clinical-biochemical parameters in the puerperal period of goats. Bulletin of the Veterinary Institute in Puławy. 47(1): 177-182.

Lekatz L.A., Wu G., Caton J.S., Taylor J.B., Reynolds L.P., Redmer D.A., Vonnahme K.A. (2011). Maternal selenium supplementation and timing of nutrient restriction in pregnant sheep: Impacts on nutrient availability to the fetus. Journal of Animal Science. 89: 59-76

Manat T. D., Chaudhary S. S., Singh V. K., Patel S. B., Puri G. (2016). Hematobiochemical profile in Surti goats during post-partum period. Veterinary world. 9(1): 19.

Nazifi S., Saeb M., Ghavami S. M., (2002). Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition, and during the post-parturition period. Journal of Veterinary Medicine. 49: 9-12.

Oikawa S., Mizunuma Y., Iwasaki Y., Tharwat M. (2010). Changes of very low-density lipoprotein concentration in hepatic blood from cows with fasting-induced hepatic lipidosis. Canadian journal of veterinary research. Revue canadienne de recherche veterinaire. 74(4): 317–320.

Pesántez-Pacheco J. L., Heras-Molina A., Torres-Rovira L., Sanz-Fernández M. V., García-Contreras C., Vázquez-Gómez M., Feyjoo P., Caceres E., Frias-Mateo M., Hernandes F., Martinez Ros P., Gonzales-Martin J.V., Gonzales-Bulnes A., Astiz S (2019): Maternal metabolic demands caused by pregnancy and lactation: association with productivity and offspring phenotype in high-yielding dairy ewes. Animals. 9(6): 295.

Piccione G., Caola G., Giannetto C., Grasso F., Runzo S. C., Zumbo A., Pennisi P. (2009). Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. Animal Science Papers and Reports. 27(4): 321-330.

Piccione G., Arfuso F., Faggio C., Casella S., Zumbo A., Panzera M. (2013). Serum proteins profile in Comisana lambs during the first month of life. Archives Animal Breeding. 56(1): 742-750.

Ramadan P., Harapin I. (1998.): Interna klinička propedeutika domaćih životinja. Pretraga krvi i krvotvornih organa. Veterinarski fakultet Sveučilišta u Zagrebu. Zagreb, 115-144.

TIBCO Statistica® 14.0.0. 2020. www.tibco.com/products/tibco-statistica

Tothova C., Nagy G., Kovac G. (2014). Acute phase proteins and their use in the diagnosis of diseases in ruminants: a review. Veterinarni Medicina. 59(4): 163-180.

Whitney T. R., Waldron D. F., Willingham T. D. (2009). Evaluating nutritional status of Dorper and Rambouillet ewes in range sheep production. Sheep Goat Res Journal. 24: 10-16.

Watson T. D. G., Burns L., Packard C. J., Shepherd J. (1993). Effects of pregnancy and lactation on plasma lipid and lipoprotein concentrations, lipoprotein composition and post-heparin lipase activities in Shetland pony mares. Reproduction. 97(2): 563-568.

Pojedini biokemijski pokazatelji gravidnih ovaca i ovaca u laktaciji

Sažetak

Cilj istraživanja bio je utvrditi biokemijske pokazatelje visoko gravidnih ovaca i ovaca u ranoj laktaciji. Istraživanje je provedeno na 10 ovaca Merinolandschaf pasmine. Većina utvrđenih pokazatelja bila je unutar referentnih vrijednosti, osim blagog odstupanja koncentracije ureje, triglicerida, albumina i LDL-a što možemo povezati s intenzivnim metaboličkim procesima, energetskim zahtjevima fetalno-placentalne jedinice u graviditetu i ranoj laktaciji, kao i obroka bogatog bjelančevinama. Značajno povečana koncentracija glukoze i triglicerida ovaca u laktaciji u odnosu na gravidne ovce je očekovana s obzirom na ranu laktaciju i krivulju laktacije.

Ključne riječi: biokemijski pokazatelji, ovce, graviditet, laktacija

IZVORNI ZNANSTVENI RAD

Linearna procjena tjelesne mase na temelju opsega prsa i visine križa crnih slavonskih svinja

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Sažetak
Cilj ovog istraživanja bio je razviti jednadžbu linearne regresije pomoću koje se jednostavno u svakodnevnoj praksi može procijeniti tjelesna masa crnih slavonskih svinja. S obzirom na malu populaciju od 2890 grla registriranih u 2020. godini, kao i činjenicu da mali broj uzgajivača posjeduje stočnu vagu, ovaj način procjene mase olakšao bi testiranje rasplodnih grla te uštedio vrijeme kontrolorima i uzgajivačima. U okviru ovog istraživanja prikupljeni su podaci o tjelesnim mjerama rasplodnih grla te je detaljnom analizom podataka utvrđeno da mjere opsega prsa i visina križa omogućuju najtočniju procjenu tjelesne mase, tj. razvijena je prediktivna jednadžba linearnom regresijom.

Ključne riječi: procjena, crna slavonska svinja, tjelesne mjere, masa, regresija

Uvod

Crna slavonska svinja autohtona je lokalna pasmina koja se uzgaja na području Republike Hrvatske, najviše u Slavoniji i Baranji. Ova pasmina poznatija pod imenom fajferica, uzgaja se na gospodarstvima ekstenzivnog tipa uzgoja i to najčešće u manjem broju u otvorenim ili poluotvorenim sustavima držanja (Lukić i sur., 2018.) Takva mala gospodarstva uglavnom su slabo opremljena, što znači da nemaju potrebnu zootehničku opremu pomoću koje bi pratili napredak i performanse svinja. Najčešće tim gospodarstvima nedostaju stočne vage, što prilikom odabira životinja za daljnji uzgoj te motrenje napretka uzgajivačima predstavlja veliki problem. Nedostatak stočne vage predstavlja problem i djelatnicima stručnih službi, odnosno kontrolorima koji na gospodarstvu provode testiranje rasplodnih grla i selekciju, sukladno uzgojnom programu. Nošenje stočne vage kontrolorima bi bilo previše komplicirano i nepraktično stoga je pronalazak drugog praktičnog rješenja za određivanje mase životinja bez korištenja vage od velike važnosti. Razvojem jednostavne i praktične metode ovaj bi se problem mogao riješiti, kako bi se posljedično i olakšao posao djelatnicima stručnih službi na terenu. Linearna regresija je statistička metoda koja omogućava procjenu pojava ili svojstava te međusobnu zavisnost. Najčešće se koristi za donošenje zaključaka o nizu slučajnih varijabli koje ovise o nekoj nezavisnoj varijabli (Freedman, 2012). U animalnim znanostima, svakodnevno se koristi u rutinskim procjenama genetskih performansi budućih generacija rasplodnjaka i plotkinja odnosno uzgojnih vrijednosti, ali i brojnim drugim slučajevima. Prema tome, linearna je regresija vrlo raširena metoda u raznim znanstvenim područjima. Koristeći ovaj pristup, znanstvenici diljem svijeta proveli su brojna istraživanja kako bi uzgajivači mogli procijeniti tjelesnu ili živu masu životinja na farmama (Groesbeck i sur., 2002.; Mutua i sur., 2010.; Walugembe i sur., 2014.; Sungirai i sur., 2014.; Steenbergen, 1989.). Iz rezultata navedenih autora vidljivo je da su tjelesne mjere, odnosno opseg prsa, visina križa i visina grebena primjereni prediktori tjelesne mase kod svinja, te da se razvojem linearnih jednadžbi masa može vrlo točno procijeniti s točnošću u rasponu od 84 do 98 %. Također, rezultati istraživanja su pokazali da se masa može procijeniti i pomoću drugih tjelesnih mjera, ovisno o njihovim razinama pouzdanosti. S obzirom na rezultate

navedenih istraživanja, provedeno je istraživanje na pasmini crna slavonska svinja. U ovom istraživanju osim regresijske jednadžbe, utvrđena je i pouzdanost procjene na temelju različitih podataka, odnosno kolika je povezanost mase tijela s ostalim tjelesnim mjerama. Cilj istraživanja bio je razviti linearnu jednadžbu pomoću koje bi kontrolori i uzgajivači na farmama, uzimanjem dvije tjelesne mjere mjernom vrpcom i mjernim štapom te unosom u računalo mogli odrediti tjelesnu masu svinja te će se testirati i njena pouzdanost.

Materijal i metode

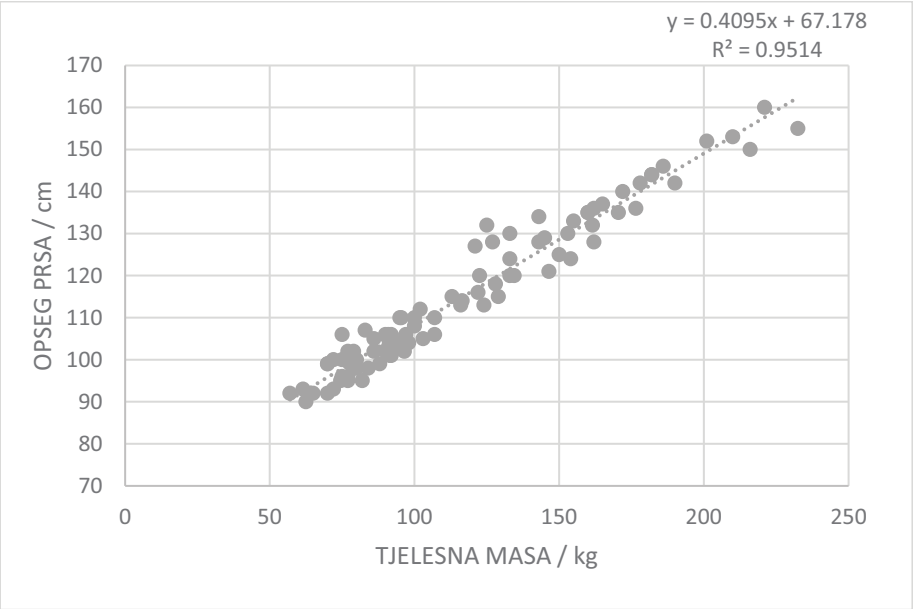
Istraživanje je provedeno na 7 gospodarstava na kojima se uzgajaju crne slavonske svinje, diljem cijele Republike Hrvatske. Uzorak su činile 102 jedinke crnih slavonskih svinja, od kojih je bilo 81 nazimica/krmača i 21 nerast. Cilj je bio analizirati što više nerastova u odnosu na ukupan broj registriranih nerastova, stoga je obuhvaćeno 8,7 % (21 analiziranih u odnosu na 242 registriranih u RH) s obzirom na njihov veći genetski doprinos u uzgoju, dok je odnos analiziranih nazimica/krmača iznosio 4,2 % (81 analiziranih u odnosu na 1930 registriranih u RH). Svim životinjama izmjereno je 11 tjelesnih mjera: tjelesna masa, visina grebena, visina križa, opseg prsa, duljina tijela, duljina trupa, širina zdjelice, duljina zdjelice, duljina glave, duljina ušiju i duljina repa. Masa je izmjerena stočnom vagom na svakom gospodarstvu. Visina grebena, visina križa i širina zdjelice izmjerena je Lydtinovim (mjernim) štapom. Opseg prsa, duljina tijela, duljina trupa, duljina zdjelice, duljina glave, duljina ušiju i duljina repa izmjerena je mjernom vrpcom. Svi podatci uneseni su u program Microsoft Excel, dok je statistička analiza izvršena u programima SAS 9.4 i Microsoft Excel. Analiza je zasebno izvršena za nazimice/krmače, a posebno za neraste kako bismo vidjeli postoje li razlike u rezultatima između spolova. Izračunate su varijance, standardne varijacije te koeficijenti korelacije za svako navedeno svojstvo. U daljnjoj analizi podataka za nazimice/krmače i nerastove određen je koeficijent regresije i koeficijent determinacije.

Rezultati i rasprava

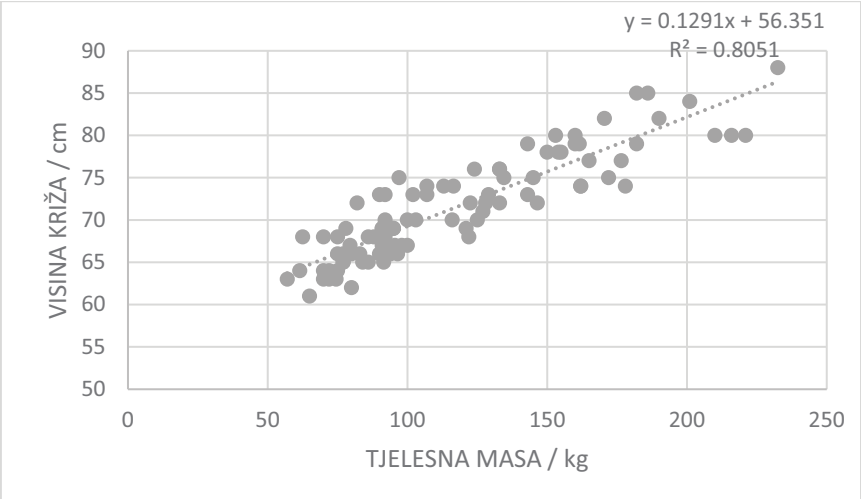
Analize su izvršene odvojeno za nerastove i nazimice/krmače, kako bi se utvrdilo je li ista tjelesna mjera u većoj ili manjoj korelaciji s masom kod različitog spola, te kolike su standardne pogreške. Koeficijent korelacije pokazuje kolika je povezanost između dva svojstva i koji je smjer korelacije, dakle je li smjer negativan ili pozitivan. Koeficijent korelacije može se opisati i kao odnos između dva svojstva koji je jasno pokazao koje je svojstvo u najvećoj povezanosti s masom (Freedman, 2012.). Regresija pokazuje za koliko se mjernih jedinica promijeni zavisno svojstvo, ako se nezavisno promijeni za jednu jedinicu, stoga regresija kao takva omogućava predviđanje kretanja neke pojave ili zavisnosti. U istraživanju Walugembe i sur. (2014.), tri su parametra korištena kao značajni izvori varijabilnosti u procjeni tjelesne mase, a to su duljina tijela, opseg prsa i širina tijela, te navode kako je opseg prsa bio najvažniji prediktor za procjenu mase (R^2>0,84). Groesbeck i sur. (2002.), također navode da je opseg prsa u gotovo potpunoj korelaciji sa tjelesnom masom (R^2= 0,98). U njihovom istraživanju interval pouzdanosti na rizini od 95 %, pokazao je procijenjenu masu od ±4,54 kg od stvarne tjelesne mase. Također, tjelesna masa može se procijeniti na temelju dobi, opsega prsa i duljine tijela, navode Sungirai i sur. (2014.), no u tom je slučaju potrebno dizajnirati skupine ovisno o starosnim kategorijama, što je više prikladno za veće populacije.

U ovom istraživanju za oba spola, korelacije za sva navedena svojstva po smjeru su pozitivna. Najniže korelacije s tjelesnom masom imaju duljina glave, te je između spolova vidljiva razlika u korelacijama, tj. kod nerastova je iznosila r = 0,83, a kod nazimica/krmača r = 0,49. Duljina ušiju kod oba spola u korelaciji s tjelesnom masom, kod krmača/nazimica iznosi r = 0,53, a kod nerastova r = 0,65, dok je za rep kod nerastova r = 0,59, a nazimica/krmača r = 0,31. Ove dvije tjelesne mjere (duljina ušiju i repa) ne bi se trebale

koristiti za procjenu tjelesne mase, jer se kod svinja često događa da si odgrizu uho i rep što je prilikom našeg istraživanja bilo dosta prisutno, stoga su ove mjere kao takve isključene iz analiza. Sve ostale tjelesne mjere su u vrlo jakoj korelaciji s tjelesnom masom. Kod svih tjelesnih mjera postoji razlika u korelaciji između muških i ženskih svinja. Za svako svojstvo korelacije s tjelesnom masom utvrđene su manje vrijednosti kod nazimica/krmača u odnosu na nerastove, osim za svojstvo duljine glave. Prilikom odabiranja tjelesne mjere, na temelju koje bi se mogla procijeniti masa, promatrano je koja tjelesna mjera ima najveću korelaciju s tjelesnom masom i najmanju razliku između spola. Utvrđeno je da najveću korelaciju s tjelesnom masom ima opseg prsa koja je iznosila 0,97 kod nerastova te 0,96 kod nazimica/krmača. Koeficijenti determinacije koji predstavljaju pouzdanost procjene u oba su spola iznosili 0,94. Pri tome je standardna pogreška relativno niska, od 10,4 kg do 11,73 kg, te se može reći da je tjelesnu masu na temelju opsega prsa moguće procijeniti relativno točno. Druga mjera koja je u jako visokoj odnosno, nerastova skoro potpunoj korelaciji je visina križa. Kod nerastova je utvrđena vrijednosti od 0,91 dok su nazimice/krmače imali vrijednost korelacije 0,86, uz standardnu pogrešku od 19,4 kg i 24,68 kg. Pomoću mjere visine križa možemo također procijeniti masu, ali sa značajno nižom pouzdanosti. Grafikoni 1. i 2. prikazuju korelaciju između tjelesne mase i opsega prsa, te tjelesne mase i visine križa na skupnim podacima. Na grafikonima su prikazane i pojedinačne jednadžbe linearne regresije, te koeficijenti determinacije za navedena svojstva.

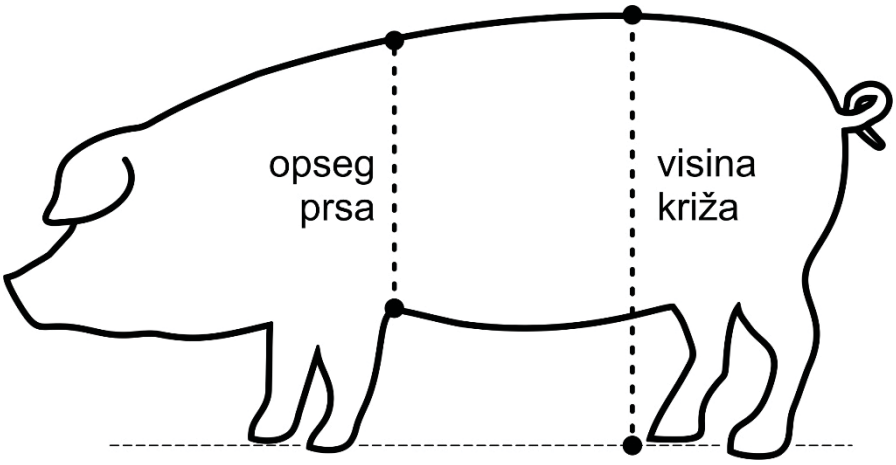


Grafikon 1. Korelacija između tjelesne mase i opsega prsa



Grafikon 2. Korelacija između tjelesne mase i visine križa

Duljina trupa imala je korelaciju 0,93 kod nerastova i 0,87 kod nazimica/krmača, dakle višu od visine križa. Također, opseg prsa i visina križa su mjere koje se lakše mogu izmjeriti na životinji, stoga su one odabrane za procjenu mase (Shema 1.). Rezultati mjerenja su precizni, a uzimanje ovih mjera je jednostavno za osobu koja je izvodi i nije toliko stresna za životinju. Uz pomoć jednadžbe linearne regresije uspješno je procijenjena tjelesna masa životinja na temelju opsega prsa i visine križa. Neke od tjelesnih mjera kao što su duljina zdjelice i visina grebena imaju manju standardnu pogrešku, ali i manju korelaciju, te je iz tog razloga naglasak stavljen na one mjere koje su u oba spola u najvećoj korelaciji.



Shema 1. Tjelesne mjere korištene u linearnoj procjeni

Razvijena linearna regresijska jednadžba, pomoću koje će se moći procijeniti tjelesna masa crnih slavonskih svinja prikazana je u tablici 1. Kako je utvrđeno da opseg prsa i visina križa imaju najvišu korelaciju, ove dvije mjere dodatno su razmatrane i kod nerastova i kod nazimica/krmača, te skupno. Jednadžba regresije za visinu križa kod nerastova glasi: y=0,1219x + 57,908. Jednadžba regresije za opseg prsa glasi: y=0,3681x + 71,386. Kod nazimica/krmača jednadžba regresije za visinu križa glasi: y=0,1204x + 57,072. Jednadžba regresije za opseg prsa kod nazimica/krmača glasi: y=0,3914x + 68,762. Uzimanjem samo jedne mjere kao prediktora omogućava se niža točnost procjene mase uz veće odstupanje.

Tablica 1. Povezanost između tjelesnih mjera i mase kod crnih slavonskih svinja

Komponenta	Jednadžba predviđanja	r	R^2
Opseg prsa	y=0,4095x + 67,178	0,98	0,95
Visina križa	y=0,1291x+ 56,351	0,90	0,81
Opseg prsa i visina križa	$y= 1,9073046x_1 +1,3949949x_2 - 202,1761812278$	0,97	0,95

Prema tome, detaljnom obradom podataka utvrđeno je da se najtočnija procjena mase uz najmanje odstupanje postiže analizom skupnih podataka (nerastovi i nazimice/krmače) višestrukom regresijom, gdje je broj promatranja viši. Ova jednadžba linearne regresije glasi:

TJELESNA MASA= 1,9073046 (opseg prsa) + 1,3949949 (visina križa) - 202,1761812

Zaključak

Rezultati ovog istraživanja pokazali su da se na temelju opsega prsa i visine križa može poprilično točno procijeniti tjelesna masa kod crnih slavonskih svinja. Analizom skupnih podataka koji uključuju nazimice/krmače i nerastove, najveću povezanost s masom imali su opseg prsa i visina križa, radi čega su ove dvije varijable korištene u procijeni tjelesne mase te su postignute najviše razine pouzdanosti. Pretpostavimo li da je u analizi bilo više nerastova, koeficijenti korelacije bili bi vjerojatno sličniji onima kod nazimica/krmača. Također, da je prikupljeni uzorak u ovom istraživanju bio viši, vjerojatno bi se postigle i više točnosti procjena. Zaključno, uzevši u obzir postignute procijenjene vrijednosti i pogreške u procjeni, rezultati ukazuju da se s ovakvim pristupom vrlo jednostavno i jeftino može procjenjivati tjelesna masa svinja u stvarnim uvjetima na farmi.

Reference

Freedman D.A. (2012). Statistical Models: Theory and Practice. p.26-36. New York, Cambridge University Press.

Lukić B., Raguž N., Karolyi D., Kranjac D., Luković Z., Mahnet Ž., Steiner Z. (2018). *Uzgoj crne slavonske svinje - Priručnik za uzgajivače i studente*. Osijek, Udruga uzgajivača crne slavonske svinje Slavonije, Baranje i zapadnog Srijema.

Groesbeck C.N., Goodband R.D., DeRouchey J. M., Tokach M. D., Dritz S.S., Nelssen J.L., Lawrence K. R., Young M. G. (2002). Using heart girth to determine weight in finishing pigs. In *Kansas Agricultural Experiment Station Research Reports*. Swine Day, p.166-168. Manhattan, Kansas, November 14, 2002.

Mutua F.K., Dewey C.E., Arimi S.M., Schelling E., Ogara W.O. (2011). Prediction of live body weight using length and girth measurements for pigs in rural Western Kenya. J Swine Health Production. 19(1): 26–33.

SAS Institute Inc. (2013). SAS® 9.4 Statements: Reference. Cary, NC: SAS Institute Inc.

Steenbergen E.J.V. (1989). Description and Evaluation of a Linear Scoring System for Exterior Traits in Pigs. Livestock Production Science. 23: 163-181.

Sungirai M, Masaka L, Benhura T.M. (2014). Validity of Weight Estimation Models in Pigs Reared under Different Management Conditions. Veterinary Medicine International. 1-5.

Walugembe M., Nadiope G., Stock J. D., Stalder K. J., Pezo D., Rothschild M. F. (2014). Prediction of live body weight using various body measurements in Ugandan village pigs. Livestock Research for Rural Development. 26(5): 96.

Linear estimation of body weight based on hearth girth and sacrum height of Black Slavonian pigs

Abstract
The aim of this research was to develop a linear regression equation, that can be used to easily estimate the body weight of Black Slavonian pigs in everyday practice. Given the small population of 2890 animals, as well as the fact that a small number of breeders have livestock scales, this method of body weight prediction would improve testing of breeding animals and save time for the controllers and breeders. In this research, data of body measurements was collected on breeding animals, while the analysis showed that heart girth and sacrum height are the most accurate predictors of body weight, which were finally used in linear regression equation.

Key words: estimation, Black Slavonian pig, live weight, body measurements, regression

PREGLEDNI RAD

Uporaba šipka (*Rosa canina L.*) u hranidbi domaćih životinja

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Sažetak
Uporaba šipka (*Rosa canina L.*) poznata je od davnina, a njegova ljekovita svojstva pripisuju se visokom sadržaju različitih fitokemikalija koje imaju široki spektar aktivnosti, kao što su antioksidativno, antimutageno, antimikrobno te antikancerogeno djelovanje. Cilj ovoga rada bio je prikazati osobine šipka te mogućnost uporabe šipka kao prirodnog dodatka u hranidbi domaćih životinja. Istraživanja su potvrdila mogućnost uporabe šipka kao dodatak u hranidbi različitih vrsta i kategorija životinja pri čemu on značajno povoljno utječe na proizvodne pokazatelje, reproduktivna svojstva, kvalitetu životinjskih proizvoda i na opće zdravstveno stanje životinja. Sukladno tome, zastupljenost šipka u suvremenoj stočarskoj proizvodnji zasigurno će u budućnosti biti još puno veća.

Ključne riječi: šipak, prirodni dodatci, hranidba domaćih životinja

Uvod

Korištenje biljaka, njihovih plodova, dijelova biljaka kao i njihovo ljekovito djelovanje poznato je još od davnina te ima dugu tradiciju, a u novije vrijeme sve se više proučavaju i same biološki aktivne tvari u njima (Rovná i sur., 2020.; Tanor i sur., 2020.; Yordanova i sur., 2020.). Otpornost patogena na antibiotike kao i njihovi ostaci u tkivima te posljedično tome i zabrana uporabe antibiotika kao promotora rasta potaknuli su brojna istraživanja te pronalaženje alternativnih tvari koje bi doprinijele poboljšanju zdravstvenog stanja životinja, rasta životinja te proizvodnih rezultata koji se očekuju od životinja (Gjorgovska i sur., 2021.; Vlaicu i sur., 2020.). Uporaba prirodnih dodataka u tu svrhu ima niz prednosti što uključuje njihovo prirodno porijeklo, izostanak njihovog rezidualnog učinka te izostanak razvoja rezistencije (Diaz–Sanchez i sur., 2015.). Jedan od bitnih razloga zašto se posljednjih godina prirodni dodatci sve više uključuju u hranidbu životinja je i potreba te potražnja potrošača za zdravijom hranom, prirodnom hranom te briga o dobrobiti životinja, što sve pridonosi primjeni upravo ovih dodataka u hranidbi životinja, a postoji i spremnost potrošača da takvu hranu plate više (Gjorgovska i sur., 2021.;Vlaicu i sur., 2020.).
Šipak (*Rosa canina L*). pripada rodu *Rosa* te je u narodu poznata kao divlja ruža, pasja ruža, šipurak, pasja drača, divlji šipak, šepurina, šipčanica, pasja roža, šibek, šip, šipak i još mnogim drugim imenima (Tomljenović i sur., 2021.; Šindrak i sur., 2012.). Šipak pripada porodice *Rosaceae* te je uspješan kolonizator raznih staništa, pokazuje veliku prilagodljivost različitim tipovima tala. Koristi se za prevenciju erozije tala, te je dobro prilagodljiv ekološkim uvjetima proizvodnje jer je otporan na različite štetnike i bolesti. Šipak je široko rasprostranjen u gotovo cijeloj Europi, zapadnoj i sjevernoj Aziji i Africi (Vasić i sur., 2020.; Pehlivan i sur., 2018.). U Hrvatskoj se nalazi u gotovo svim krajevima, od rubova cesta i putova, preko livada, pašnjaka i šuma pa sve do brdskih i planinskih područja (nalazimo je na preko 1400 metara nadmorske visine). Zbog velike rasprostranjenosti pripada u najraširenije samonikle pripadnike roda *Rosa* (Šindrak i sur., 2012.). Ljekovita fiziološka

svojstva šipka uglavnom se pripisuju visokom sadržaju fitokemikalija uključujući fenole, flavonoide, tokoferole i triterpenske kiseline, s posebno velikim količinama vitamina C i karotenoida koji imaju široki spektar aktivnosti, kao što su antioksidativno, antimutageno, antimikrobno te antikancerogeno djelovanje (Rovná i sur., 2020.; Vlaicu i sur., 2020.). Prvi spomeni ove biljke datiraju od Plinija Starijeg (23–79.pr.Kr.) koji je prvi opisao njena ljekovita svojstva kada je njenu uporabu uočio kod francuskih plemena u liječenju ugriza pasa. Ovaj opis naknadno je iznjedrio naziv vrste (*Rosa canina*) pasja ruža (Tolekova i sur., 2020.; Winther i sur., 2016.). Prema makroskopskim ostacima britanske flore, kao hrana korišteni su plodovi šipka u kasnom neolitu, željeznom dobu, starijem i srednjem vijeku (Tomljenović i sur., 2021.). U Europi je šipak opisala i dobro poznata časna sestra Hildegarda iz Bingena (1098.–1179.po.Kr.), koja ga je koristila kao čaj za jačanje u svojim tretmanima liječenja (Selahvarzian i sur., 2018.; Winther i sur., 2016.). Zbog visokog sadržaja vitamina C, šipak je postao glavni izvor vitamina C i u Velikoj Britaniji tijekom Drugog svjetskog rata. Zbog pomorske blokade tijekom koje su njemačke podmornice potapale trgovačke brodove koji su plovili prema Velikoj Britaniji opskrba Britanije voćem, posebno agrumima, bila je u potpunom prekidu. Tadašnje Ministarstvo hrane donijelo je naredbu o prikupljanju šipka i masovnoj proizvodnji šipkovog sirupa. Štoviše, i veliki iranski liječnik Avicena (980.–1037. godine) spomenuo je šipak u svojoj knjizi "Kanon medicine" kao mogući lijek za brojne bolesti (Tolekova i sur., 2020.; Selahvarzian i sur., 2018.). Cilj ovoga rada bio je prikazati osobine šipka te mogućnost uporabe šipka kao prirodnog dodatka u hranidbi domaćih životinja.

Šipak (*Rosa canina L.*)

Šipak (*Rosa canina L.*) pripada rodu *Rosa*, porodici *Rosaceae* koja obuhvaća više od 115 rodova i oko 3000 vrsta, a rod *Rosa* obuhvaća između 100 i 250 vrsta. U hrvatskoj flori zastupljeno je nekoliko desetaka vrsta i međuvrsnih križanaca šipka (Šindrak i sur., 2012.). Šipak je višegodišnji listopadni grm u rasponu visine 2–3 metra koji ima vitke tanke uspravne ili povijene grane s trnovima koji su prošireni pri dnu. Korijenov sustav biljke je jak i prodire duboko u tlo (Pehlivan i sur., 2018.; Selahvarzian i sur., 2018.). Listovi su dugi 6–10 cm, sastavljeni od 5–7 listića. Listići su obično jajolikog oblika oštro nazubljeni, s gornje strane su sjajni i goli dok su s donje strane obrasli dlačicama (Miraj, 2016.; Šindrak i sur., 2012.). Cvjetovi su veliki, ružičasto–bijele boje nježnog i ugodnog mirisa, a sastoje se od 5 latica koje mogu stajati pojedinačno ili u skupini po nekoliko cvjetova. Cvjetovi imaju visok sadržaj nektara i peluda pa ih rado posjećuju mnoge vrste kukaca. Vrijeme cvatnje je od svibnja do srpnja nakon čega latice otpadaju, a ostaje zeleni mesnati ovoj prepun sitnih dlačica s tvrdim sjemenkama–plod. Plod je jajolikog oblika dužine oko 1,5–2 cm obično žuto narančaste do tamno crvene boje na površini sjajan te se na vrhu ploda uočavaju ostaci tučka. Šipak dozrijeva u rujnu i listopadu, a udio mezokarpa u šipku iznosi oko 75 % (Selahvarzian i sur., 2018.; Miraj, 2016.; Šindrak i sur., 2012.). Šipak je bogat brojnim biološko aktivnim tvarima kao što su vitamin C, B_1, B_2, K, E, karoten posebice β-karoten i likopen, antocijan, brojni flavonoidi (kamferol, kvercetin, rutin)), pektin, tanin, mineralne tvari K, P, Ca, Mg, Mn, Na, Zn, Se i Fe, voćne kiseline, šećeri, masti, organske kiseline (jabučna i limunska), aminokiseline i eterična ulja (Aldemir i sur., 2021.; Konca i sur., 2021.; Kwatra i sur., 2021.). Što se tiče masnih kiselina, poznato je da šipak ima u svom sastavu zastupljene uglavnom palmitinsku, linoleinsku i α-linolensku kiselinu kao glavne masne kiseline te niske količine stearinske i oleinske kiseline (Gjorgovska i sur., 2021.; Fan i sur., 2014.).

Primjena šipka u hranidbi životinja

Do danas je objavljen veliki broj istraživanja koja su ispitivala utjecaj hranidbe uz dodatak šipka na proizvodne pokazatelje, biokemijske pokazatelje, reproduktivne pokazatelje te

općenito zdravstveno stanje različitih vrsta i kategorija životinja, a u nastavku su detaljnije opisana najznačajnija otkrića.

Aldemir i sur. (2021.) u svom su istraživanju željeli utvrditi moguće količine šipka (5g/kg, 10g/kg, 15 g/kg hrane) koje bi mogle zamijeniti komercijalne pripravke askorbinske kiseline kako bi zadovoljili potrebe za vitaminom C kod kokoši nesilica i pijetlova te utjecaj spomenutog dodatka na stopu plodnosti, vijabilnost izvaljenih pilića, neke krvne parametre kokoši i pijetlova uzgajanih pod stresom velike nadmorske visine i hladnoće. Utvrdili su kako niske količine šipka (5 g/kg hrane) pozitivno utječu na stopu valjenja pilića (P<0,05), a i koncentracija vitamina C u plazmi bila je značajno viša (P<0,0001) kod pokusnih životinja u odnosu na kontrolnu skupinu, dok je razina triglicerida te mokraćne kiseline bila značajno manja (P<0,05). Zaključili su kako dodatak ploda šipka u količini od 5 g/kg u obrocima kokoši nesilica i pijetlova izloženih visokoj nadmorskoj visini i hladnoći može uspješno zamijeniti komercijalni dodatak vitamina C. Criste i sur. (2017.) željeli su utvrditi utjecaj dodatka 2% šipka u krmnu smjesu za brojlere koji su uzgajani pod toplinskim stresom na njihove performanse rasta, razvoj unutarnjih organa te na mikrobnu populaciju crijeva. Dodatak šipka nije utjecao na tjelesnu masu, dnevni prirast i konzumaciju hrane, ali je pozitivno utjecao na povećanje mase jetre i na kolonizaciju crijeva korisnim bakterijama (*Lactobaccilus spp.*) (P<0,05) te inhibiranje patogenih bakterija (*E. coli*), pa se može zaključiti kako dodatak šipka povoljno djeluje na održavanje zdravlja probavnog trakta očuvanjem ravnoteže populacije mikroorganizama koji koloniziraju crijeva pilića.

Grigorova i sur. (2021.) u svom su istraživanju željeli istražiti utjecaj dodatka šipka (0,5 %) krmnim smjesama kokoši nesilica na proizvodnju i kvalitetu jaja, morfološke karakteristike jaja, oksidaciju žumanjka jaja i odabrane krvne parametre. Dodavanje šipka smjesama značajno je utjecalo na razine triglicerida kao i na razinu malondialdehida žumanjka (P<0,05), te je dovelo do značajnog povećanja pigmentacije žumanjka (P<0,001). Varzaru i sur. (2021.) istraživali su učinke dodatka šipka (2 %) krmnim smjesama kokoši nesilica na antioksidativni profil te peroksidaciju lipida u pohranjenim jajima. Uočene su povećane koncentracje vitamina A u jajima (P<0,05) te značajno usporavanje procesa lipidne peroksidacije tijekom skladištenja. Konca i sur. (2021.) željeli su utvrditi utjecaj različitih koncentracija šipka (2,5; 5; 10 i 15 %) dodanih u krmne smjese prepelica na performanse rasta, kvalitetu jaja i mesa te antioksidativni status. Uočeno je kako je masa jaja porasla u tretmanima s 5 % i 10 % šipka. Suplementacija šipkom uzrokovala je smanjenje L* vrijednosti boje žumanjka, međutim, vrijednosti crvenila (a*) i žute boje (b*) žumanjka bile su veće od vrijednosti kontrolne skupine (P<0,01). Dodatak šipka u koncentraciji od 2,5 i 5% smanjio je serumske koncentracije malondialdehida (MDA) i povećao razine serumske superoksid dismutaze (SOD) (P<0,01).

Yordanova i sur. (2020.) istraživali su učinak dodatka šipka i koprive (20 g/kg) te njihov utjecaj na zdravstveno stanje nerastova, količinu i kvalitetu sjemena nerastova. Ispitani dodatci ne utječu na glavne osobine kvalitete sjemena–volumen, gustoću i pokretljivost, ali značajno smanjuju postotak aglutinacije (P=0,043) te imaju tendenciju smanjenja broja patoloških spermatozoida za 14,56 %. Yordanova i sur. (2020a.) istraživali su suplementaciju biljnim dodatkom (50 % brašna od šipka, 30 % listova koprive, 10 % trave, 5 % maslačka, 5 % gloga) u koncentraciji 20 g/kg na klaoničke parametre i kvalitetu mesa svinja u tovu. Spomenuti dodatak imao je značajan utjecaj na debljinu masnog tkiva leđa (P=0,016). Untea i sur. (2009.) proveli su istraživanje na odbijenoj prasadi te pratili učinke dodatka šipka (3 %) preko eliminacije bakra i cinka putem fecesa. Uočeno je kako su se smanjile koncentracije bakra za 13,45 % odnosno cinka za 4,93 % .

Szumachher–Strabel i sur. (2011.) istraživali su učinke dodatka ulja šipka (5 %) s ciljem smanjivanja stvaranja metana u buragu *in vivo* te su zaključili kako ulje šipka ima potencijal smanjiti stvaranje metana u buragu. Esenbuğa i sur. (2011.) istraživali su učinke dodatka

šipka (5 % i 15 %) u obrocima muške janjadi na performanse rasta, klaoničke pokazatelje te kvalitetu mesa. Dodatak šipka nije imao utjecaj na performanse rasta janjadi niti u boji mesa niti u pH vrijednostima no uočene su značajne razlike u masi (P<0,05) kod toplih i hladnih polovica trupova janjadi.

Zaključak

Recentna istraživanja potvrdila su mogućnost uporabe šipka kao dodatka u hranidbi različitih vrsta i kategorija životinja pri čemu su posebno značajni učinci ovog dodatka na proizvodne pokazatelje, reproduktivna svojstva, kvalitetu životinjskih proizvoda (jaja, meso) te na opće zdravstveno stanje životinja. Slijedom toga, za očekivati je kako će spomenuti dodatak u budućnosti biti još više zastupljen u suvremenoj stočarskoj proizvodnji.

Literatura

Aldemir R., Tekeli A., Demirel M., Yıldız S., Yörük İ.H., Belhan S., Koşal V. (2021). The effects of different levels of rosehip fruit added in the rations of laying hens raised under high altitude and cold stress on some blood parameters, rectal temperature, fertility rate and chick quality. Livestock Studies. 61(1):14–21.

Criste R.D., Panaite T.D., Tabuc C., Sărăcilă M., Șoica C., Olteanu M. (2017). Effect of oregano and rosehip supplementson broiler (14-35days) performance, carcass and internal organs development and gut health. AgroLife Scientific Journal. 6(1): 75–83.

Diaz–Sanchez S., D'Souza D., Biswas D., Hanning I. (2015). Botanical alternatives to antibiotics for use in organic poultry production. Poultry Science. 94:1419–1430.

Esenbuğa N., Macit M., Karaoglu M., Aksakal V., Yoruk M.A., Gül M., Aksu M. İ., Bilgin Ö.C. (2011). A study on possibility of Rosa canina seed use as feed ıngredient in diets of Morkaraman male lambs. Tropical Animal Health and Production. 43:1379–1384.

Fan C., Pacier C., Martirosyan D.M. (2014). Rose hip (*Rosa canina L*): A functional food perspective. Functional Foods in Health and Disease. 4(11): 493–509.

Gjorgovska N., Grigorova S., Levkov V. (2021). Application of rosehip fruits as feed supplement in animal nutrition. Journal of Agriculture Food and Development. 7:12–15.

Grigorova S., Gjorgovska N., Levkov V. (2021). Effects of rosehip feed supplementation on egg quality parameters, yolk lipid oxidation, and blood parameters of laying hens. Iranian Journal of Applied Animal Science. 11(4): 827–833.

Konca Y., Kaliber M., Uzkulekci H.H., Cimen B., Yalcin H. (2021). The effect of rosehip (*Rosa canina L.*) supplementation to diet on the performance, egg and meat quality, antioxidant activity in laying quail. Sains Malaysiana. 50(12): 3617–3629.

Kwatra B., Solanki A., Pal M., Jasdanwala S.S., Pathak T. (2021). Reviewing effects of rosehip, curcumin, piperine and chondroitin sulfate on collagen. International Journal of Pharmaceutical Sciences Review and Research. 68(25): 148–164.

Miraj, S. (2016). Phytochemical composition and in vitro pharmacological activity of rose hip (*Rosa canina* L.). Der Pharma Chemica. 8(13): 117–122.

Pehlivan M., Mohammed F.S., Sevindik M., Akgul H. (2018). Antioxidant and oxidant potential of *Rosa canina*. Eurasian Journal of Forest Science. 6(4): 22–25.

Rovná K., Ivanišová E., Žiarovská J., Ferus P., Terentjeva M., Łukasz Kowalczewski P.L., Kačániová M. (2020). Characterization of *Rosa canina* fruits collected in urban areas of Slovakia. Genome size, iPBS profiles and antioxidant and antimicrobial activities. Molecules. 25(8): 1888.

Selahvarzian A., Alizadeh A., Baharvand P.A., Eldahshan O.A., Rasoulian B. (2018). Medicinal properties of *Rosa canina L.*. Herbal Medicines Journal. 3(2): 77–84.

Szumacher–Strabel M., Zmora P., Roj E., Stochmal A., Pers-Kamczyc E., Urbańczyk A., Oleszek W., Lechniak D., Cieślak A. (2011). The potential of the wild dog rose (*Rosa*

canina) to mitigate in vitro rumen methane production. Journal of Animal and Feed Sciences. 20: 285–299.
Šindrak Z., Jemrić T., Baričević L., Han Dovedan I., Fruk G. (2012). Fruit quality of dog rose seedlings (*Rosa canina L*). Journal of Central European Agriculture. 13(2):321–330.
Tanor E.B., Matamane R.P., Hapazari I., Magama S. (2020). Phytochemical screening and antioxidant analysis of the ethanolic extract of rosehip seed press cake. Current Journal of Applied Science and Technology. 39(35): 57–67.
Tolekova S., Sharmanov T., Sinyavskiy Y., Berzhanova R., Mammadov R., Aksoy O.K., Yusifli R. (2020.). Antioxidant, pharmacological, medical properties and chemical content of Rosa L. extracts. International Journal of Secondary Metabolite. 7(3):200–212.
Untea A., Criste R.D., Panaite T., Radutoiu D. (2009). Evaluation of the phytogenic potential (I-*Rosa canina*)of the weaned piglets diets on copper and zinc absorption and elimination through faeces. Bulletin of university of agricultural sciences and veterinary medicine cluj-napoca. Animal Science and Biotechnologies. 66(1–2): 80–85.
Tomljenović N., Jemrić T., Vuković M. (2021). Variability in pomological traits of dog rose (*Rosa canina L.*) under the ecological conditions of the Republic of Croatia. Acta Agriculturae Serbica. 26(51): 41–47.
Vasić D., Paunović D., Špirović Trifunović B., Miladinović J., Vujošević L., Đinović D., Popović–Đorđević J. (2020). Fatty acid composition of rosehip seed oil. Acta Agriculturae Serbica. 25(49): 45–49.
Varzaru I. , Untea A.E. , Panaite T., Olteanu M. (2021). Effect of dietary phytochemicals from tomato peels and rosehip meal on the lipid peroxidation of eggs from laying hens. Archives of Animal Nutrition. 75(1): 18–30.
Vlaicu A.P., Turcu R.P., Panaite D.T. (2020). Rosehip (*Rosa canina*) as a beneficial dietary feed in poultry nutrition: Review. Advanced Research in Life Sciences. 4:11–15.
Winther K., Vinther Hansen A. S., Campbell–Tofte J. (2016). Bioactive ingredients of rose hips (*Rosa canina L*) with special reference to antioxidative and anti-inflammatory properties: in vitro studies. Botanics: Targets and Therapy. 6: 11–23.
Yordanova G., Kanev D., Marchev Y., Nedeva R., Nikolova T., Palova N. (2020). Effect of herbal supplements (nettle and rosehip) on the sperm quality in Danube White breed boars. Bulgarian Journal of Agricultural Science. 26(5): 1020–1023.
Yordanova G., Kanev D., Nedeva R., Eneva K. (2020a). Influence of herbal supplements on the productivity and slaughter qualities in fattening pigs. Scientific Papers. Series D. Animal Science. 63(2): 136–142.

The usage of rose hip (*Rosa canina L.*) in feeding of domestic animals

Abstract
The use of rose hips (*Rosa canina L.*) has been known since ancient times, and its medicinal properties are attributed to the high content of various phytochemicals that have a wide range of activities, such as antioxidant, antimutagenic, antimicrobial and anticancer effects. The aim of this paper was to present the properties of rose hips and the possibility of using rose hips as a natural supplement in the diet of domestic animals. Researches have confirmed the possibility of using rose hips as a feed additive for various species and categories of animals, where it has a significant beneficial effect on production indicators, reproductive properties, quality of animal products and general health of animals. Accordingly, the presence of rose hips in modern livestock production will certainly be much higher in the future

Key words: rose hips, natural supplements, domestic animals feeding

PREGLEDNI RAD

Utjecaj konformacije na performans dresurnoga konja

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Sažetak
Konjička industrija bilježi porast potražnje i širenja tržišta visokovrijednih elitnih dresurnih konja. Dresurno jahanje olimpijski je sport za koji se koriste elitna grla. Osobitosti uzgojnoga i sportskog tipa posljedica su intenzivnoga uzgojno-selekcijskog rada koji se temelji na raznim znanstvenim spoznajama: o genetici, biomehanici i konformaciji konja. Konformacija utječe na kretnje i performans. Znanstvenici razvijaju kvantitativne analize koje bi poboljšale performans s pomoću selekcije poželjnih morfoloških obilježja dresurnih konja. Trenutačne spoznaje o konformaciji i nasljednosti konformacijskih osobina korisne su za uzgojno-selekcijski rad i individualizaciju treninga sportskih konja.

Ključne riječi: konformacija, performans, dresurno jahanje

Uvod

Dresurno jahanje konjička je disciplina koja je intenzivno popularizirana nekoliko zadnjih desetljeća. Posljedično se selekcijski rad usmjerio na produkciju visokovrijednih grla s velikim potencijalom za konkuriranje u ovome olimpijskom sportu. Elitne dresurne konje karakteriziraju specifična konformacijska obilježja koja im omogućuju izvedbu zahtjevnih dresurnih radnja. Konformaciju čine neizmjenjive dimenzije i proporcije tijela određene mišićno-koštanim sustavom. Primarno utječe na prirodne kretnje konja, a sekundarno na potencijal uspjeha u sportu. S druge strane, performans nekoga sportskog konja nije uvjetovan samo konformacijom, nego i pravilno prilagođenim treningom, hranidbom i uvjetima držanja. Konformacija i kretnje dva su neodvojiva pojma koja se uzimaju kao činitelji uzgojne i sportske vrijednosti konja u svim uzgojnim organizacijama. Poznata i aktualna metoda za procjenu konformacije i kretnja zove se „linearno ocjenjivanje“, a zadnjih se nekoliko desetljeća radi na razvitku i popularizaciji digitalne kvantitativne analize.

Procjena konformacije konja

Procjena konformacije koristi se za predviđanje sposobnosti konja za sport, a čimbenici kao što su trening i uvjeti držanja uvelike utječu na performans dresurnoga konja, pa Barrey i sur. (2010.) ranu selekciju temeljem ocjenjivanja kretnja i konformacije smatraju puno korisnijom negoli ostvarene rezultate u sportu. Erck-Westergren i Foreman (2014.) ističu kako selekcija samo na temelju konformacije nije poželjna zbog niske korelacije s budućim performansom, a kretnje konja su korisnije, ali ih je teško precizno analizirati bez uređaja za detaljnu analizu. Koenen i sur. (1995.) su istraživanjem na dresurnome i preponskom tipu kraljevskog nizozemskog toplokrvnjaka (KWPN - Koninklijk Warmbloed Paardenstamboek Nederland) potvrdili niske genetske korelacije između konformacije i performansa, iako su određena konformacijska svojstva korisna. Linearno ocjenjivanje je trenutačno najzastupljenija metoda, koja obuhvaća subjektivnu procjenu konformacije, kretnja i karaktera konja. Po prvi put je uvedena 1989. godine u uzgojnoj organizaciji KWPN pasmine (Koenen i sur., 1995.), a bazira se na procjeni niza osobitosti grla te njihovu odstupanju od

prosječne populacije i idealnoga pasminskog tipa. Iako je ocjena konja proizvod subjektivnoga doživljaja, Back i Clayton (2013.) navode kako interpretacijom i analiziranjem svojstava ocjena postaje objektivnija. Za Kuhnke i sur. (2019.) linearno ocjenjivanje također je objektivnija metoda od drugih, i to osobito za procjenu svojstava koja su slabo vidljiva, a varijabilnost njihovih ocjena prikaz je fenotipske varijacije. Za Van Weerena i Crevier-Denoixa (2006.) većina je uzgajivača tradicionalno orijentirana ali smatraju kako će napredak tehnologije u konjičku industriju uvesti kvantitativnu analizu konformacije. Back i Clayton (2013.) ističu sporu proceduru kao najveći nedostatak kvantitativne analize pri njezinu uvođenju u službene protokole ocjenjivanja konja. Druml i sur. (2016.) analizirali su pak konformaciju lipicanskih konja pomoću kvantitativnih i kvalitativnih metoda. Koristili su statističku metodu analize pouzdanosti i linearne regresije, a njihov rad u budućnosti bi mogao koristiti obrazovanju sudaca te ujednačenju rezultata i protokola ocjenjivanja. Sánchez i sur. (2013.) ukazuju na buduća istraživanja koja će se orijentirati prema kombinaciji genetski prenosivih svojstava radi razvijanja selekcijskoga indeksa, koji bi pomogao poboljšanju performansa putem selekcije temeljene na poželjnim morfološkim obilježjima dresurnih konja.

Kretnje i trening dresurnoga konja

Za dresurnog konja je uz samonosivost i fleksibilnost, važna pravilnost njegovih slobodnih kretnji. Byström i sur. (2019.) navode simetriju i ujednačenost kao važan kriterij za ocjenu kretnji i performans u dresurnome sportu. Osnovne kretnje koje se ocjenjuju su hod, kas i galop. Hod je najsporija i četverotaktna kretnja, kas je dvotaktna simetrična kretnja, a galop je trotaktna kretnja (Barrey, 2014.). Ovaj autor passage i piaffe naziva derivatima prikupljenoga kasa te ističe specifičnost passagea zbog pretvorbe sile i povećanja suspenzije. Unutar kasa sila koja potiskuje konja prema naprijed, tijekom passagea potiskuje ga prema gore. Upravo radi povećanja suspenzije, koja u kasu predstavlja trenutak koji odvaja dijagonalni par nogu, pokreti postaju ekspresivniji. Erck-Westergren i Foreman (2014.) smatraju dresurno jahanje umjetnošću u kojoj trening konja dolazi do izražaja više negoli u svim ostalim konjičkim disciplinama. Usvojena i pravilna dresurna skala sastoji se od šest sljedova koji opisuju kvalitetu položaja tijela i kretnja konja. Ona uključuje ritam (ravnomjerno izmjenjivanje kontakta kopita s tlom unutar jednoga koraka), opuštenost (smiren i zadovoljan pogled konja, dobra aktivnost leđa, zatvorena i opuštena usta, mirno njihanje repa, opušteno frktanje), kontakt (kada je kopito u kontaktu s tlom), zamah (kada kopito nije u kontaktu s tlom) i potisak (pokretačka sila), izravnatost (kada stražnje noge prate tragove prednjih nogu) i prikupljenost (točka težišta mu se pomiče prema natrag i prema dolje tako da sve veći teret pada na stražnje noge). Byström i sur. (2019.), praćenje ovoga smislenog načina treninga smatraju bitnim za postupno povećanje kvalitete performansa. Barrey (2014.) ističe prijelaze između osnovnih kretnji konja, koji su ključni u svakome sustavu izobrazbe dresurnih konja, kao jednu od najtežih osnovnih vježba zbog zahtijevanja specifične koordinacije i snage konja. Promatrajući konje s iznadprosječnim kretnjama, Back i sur. (1994.) su zaključili kako su njihovi koraci dulji i kako je frekvencija njihovih kretnja sporija, uz veliku ekstenziju u prednjim nogama i veliku fleksiju u stražnjim nogama, dok Barrey (2014.) uočava kako u elitnih dresurnih konja stražnja noga dapače dolazi u kontakt sa zemljom 20 – 30 m/s prije dijagonalne prednje noge. Ipak, za uspješan performans u dresurnome sportu nije važna samo kvaliteta kretnja, već i sposobnost konja za prikupljanje, koja je određujući čimbenik dresurnoga potencijala, jer u slučaju njezina izostanka teže radnje ne mogu izvesti pravilno (Barrey, 2014.). Prema službeno propisanome pravilniku Međunarodne konjičke federacije (FEI, 2022.), konj koji ne želi ili se ne može pravilno koristiti svojim stražnjim trupom nikada se neće moći kretati s lakoćom i samonosivošću uz zamah i potisak, odnosno nikada neće biti pravilno prikupljen.

Nemogućnost pravilne prikupljenosti može biti posljedica konformacije, a konformacija je većim dijelom pasminski predodređena. Barrey i sur. (2010.) u istraživanju kretnja i konformacije trogodišnjih dresurnih konja pokazali su kako su njemačke toplokrvne pasmine konja superiornije u usporedbi sa Sel Franse (Selle Français) i punokrvnim španjolskim konjima. S obzirom na dužinu koraka i njihovu sporiju frekvenciju, zaključeno je kako njemačke toplokrvne pasmine (hanoveranci, oldenburzi, vestfalci) imaju veće predispozicije za uspjeh u kompetitivnome dresurnom sportu (Barrey i sur., 2010.). Konformacijske razlike između španjolskih punokrvnjaka i nizozemskih toplokrvnjaka su u većim kutovima u laktovima i karpalnim zglobovima u korist španjolskih konja. Međutim, ti veći kutovi uzrokuju kraće korake, veću frekvenciju kretnje i manju aktivnost pri kretanju, i to zbog fleksije koja je veća od ekstenzije u karpalnome i tarzalnom zglobu konja španjolskih pasmina. Upravo ih te eksterijerne karakteristike razlikuju u usporedbi izdašnih kretnji prema naprijed u odnosu na ostale sportske dresurne pasmine konja (Barrey, 2014.).

Konformacijske osobitosti dresurnih konja

Love i sur. (2006.) ukazali su na vjerojatnost variranja konformacijskih osobitosti koje utječu na performans i zdravlje konja unutar različitih konjičkih disciplina, a Back i Clayton (2013.) naglasak stavljaju na međupasminske konformacijske različitosti. Holmström i Philipson (1993.) smatraju tip, sklad te oblik glave, vrata i cijeloga tijela nemjerljivima. Kratak vrat se smatra manje fleksibilnim, dok se dug vrat povezuje s hipermobilnošću i negativnim utjecajem na raspodjelu težine u tijelu, iako ga Ducro i sur. (2009.a) genetski povezuju upravo s konformacijom specifičnom za dresurne konje. Istraživanjem su Caspar i sur. (2015.) ispitali ukuse jahača i trenera te zaključili kako se zbog estetskoga učinka preferiraju specifični oblici vrata koji su skloniji hiperfleksiji. Iako je hiperfleksija primarno pojam koji se odnosi na kontroverzne metode u treningu, konformacijske karakteristike konja čine sklonijim savijanju u vratu u prevelikoj mjeri. Van Weeren i Crevier-Denoix (2006.) ističu udaljenost krila prvog cervikalnog kralješka i mandibule kao činitelja fleksibilnosti vrata. Evolucija dresurnoga sporta smatra se prekretnicom u promjeni standarda građe konja. Moderni dresurni konji pravokutne su građe tijela, a španjolske pasmine, luzitanski konji (Lusitano) i lipicanci su zadržali kvadratičan oblik tijela. Uzgojna organizacija KWPN pasmine dresurni tip opisuje uzbrdno i finije građenim konjem pravokutnoga oblika tijela duljih proporcija, s finijim spojem glave i vrata koji je visoko nasađen, s razvijenim mišićima u gornjem dijelu. Johnston i sur. (2010.) duža leđa smatraju lošima za zdravlje konja, a Ducro i sur. (2009.a) visinu konja povezuju s kraćim sportskim vijekom. Istraživanje Mayakija i sur. (2019.) pokazalo je kako se broj jahaćih konja oboljelih od specifične bolesti leđa (Equine Back Disorder, EBD) povećava. Bolest je uzrokovana abnormalnim kralješcima i promjenama u strukturi kralježnice, a glavni su simptomi bol u leđima i lošiji performans. Iako ne postoji dobna ili spolna predispozicija za bolest, utvrđeno je kako se u specifičnih pasmina pojavljuje češće. Pasmine kod kojih je zabilježena su toplokrvnjaci, engleski punokrvnjaci, kvorteri (quarters) i arapski konji. Stoga se zasada može pretpostaviti kako težnja k idealnoj konformaciji nije jedini indikator za funkcionalnost i performans sportskoga konja. Važne karakteristike svakoga sportskog konja su stav i kvaliteta nogu. Stav nogu utječe na putanju nogu odnosno ispravnost samih kretnji, a time i na sile koje se stvaraju pri kretanju. Back i sur. (1994.) slabije i duže putice s većim nagibom povezuju s elastičnijim pokretima, ali Ducro i sur. (2009.b) zaključuju kako konji s ravnije postavljenim puticama imaju duži sportski vijek. Nepravilni stavovi nogu ili različit oblik i većina prednjih nogu, odnosno asimetrične prednje noge u dresurnih konja skraćuju sportski vijek (Ducro i sur., 2009.b). Za dresurnoga konja poželjna je karakteristika veća lopatica, koja je zabačenija unatrag. Lopatica zabačenija unatrag utječe na veći domet prednjih nogu i na podizanje u prednjem trupu u zadnjoj fazi kasa, iako su dobra ravnoteža i kapacitet stražnjih nogu za

nošenje težine ključni za slobodu u prednjem dijelu tijela pri kretanju (Holmström i sur., 1994.). S druge strane, Back i Clayton (2013.) lopaticu smatraju manje važnom u performansu dresurnoga konja zbog drugih važnijih i funkcionalnijih konformacijskih osobitosti, a duži humerus drže boljom podlogom za triceps, koji utječe na domet prednjih nogu. Većina autora dresurnih priručnika i knjiga o konformaciji ističe konformaciju stražnjega dijela trupa kao najvažniju za dresurnoga konja. Holmström i sur. (1994.) ukazuju na položaj i veličinu femura kao faktor za stvaranje potiska iz stražnjega dijela tijela. Upravo manji kut koljenog zgloba i veći femur u korelaciji su s kvalitetom kretanja konja u kasu i hodu (Holmström i sur., 1993.). Za prikupljene radnje bitan je i položaj zdjelice. Ravnija zdjelica podložnija je rotaciji u kretanju osobito pri izvođenju kvalitetnoga passagea (Back i Clayton, 2013.). S druge strane, donekle spušteniji zadnji dio tijela je, prema Koenenu i sur. (1995.), u pozitivnoj korelaciji s kvalitetom kretnja konja. Kukovi su vrlo važni u pomicanju stražnjih nogu prema naprijed i ograničeno sa strane, a što su postavljeni više, time su i uži što nije poželjno za prikupljene radnje. Weller i sur. (2006.) veći kut kuka smatraju važnim za performans i pravilno nasađene mišiće koji stvaraju potisak. Back i Clayton (2013.) tvrde kako različite kombinacije kutova u stražnjim nogama mogu rezultirati kvalitetnim sportskim konjem, iako ravniju zdjelicu, femur s nagibom i normalan ili nešto manji kut femura i tarzalnoga zgloba smatraju poželjnima. Problem u ovakvoj analizi postavljenosti kostiju i kutova leži u nemogućnosti njihova povezivanja s duljinom koraka konja.

Zaključak

Konformacija je neizmjenjiva osobitost konja na koju se treningom i rehabilitacijom može pozitivno ili negativno utjecati. Utječe na konceptualizaciju treninga te je značajna u uzgojno-selekcijskome radu. Dresurna grla sportske ili uzgojne svrhe imaju specifična konformacijska obilježja, koja se razlikuju unutar uzgojnih organizacija, a pažljivim se križanjima različitih pasmina ili tipova unutar pasmina stvaraju vrhunska sportska grla. Performans je skup različitih genetskih i paragenetskih čimbenika koji izuzimanjem ne ostavljaju mogućnost važeće procjene potencijala sportskoga konja. Vidljiv je tek u zrelijoj dobi i fazi treninga dresurnoga konja kroz rezultate ostvarene u visokim kategorijama kompetitivnoga sporta. Konformacija i kretnje osnovni su činitelji potencijala mladih grla za sport te se njihova procjena provodi u obliku linearnoga ocjenjivanja, a zadnjih se godina djeluje na razvoj i popularizaciju metode digitalne kvantitativne analize, koja povećava pouzdanost procjene budućega performansa. Unatoč istraživanjima o povezanosti konformacije i performansa, nema puno znanstveno opravdanih činjenica koje povećavaju pouzdanost predviđanja uspješnosti konja u sportu.

Napomena

Rad je izvod iz prijavljenoga završnog rada Petre Prlić pod nazivom Utjecaj konformacije na performans dresurnog konja na Fakultetu agrobiotehničkih znanosti Osijek.

Literatura

Back W., Barneveld, A., Bruin G., Schamhardt H. C., Hartman W. (1994). Kinematic detection of superior gait quality in young trotting warmbloods. Veterinary Quarterly. 16(2): 91–96.

Back W., Clayton H.M. (2013.). Equine locomotion (Second Edition). Edinburgh. New York. Saunders Elsevier.

Barrey, E. (2014). Biomechanics of locomotion in the athletic horse. Equine Sports Medicine and Surgery. 189–211.

Barrey E., Desliens F., Poirel D., Biau S., LeMaire S., Rivero J.-L. L., Langlois B. (2010). Early evaluation of dressage ability in different breeds. Equine Veterinary Journal. 34(34): 319–324.

Byström A., Clayton H. M., Hernlund E., Rhodin M., Egenvall, A. (2019). Equestrian and biomechanical perspectives on laterality in the horse. Comparative Exercise Physiology. 1–12.

Caspar G. L., Dhand N. K., McGreevy P. D. (2015). Human Preferences for Conformation Attributes and Head-And-Neck Positions in Horses. PLOS ONE. 10(6): e0131880

Druml T., Dobretsberger M., Brem G. (2016). Ratings of equine conformation – new insights provided by shape analysis using the example of Lipizzan stallions. Archives Animal Breeding. 59: 309–317.

Ducro B. J., Bovenhuis H., Back W. (2009a). Heritability of foot conformation and its relationship to sports performance in a Dutch Warmblood horse population. Equine Veterinary Journal. 41(2): 139–143.

Ducro B. J., Gorissen B., Eldik P., Back W. (2009b). Influence of foot conformation on duration of competitive life in a Dutch Warmblood horse population. Equine Veterinary Journal. 41(2): 144–148.

Erck-Westergren E., Foreman J.H. (2014). Veterinary aspects of training dressage horses. Equine Sports Medicine and Surgery. (Sedond Edition).

Holmström M., Philipsson J. (1993). Relationships between conformation, performance and health in 4-year-old swedish warmblood riding horses. Livestock Production Science. 33(3-4): 293–312.

Holmström M., Fredericson I., Drevemo S. (1994). Biokinematic differences between riding horses judged as good and poor at the trot. Equine Veterinary Journal. 26(S17): 51–56.

Johnston C., Holm K., Faber M., Erichsen C., Eksell P., Drevemo S. (2010). Effect of conformational aspects on the movement of the equine back. Equine Veterinary Journal. 34(34): 314–318

Koenen E. P. C., van Veldhuizen A. E., Brascamp E. W. (1995). Genetic parameters of linear scored conformation traits and their relation to dressage and show-jumping performance in the Dutch Warmblood Riding Horse population. Livestock Production Science. 43(1): 85–94.

Kuhnke S., Bär K., Bosch P., Rensing M., Borstel U. K. V. (2019). Evaluation of a Novel System for Linear Conformation, Gait, and Personality Trait Scoring and Automatic Ranking of Horses at Breed Shows: A Pilot Study in American Quarter Horses. Journal of Equine Veterinary Science. 78: 53–59.

Love S., Wyse C. A., Stirk A. J., Steat M. J., Calver P., Voute L. C., Mellor D. J. (2006). Prevalence, heritability and significance of musculoskeletal conformational traits in Thoroughbred yearlings. Equine Veterinary Journal. 38(7): 597–603.

Mayaki A.M., Intan-Shameha A.R., Noraniza M.A., Mazlina M., Adamu L., Abdullah R. (2019.). Clinical investigation of back disorders in horses: A retrospective study (2002-2017).

Sánchez M. J., Gómez M. D., Molina A., Valera M. (2013). Genetic analyses for linear conformation traits in Pura Raza Español horses. Livestock Science. 157(1): 57–64.

Weeren P. R., Crevier-Denoix, N. (2006). Equine conformation: clues to performance and soundness? Equine Veterinary Journal. 38(7): 591–596.

Weller R., Pfau T., Verheyen K., May S. A., Wilson A. M. (2006). The effect of conformation on orthopaedic health and performance in a cohort of National Hunt racehorses: preliminary results. Equine Veterinary Journal. 38(7): 622–627.

https://inside.fei.org/sites/default/files/FEI_Dressage_Rules_2022_Clean_Version_V2.pdf

The Impact of Conformation on the Dressage Horse Performance

Abstract
Equestrian sports industry has been registering an increase in demand and expansion of the market for high-value, elite dressage horses . Dressage riding is an Olympic sport for which elite horses are used. The particularities of a breeding and sport type are the result of intensive breeding and selection work, based upon various scientific knowledge: genetics, biomechanics, and horse conformation. Conformation influences the movements and performance. Scientists are develop quantitative analyses to improve performance while selecting favorable morphological characteristics of dressage horses. The present-day knowledge about conformation and heritability of conformation traits are useful for breeding and selection work and for individualization of sport horse training.

Keywords: conformation, performance, dressage riding

IZVORNI ZNANSTVENI RAD

Dobrobit životinja u uzgoju i njezin utjecaj na kupce životinjskih proizvoda

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Sažetak

Važnost utjecaja poljoprivredne prakse na dobrobit životinja te njezin povezani utjecaj na javno zdravlje i zdravlje životinja, u novije vrijeme sve je bitniji čimbenik na koji kupci obraćaju pozornost prilikom kupovine proizvoda animalnog podrijetla. Ipak stavovi potrošača prema dobrobiti životinja na farmama nisu ujednačeni, oni podrazumijevaju različite stavove i društvene dimenzije vezane uz etničku pripadnost, poljoprivredno-prehrambenu kulturu, etiku, kupovnu moć i vjerovanja. Stoga je ovaj rad imao za cilj pokazati stavove hrvatskih kupaca, prema tome koliko im je bitan utjecaj dobrobiti životinja u uzgoju prilikom kupovine proizvoda animalnog podrijetla. Za prikupljanje podataka korištena je metoda ankete, a kao instrument korišten je anketni upitnik (n = 100). Ispitivanjem su utvrđene statistički značajne razlike u stavovima ispitanika vezanim za dobrobit životinja prema spolu, obrazovanju i prihodu ispitanika te da su kupci animalnih proizvoda zabrinuti za dobrobit životinja na farmama.

Ključne riječi: dobrobit, životinje, animalni proizvodi, potrošači, kupci

Uvod

Dobrobit životinja je pojam koji se koristi za iskazivanje etičke zabrinutosti u vezi s kvalitetom življenja koju životinje imaju, osobito životinje koje se koriste u proizvodnji poljoprivrednih proizvoda (Hansen i Osteras 2019.; Fraser i Weary, 2004.). Krajem 20. stoljeća došlo je do velikih promjena u stočarskoj proizvodnji (Fraser, 2008.). Broj životinja na farmama znatno se povećavao, farme su postale visoko specijalizirane, a proizvodnja se intenzivirala (Porcher, 2001.). Stočarska proizvodnja postala je visoko industrijalizirana, posljedično čega se kvantiteta stavila ispred kvalitete, što je utjecalo na kvalitetu života životinja na farmama. Kroz godine, kulturološki i komercijalni stavovi ometali su konstruktivnu komunikaciju između poljoprivrednika i kupaca, ali s vremenom je došlo do buđenja svijesti i danas je dobrobit životinja važno pitanje za kupce diljem Europe. Zabrinutost javnosti prema dobrobiti životinja raste (Bennet i Blaney 2003.; Mc Eachern i sur., 2007.), a istraživanja pokazuju kako su pozitivni stavovi povezani s višom razinom zabrinutosti i empatije za dobrobit životinja (Mc Kendree i sur. 2014.; Tonsor i Olynk 2011.). Postoji jasna potražnja kupaca u Europskoj Uniji za višim standardima dobrobiti kod domaćih životinja (Kjaernes i Lavik, 2008.) i o informacijama o istoj. Utjecaj poljoprivredne prakse na dobrobit životinja i njezin povezani učinak na javno zdravlje i zdravlje životinja pitanja su kojima građani EU-a pridaju sve veću pozornost. Zbog toga proizvođači i trgovci prepoznavši ove potrebe potrošača, dobrobit životinja koriste i kao sastavni dio strategije izdvajanja takvih proizvoda i stvaranja prednosti s njima na tržištu (Miele i sur., 2005.). Potražnja za informacijama o dobrobiti životinja prilikom kupovine životinjskih proizvoda ipak varira od zemlje do zemlje i čini se da često odražava samo opći interes, ali ne i onaj koji utječe na samu kupnju (Kjaernes i Lavik, 2008.). Po pitanju dobrobiti životinja,

Europska Unija u posljednja tri desetljeća kontinuirano unaprjeđuje zakonske propise, upravo s naglaskom na zaštiti životinja koje se uzgajaju za proizvodnju hrane (Pavičić i Ostović, 2013.). Europska Unija ima neke od najviših standarda dobrobiti životinja na svijetu, koji uključuju opće zahtjeve u pogledu uzgoja, prijevoza i klanja domaćih životinja te posebne zahtjeve za određene vrste.

Materijal i metode

Primarni podatci prikupljeni su putem metode ankete, a kao instrument istraživanja korišten je online anketni upitnik (n = 100). Ciljana skupina ispitanika bili su ispitanici s područja Republike Hrvatske koji konzumiraju meso, jaja i barem djelomično su odgovorni za kupnju prehrambenih proizvoda u domaćinstvu. Istraživanje o stavovima potrošača prema dobrobiti životinja koje se koriste za proizvodnju hrane provedeno je u razdoblju od 1. siječnja do 20. veljače 2022. putem online anketnog softvera tvrtke Qualtrics LLC. Cjelokupnim anketnim upitnikom istražuju se potrošačke preference prema mesu i jajima te stavovi ispitanika prema dobrobiti životinja, upitnik sadrži ukupno 63 pitanja otvorenog i zatvorenog tipa podijeljenih u nekoliko skupina, a na kraju anketnog upitnika dodana su pitanja koja se odnose na sociodemografska obilježja ispitanika. Zbog velike opširnosti anketnog upitnika za potrebe ovog rada prikazani su samo dijelovi vezani za potrošnju mesa i jaja, pitanja vezana za stavove ispitanika o dobrobiti životinja (mjereno korištenjem Likertove ljestvice od 5 stupnjeva: 1 – uopće se ne slažem, 5 – u potpunosti se slažem) i sociodemografska obilježja (spol, zanimanje i prihod). Anketnim istraživanjem prikupljeno je ukupno 120 odgovora, a od ukupnog broja ispitanika iz daljnje analize isključeno je 10 ispitanika zbog nepotpuno ispunjenog anketnog upitnika te 10 ispitanika nije odgovaralo ciljanoj skupini. Analiza podataka provedena je u statističkom programskom paketu IBM SPSS Statistic V26. U analizi podataka korištena je deskriptivna statistika (postotci, frekvencija, aritmetička sredina, standardna devijacija) kako bi se opisao uzorak, a od parametrijskih testova korišten je Studentov t – test (independent t- test) i jednosmjerna analiza varijance (oneway ANOVA) u svrhu utvrđivanja razlika u stavovima ispitanika vezanim za dobrobit životinja.

Rezultati i rasprava

Od ukupnog broja ispitanika (n = 100) 65,0 % su bile žene, a 35,0 % muškarci. Najveći broj ispitanika (34,0 %) bio je u rasponu godina od 18 do 24 godine, zatim, 26,0 % ispitanika je 35 – 44 godine starosti. Najveći broj ispitanika kao mjesto stanovanja navodi grad (68,0 %) zatim njih 19,0 % selo i 13,0 % prigradsko naselje. U istraživanju su većinom sudjelovali zaposleni (59,0 %) ispitanici i studenti (41,0 %) s mjesečnim prihodom kućanstva većim od 13.000,00 kn (42,0 %). Najveći broj ispitanika (66,0 %) izjasnio se da živi u kućanstvu s 3-5 članova obitelji bez djece mlađe od 15 godina starosti (61,0 %) te njih 66,0 % ima kućnog ljubimca. Na pitanja vezana za konzumaciju mesa i jaja ispitanici su se izjasnili na sljedeći način. Najveći broj ispitanika konzumira meso četiri do šest puta tjedno (48,0 %), a meso većinom kupuju u mesnici (47,0 %) ili u supermarketu (33,0 %). Za uobičajene potrebe svoje obitelji većinom kupuju 0 – 1 kg mesa (71,0 %) te velika većina ispitanika nije smanjila konzumaciju mesa u prethodnim godinama (75,0 %) i ne planira je smanjiti u budućnosti (74,0 %). Na pitanja vezana za konzumaciju jaja ispitanici su se izjasnili da jaja konzumiraju u najvećoj mjeri dva do tri puta tjedno (36,0 %), 89,0 % ispitanika na mjesečnoj bazi kupuje od 10 do 50 komada jaja u najvećoj mjeri u supermarketu (45,0 %) i direktno od proizvođača (23,0 %). Istraživanja u Kanadi pokazuju kako su na preferencije potrošača prilikom kupnje jaja utjecali između ostalog i demografski pokazatelji. Došli su do spoznaje da kupci nižeg stupnja obrazovanja, stariji te kupci niže platežne moći kupuju pretežito u velikim trgovačkim lancima, a kupci koji su više iskazali zabrinutost za dobrobit životinja, su kupovali jaja iz slobodnog sustava i manje ih je brinula cijena proizvoda te su oni

prvenstveno kupovali na poljoprivrednim tržnicama i kod lokalnih prodavača i proizvođača ekoloških proizvoda (Bejaei i sur. 2015.). Kao i kod mesa velika većina ispitanika nije smanjila konzumaciju jaja (81,0 %) niti planira isto u budućem razdoblju (89,0 %). Potrošači su zatim izrazili svoje mišljenje na sljedećih deset tvrdnji: životinjama na farmama je bolje nego u prirodi i nije bitan način držanja životinja na farmama jer one ne znaju za bolje (T1), ne osjećam se krivim kada jedem meso i jaja (T2), većina poljoprivrednih proizvođača tretira životinje s poštovanjem (T3), životinjama je potrebno omogućiti što više prostora i poboljšati uvjete držanja na farmama (T4), smatram da je klasični način uzgoja (intenzivni u zatvorenome) okrutan (T5), rezanje repa kod svinja i vrha kljuna kod kokoši je okrutno (T6), životinje na farmama trebaju imati mogućnost izražavanja vrsti svojstvenog ponašanja (T7), prilikom transporta životinja potrebno je smanjiti vrijeme transporta i omogućiti im više prostora (T8), smatram da je važno znati mjesto i način proizvodnje mesa i jaja (T9), poštivanje pravila i zakona o zaštiti životinja na farmama trebalo bi strože kontrolirati (T10). Na temelju odgovora ispitanika izračunate su aritmetičke sredine (M) i standardna devijacija (SD). Ispitivani potrošači su se u najvećoj mjeri složili s tvrdnjama prilikom transporta životinja potrebno je smanjiti vrijeme transporta i omogućiti životinjama više prostora (M = 4,36, SD = 0,759), životinjama je potrebno omogućiti što više prostora i poboljšati uvijete držanja na farmama (M = 4,26, SD = 0,774), smatram da je važno znati mjesto i način proizvodnje mesa i jaja (M = 4,26, SD = 0,872) i poštivanje pravila i zakona o zaštiti životinja na farmama trebalo bi strože kontrolirati (M = 4,25, SD = 0,857). Najmanje slaganje ispitanici su izrazili prema tvrdnjama životinjama na farmama je bolje nego u prirodi i nije bitan način držanja životinja na farmama jer one ne znaju za bolje (M = 2,19, SD = 0,992) i većina poljoprivrednih proizvođača tretira životinje s poštovanjem (M = 3,09, SD = 0,854).

Tablica 1. Testiranje razlika aritmetičkih sredina tvrdnji s obzirom na spol ispitanika

Tvrdnje	Muško		Žensko		t-test	P
	M	SD	M	SD		
T(1)	2,29	0,893	2,14	1,044	0,706	0,482
T(2)	3,83	1,150	3,82	0,950	0,061	0,951
T(3)	3,11	0,900	3,08	0,835	0,208	0,836
T(4)	4,09	0,951	4,35	0,648	-1,668	0,098
T(5)	3,46	1,094	3,98	0,944	-2,520	0,013**
T(6)	3,63	1,140	4,23	0,948	-2,819	0,006**
T(7)	3,86	1,089	4,42	0,659	-3,195	0,002**
T(8)	4,29	0,860	4,40	0,703	-,716	0,475
T(9)	4,00	1,085	4,40	0,703	-2,232	0,028*
T(10)	3,97	0,954	4,40	0,766	-2,444	-2,444

** P<0,01; * P<0,05

Prema rezultatima t-testa prikazanima u Tablici 1. vidljivo je da postoji statistički značajna veza između ponuđenih tvrdnji i spola ispitanika. Statistički značajna razlika uočena je kod tvrdnji da je životinjama potrebno omogućiti što više prostora i poboljšati uvjete držanja na farmama (t = -2,520, P<0,01), rezanje repa kod svinja i vrha kljuna kod kokoši je okrutno (t = -2,819, P<0,01), životinje na farmama trebaju imati mogućnost izražavanja vrsti svojstvenog ponašanja (t = -3,195, P<0,01) i smatram da je važno znati mjesto i način proizvodnje mesa i jaja (t = -2,232, P<0,05). Ostale tvrdnje nisu se pokazale statistički značajne u odnosu na ponuđene tvrdnje. Prema srednjim vrijednostima prikazanim u Tablici 1. vidljivo je da se ispitanici ženskog spola više slažu s navedenim tvrdnjama te da više obraćaju pažnju na dobrobit životinja od muških ispitanika. Da je spol dosljedan prediktor

brige za životinje te da one pokazuju više zabrinutosti za životinje navode u svojim istraživanjima i Clark i sur. (2016.); Albert i sur., (2020); Estevet-Moreno i sur. (2021.). Neke studije pretpostavljaju da je tome tako, ne zbog samog spola koliko zbog ženske orijentacije prema spolnoj ulozi Herzog i sur. (1991.); Peek i sur. (1997.). Prema podatcima u Tablici 2. vidljivo je da postoji statistički značajna veza između tvrdnji ne osjećam se krivim kada jedem meso i jaja (F = 6,439, P<0,01) većina poljoprivrednih proizvođača tretira životinje s poštovanjem (F = 4,429, P<0,05), životinjama je potrebno omogućiti što više prostora i poboljšati uvijete držanja na farmama (F = 6,353, P < 0,01) i životinje na farmama trebaju imati mogućnost izražavanja vrsti svojstvenog ponašanja (F = 4,548, P<0,05) u odnosu na zanimanje ispitanika. Ispitanici koji su se izjasnili kao zaposleni više se slažu se tvrdnjom većina poljoprivrednih proizvođača tretira životinje s poštovanjem i potrebno je smanjiti vrijeme transporta i omogućiti im više prostora (M = 4,41, SD = 0,746) od studenata. Neka istraživanja upućuju da su ovakve razlike u mišljenjima u biti zbog dobi, tj. zaposlena populacija je najčešće i starije dobi od studenata, a kod starije populacije postoji taj kohortni utjecaj zajedničke povijesti (Kendal i sur., 2007.), dok Baron-Cohen (2003.) implicira da je to zbog mentalnih atributa koji kod ljudi s godinama postaju složeniji.

Tablica 2. Testiranje razlika aritmetičkih sredina tvrdnji s obzirom na zanimanje potrošača

Tvrdnje	Student		Zaposlen		F	P
	M	SD	M	SD		
T(1)	2,24	1,113	2,15	0,906	0,204	0,653
T(2)	4,12	0,872	3,61	1,067	6,439	0,013**
T(3)	2,88	0,927	3,24	0,773	4,429	0,038*
T(4)	4,49	0,553	4,10	0,865	6,353	0,013**
T(5)	3,98	0,987	3,68	1,041	2,062	0,154
T(6)	4,15	1,014	3,93	1,081	0,999	0,320
T(7)	4,44	0,673	4,07	0,962	4,548	0,035*
T(8)	4,29	0,782	4,41	0,746	0,544	0,463
T(9)	4,41	0,741	4,15	0,943	2,213	0,140
T(10)	4,32	0,789	4,20	0,906	0,423	0,517

** P<0,01; * P<0,05

U tablici 3. prikazani su rezultati testiranja aritmetičkih sredina tvrdnji s obzirom na mjesečni prihod obitelji te je vidljivo da prihod obitelji ima utjecaj na mišljenje ispitanika o dobrobiti životinja po pojedinim tvrdnjama. Statistički značajna razlika uočena je kod tvrdnji životinjama na farmama je bolje nego u prirodi i nije bitan način držanja životinja na farmama jer one ne znaju za bolje (F = 3,257, P<0,05), većina poljoprivrednih proizvođača tretira životinje s poštovanjem (F = 4,674, P<0,01), smatram da je klasični način uzgoja (intenzivni u zatvorenome) okrutan (F = 3,350, P<0,01) i prilikom transporta životinja potrebno je smanjiti vrijeme transporta i omogućiti im više prostora (F = 3,007, P<0,05). Ispitanici s većim prihodom (13000,00 kn) i manjim (6999,99 – 10999,99 kn) u većoj mjeri se slažu s tvrdnjama da je bitno znati mjesto i način proizvodnje mesa i jaja, da je životinje bitno uzgajati prema većim standardima dobrobiti te da se propisi i zakoni o zaštiti životinja na farmama trebaju strože kontrolirati. Za razliku od ispitanika s prihodom (11000,00 – 12999,99 kn) koji se više slažu s tvrdnjama da je životinjama bolje na farmama nego u prirodi te da ne osjećaju krivnju kada jedu meso i jaja. Neka istraživanja upućuju na to da ljudi s nižim stupnjem obrazovanja (nižim primanjima), imaju i nižu razinu svijesti i brige o dobrobiti životinja (Clark i sur., 2016.; Estevez-Moreno i sur., 2021.; Miranda-de-la-Lama i sur. i 2019.).

Tablica 3. Testiranje razlika aritmetičkih sredina tvrdnji s obzirom na mjesečni prihod obitelji

	< 6.999,99		7.000,00–8.999,99		9.000,00 – 10.999,99		11.000,00 – 12.999,99		> 13.000,00		F	P
	M	SD	M	SD	M	SD	M	SD	M	SD		
T(1)	1,74	0,806	2,13	0,957	2,53	1,179	3,17	0,983	2,14	0,899	3,257	0,015*
T(2)	3,63	0,831	3,75	1,065	4,00	1,275	4,50	0,548	3,76	1,008	1,017	0,403
T(3)	2,68	0,749	2,63	1,025	3,24	0,752	3,83	0,983	3,29	0,708	4,674	0,002**
T(4)	4,63	0,597	4,25	1,065	4,12	0,857	4,17	0,408	4,17	0,696	1,440	0,227
T(5)	4,26	0,733	3,94	1,124	3,59	1,064	2,67	1,211	3,79	0,951	3,350	0,013**
T(6)	4,42	0,838	3,88	1,258	4,00	1,000	3,33	1,366	4,00	1,012	1,430	0,230
T(7)	4,26	1,046	4,06	1,124	4,06	0,748	4,00	0,894	4,36	0,727	0,634	0,639
T(8)	4,47	0,697	4,00	1,155	4,06	0,659	4,17	0,753	4,60	0,544	3,007	0,022*
T(9)	4,26	1,046	4,19	1,047	4,18	0,636	3,67	1,033	4,40	0,767	1,053	0,384
T(10)	4,32	0,946	4,25	1,125	4,00	0,791	4,17	0,753	4,33	0,754	0,493	0,741

** P<0,01; * P<0,05

Zaključak

Prema rezultatima ovog istraživanja, možemo zaključiti da potrošači nisu smanjili količinu konzumacije mesa i da je ne planiraju smanjiti u budućnosti, te da je konzumacija jaja 2-3 puta tjedno. Smatraju da je potrebno smanjiti vrijeme trajanja transporta životinja i omogućiti im više prostora te poboljšati uvijete držanja na farmama. Također, može se zaključiti kako je potrošačima bitno znati mjesto i vrijeme proizvodnje animalnih proizvoda koje konzumiraju te da smatraju kako bi poštivanje pravila i zakona o zaštiti životinja na farmama trebalo strože kontrolirati. Stoga, može se zaključiti kako je potrošačima stalo do dobrobiti životinja u uzgoju prilikom kupovine proizvoda animalnog podrijetla. Rezultati provedenog istraživanja od velike važnosti su proizvođačima kako bi proizveli proizvode koje su potrošači spremni platiti, jer u današnje vrijeme nije bitno samo proizvesti što veće količine, nego zadovoljiti specifične potrebe i preferencije potrošača. Kao i nositeljima gospodarske politike prilikom kreiranja novih zakona i uvođenju oznaka na proizvodima o dobrobiti životinja te marketing stručnjacima u svrhu kreiranja i provođenja promotivnih aktivnosti.

Literatura

Albert B., Kota M., Boaitey A., Minegishi K. (2020). Who are farm animal welfare conscious consumers? British Food Journal. 122(12): 3779-3796.

Baron-Cohen S. (2003). The essentional Difference: Men, Women and the Extreme Male Brain; Allen Lane: London, UK.

Bejaei M., Wiseman K., Cheng K. M. (2015). Developing logistic regression models using purcase attributes and demographics to predict the probabolity of purchases of regular and speciality eggs. British Poultry Science. 56: 426.-435.

Bennett R. M., Blaney R. J., (2003) Estimating the benefits of farm animal legislation using the contigent valuation method. Agricultural Economics. 29: 85-98.

Clark B., Stewart G. B., Panzone I. A., Kyriazakis I., Frewer I. J. (2016). A systematic review of public attitudes, perceptions and behaviours towards production diseases associated with farm animal welfare. Journal of Agricultural and Enviromental Ethics. 29(3): 455-478.

Estèvez-Moreno L. X., Marìa G. A., Sepúlveda W. S., Villarroel M., Miranda-de-la Lama G. C. (2021). Attitudes of meat consumers in Mexico ans Spain about farm animal welfare: A cross-cultural study. Meat Science. 173: Article 108377.

Fraser D., Weary D. M. (2004). Quality of life for farms animals: linking science, ethics, and animal welfare. In: Appleby, M. C., Hughes, B. O. (Eds.), The well-Being of farm Animals: Challenges and Solutions. Blackwell, Ames, Iowa, pp. 39-60.
Fraser D. (2008). Understanding animal welfare: The science in its cultural contex. Oxford: Wiley. Blackwell, 324 pp.
Hansen B. G., Østeras O. (2019). Farmer welfare and animal welfare-Exploring the relationship between farmer`s occupational well-being and stress, farm expansion and animal welfare. Preventive Veterinary Medicine. 170: 104741.
Herzog H. A., Betchart N. S., Pittman R. B. (1991). Gender, seks role orientation, and attitudes toward animals. Anthrozoös. 4: 184-191.
Kjaernes U., Lavik R. (2008). Opinions on animal welfare and food consuption in seven European countries. In: U. Kjaernes, U., Bock, B., Roe, E., Roex, J. (eds.) Consuption, distribution and production of farm animal welfare-opinions and practies within the supply chain, Welfare Quality® Reports No. 7, pp. 1-126. Cardiff: University of Cardiff.
Mc Eachern M. G., Schröder M. J., Willock J., Mason R. (2007). Exploring ethical brand extensions and consumer buying behaviour: The RSPCA and the „Freedom Food" brand. 16: 168-177.
Miele M., Murdoch J., Roe E. (2005). Animals and ambivalence, governing farm animal welfare in the European food sector. In: Higgins, V., Lawrence, G. (eds.) Agricultural Governance, Routledge, London, pp. 169-185.
Miranda-de-la Lama, G. C., Estèvez-Moreno, L. X., Villarroel, M., Rayas-Amor, A. A., Maria, G. A., Sepúlveda, W. S. (2019). Consumer attitudes toward animal welfare-friendly products and willingness to pay: Exploration of Mexican market segments. Journal of Applied Animal Weffare Science. 22(1): 13-25.
Pavičić, Ž., Ostović, M. (2013): Dobrobit farmskih životinja. Hrvatski veterinarski vjesnik. 21: 7-8.
Peek C. W., Dunham C. C., Dietz B. E. (1997). Gender relational role orientation, and affinity for animal rights. Seks Roles. 37: 905-920.
Porcher J. (2001): Le travail dans l`èlevage industriel des porcs. Souffrance des animaux, souffrance des hommes. In: Burgat, F., Dantzer, R. (eds.) Un point … Les aniumaux d`èlevage ont-ils droit au bienètre? (Labour in industrial pig farming: animal suffering human suffering. In Are Farm Animal Entitled to their Well-being?) pp. 23-64. Paris: INRA Editions.
SPSS (2019). IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.
Tonsor G. T., Olynk N. J. (2011). Impact of Animal Well-Being and Welfare Media on Meat Demand. Journal of Agricultural Economics. 62: 59-72.
Qualtrics (2021). Qualtrics LLC. Released 2005. Provo, Utah, USA. Version 20/4/2021-19/4/2022.

Animal welfare in breeding and its impact on buyers of animal products

Abstract
The importance of the agricultural practices impact on animal welfare and its associated impact on public and animal health has recently become an increasingly important factor that customers pay attention to when buying products of animal origin. However, consumer attitudes towards animal welfare on farms are not uniform, they imply different attitudes and social dimensions related to ethnicity, agri-food culture, ethics, purchasing power and beliefs. Therefore, the aim of this paper was to show the attitudes of Croatian consumers, according to how important the impact of animal welfare in breeding when buying products of animal origin.

Key words: welfare, animals, animal products, consumers, customers

PREGLEDNI RAD

Od ishodišnih oblika domaćih životinja do suvremenih pasmina

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Sažetak
Od početka domestikacije, od divljih vrsta do nastanka lokalnih te suvremenih pasmina, odvili su se su brojni i složeni procesi. To se najbolje pokazuje promjenom životinjskih vrsta i ogromnom genetskom i fenotipskom raznolikošću. Prirodna selekcija je proces pod utjecajem prirodnih zakonitosti, gdje okolina potiče odabir jedinki. Za razliku od prirodne selekcije, uzgajivač u populacijama domaćih životinja planski odabire najbolje životinje za daljnju reprodukciju. Primjenom genomske selekcije u posljednjih 10 godina olakšan je izbor najboljih životinja za reprodukciju. Kao rezultat, danas je u svijetu poznato nekoliko tisuća pasmina, koje je zbog očuvanja biraznolikosti i klimatskih promjena, neophodno očuvati.

Ključne riječi: domestikacija, pasmine, genetska raznolikost, genetski markeri, genomika

Uvod

Čovjek je počeo stvarati pasmine koristeći principe umjetne selekcije prije približno 250 godina (Olenbroek i van der Waaij, 2015.). Kao rezultat, danas postoji nekoliko tisuća pasmina domesticiranih životinja, u koje ubrajamo pasmine goveda, svinja, ovaca, koza i kokoši. U današnje vrijeme, uzgoj visoko produktivnih životinja, primjerice svinja i kokoši u rukama je multinacionalnih kompanija koje ulažu velika financijska sredstva u njihove uzgojne programe. Ono što je zajedničko prvotnim praksama u uzgoju domaćih životinja prije nekoliko stotina godina i današnjim suvremenim uzgojnim programima je uzgojni cilj, odnosno selekcijom unaprijediti važna proizvodna svojstva, poput količine mlijeka, količine mesa, nesivosti itd. Te osobine definirane su uvjetima i željama ljudskih zajednica, a često su se mijenjale kroz vrijeme.

Domestikacija divljih vrsta

Pripitomljavanje brojnih vrsta biljaka i životinja na odvojenim lokacijama svijeta, započelo je prije približno 12000 godina o čemu osim genetskih istraživanja, postoji i puno arheoloških istraživanja. O tome najbolje govore navodi povjesničara o neolitskoj te narednim revolucijama. Domestikacija podrazumijeva proces pretvorbe divljih, te nakon procesa pripitomljavanja u domaće životinje. Prva domesticirana životinja bio je pas i to prije 12000 godina (Olenbroek i van der Waaij, 2015.). Nakon psa domesticirane su ovce, koze, svinje i goveda, dok su konji i kokoši domesticirani kasnije. Složenim procesima kroz dugo vremensko razdoblje od više tisuća godina, uzgojem domaćih životinja stvoreni su različiti fenotipovi i pasmine. U današnje vrijeme kada su genomske sekvence domaćih životinja postale dostupne i omogućile identifikacije mutacija, stvorena je podloga za egzaktno utvrđivanje pretvorbi divljih u domaće vrste životinja.

Uzgajane vrste i pasmine

Prema Loftusu i sur. (1994.) postoje dvije podvrste goveda: *Bos taurus* (europska ili taurina goveda) i *Bos indicus* (azijska ili zebu goveda). Njihov zajednički predak bio je *Bos*

primigenius Bojanus od kojeg su u dva, ili čak tri (Pitt i sur., 2017.) neovisna događaja razdvojile podvrste taurus i indicus. Razlikujemo 1019 pasmina goveda u svijetu (FAO, 2015.). Prema FAO bazi podataka (Domestic Animal Diversity Information System (DAD-IS), u Hrvatskoj su najzastupljenije simentalska (procijenjena veličina populacije u svijetu je 2.718.236), holštajn-frizijska (procijenjena veličina populacije u svijetu je 23398559) i smeđa pasmina (procijenjena veličina populacije u svijetu je 1000212). Prema najnovijim spoznajama Frantza i sur. (2019.) o porijeklu svinje (*Sus scrofa*) prihvaćeno je da su u Europu prve domesticirane svinje uvezene iz Bliskog istoka dolaskom prvih stočara početkom neolitika prije ~10500 godina, te da je Europska domaća svinja potomak ovih Azijskih domesticiranih svinja i Europskih divljih svinja. Danas je poznato 543 pasmina svinja u svijetu (FAO, 2015.), dok je proizvodnja organizirana na svega nekoliko najutjecajnijih suvremenih pasmina i njihovih hibrida, a to su prema FAO bazi podataka (Transboundary breed summary. Domestic Animal Diversity Information System (DAD-IS). Food and Agriculture Organization of the United Nations): veliki jorkšir (procijenjena veličina populacije je 458646), landras (procijenjena veličina populacije je 252551), pietren (procijenjena veličina populacije je 23116), durok (procijenjena veličina populacije je 160109) i hempšir (procijenjena veličina populacije zajedno sa populacijom američkog hempšira je 27319).

Kokoš (*Gallus domestica*) nastala je od svog ishodišnog oblika divlje kokoši (*Gallus gallus*) (Linnaeus, 1758.) na području Azije. Prema FAO izvješću (2015.) postoji 1514 pasmina kokoši koje se dijele na: ukrasne i patuljaste pasmine, borce, lake pasmine, teške pasmine i kombinirane. Danas se suvremena peradarska proizvodnja temelji na proizvodnji hibrida.

Prema Larsonu i Fulleru (2014.) ishodišni oblik današnje ovce (*Ovies aries*), je Azijski muflon (*Ovis musimon*) čija je domestikacija započela u jugozapadnoj Aziji prije otprilike 11000 godina. Danas postoji 1155 pasmina ovaca (FAO, 2015.), a u Hrvatskoj se uzgaja 15 pasmina, što izvornih što uvoznih.Koza (*Capra hircus*) potječe od bezoar koze *(Capra aegagrus)* (Gootwine, 2020.), danas je u svijetu poznato 576 različitih pasmina koza (FAO, 2015.) koje se dijele prema tipovima i pasminskim skupinama.S obzirom da je divlji ishodišni predak konja izumro, pretpostavlja se da su današnji konji nastali od divljih konja na području današnje Ukrajine i Kazahstana (Librado, 2016.). Danas u svijetu postoji 694 pasmina konja (FAO, 2015.), koje se dijele u čistokrvne, križance, hladnokrvne i toplokrvne. Tijekom povijesti se uloga konja značajno promijenila. U prošlosti je konj služio kao radna životinja i kao takav bio je neophodan za život. Danas se konji koriste za sport i rekreaciju, dok se posjedovanje ove životinje smatra luksuzom.

Poljoprivredne revolucije i začeci kontroliranog uzgoja

Domestikacija domaćih životinja započela je u fazi Neolitske ili prve revolucije na Bliskom istoku, prije otprilike 12000 godina (Hole, 1984.). Neolitsku revoluciju obilježava promjena načina života iz lovačkog i sakupljačkog u početak bavljenja poljoprivredom, gradnja trajnih naselja, razvoj miješane ekonomije, domestikacija biljaka i životinja, rast populacije te kolonijalizam. U interesu čovjeka, od domesticiranih životinja na prvom su mjestu bile koze, ovce i goveda i to zbog njihovih kombiniranih proizvodnih osobina (mlijeko i meso). Završetak neolitske revolucije označava nastanak sumeranskih gradova i pojava prvog pisma iz razdoblja 3500 god. pr. Kr. Druga poljoprivredna revolucija u kojoj dolazi do značajnih promjena u uzgoju domaćih životinja je Britanska revolucija u vremenskom razdoblju između sredine 17. i kraja 19. stoljeća. Značajne promjene u stočarstvu nastaju sredinom 18. stoljeća kada Robert Bakewell i Thomas Coke uvode selekciju i planski uzgoj kao znanstveno-stručne discipline. Njihov rad se temeljio na korištenju inbridinga u svrhu ujednačavanja pojedinih svojstava i kvalitete te smanjenja genetske raznolikosti. Robert Bakewell stvorio je okvir za suvremeni model uzgoja životinja, te se smatra utemeljiteljem

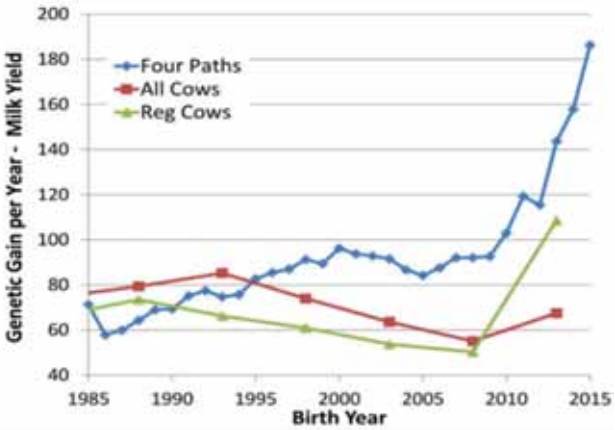
planskog uzgoja životinja (Lush, 1943.). Tijekom Britanske revolucije počinje rasti potreba za praćenjem uzgoja životinja, te je u to vrijeme nastala i prva matična knjiga. Nakon prve matične knjige koja se odnosila na konje počinju se razvijati i za ostale vrste i pasmine koje su imale utjecaja na nastanak suvremenih matičnih knjiga. Treća poljoprivredna ili Zelena revolucija trajala je između 1950. i 1960. godina prošlog stoljeća. Zelenu revoluciju karakterizira pojava kemijskih gnojiva, agrokemikalija, navodnjavanja, te novije metode kultiviranja uključujući mehanizaciju. Ključni elementi koje ova revolucija uključuje su: korištenje najnovije tehnologije i kapitalnih ulaganja u farmski uzgoj životinja i mehanizaciju, korištenje modernih znanstvenih metoda u poljoprivredi, korištenje visokoprinosnih sorti i pasmina kao i okrupnjavanje zemljišnih posjeda. Zelena revolucija specifično se odnosi na povećanje prinosa nekoliko vrsta žitarica razvijenih 1960. godine zajedno s korištenjem pesticida i gnojiva te označava početak revolucije proizvodnje hrane. Paralelno s razvojem sorti razvijaju se i visokoproizvodne pasmine životinja. Korištenjem znanja iz znanstvenih istraživanja, stvoreni su neophodni temelji za napredak stočarstva te samim time dolazi i do razvoja u područjima genetike i selekcije najboljih rasplodnjaka i plotkinja, njihovoj reprodukciji i dijagnosticiranju bolesti potrebnih za povećanje produktivnosti te hranidbi životinja.

Prvi uzgojni programi i registri

Korištenje rodovnika ili pedigrea u uzgoju započelo je u ruralnom dijelu Engleske, krajem 18. stoljeća, dok su se općenito evidencije i registri pasmina razvili tek sredinom 19. stoljeća (Lush, 1943.) Lokalnim uzgajivačima, privatni zapisi o uzgoju bili su dovoljni i s tim su mogli zadovoljiti ondašnje potrebe tržišta, dok je genetski napredak bio neznatan. S vremenom je rasla potreba za osnivanjem evidencije u svrhu praćenja rodovnika i sigurnosti prilikom kupovine i prodaje životinja. Prva matična knjiga bila je „*An Introduction to the General Stud Book*“ James Weatherbya za punokrvne konje 1791. godine, u kojoj su zabilježeni rodovnici konja koji su bili pobjednici važnih utrka (Lush, 1943.), te je osim za uzgojne potrebe, knjiga koristila za vlasničke odnose i promet životinja. Matična knjiga za Shorthorn govedo, nastala 1822. godine oblikovana je kao primjer suvremenih matičnih knjiga, u koje su bili upisani rodovnici svih životinja uzgojenih u čistoj pasmini. Prve matične knjige sadržavale su samo rodoslovlje i službene odredbe usmjerene na uzgoj u čistoj pasmini, dok se s vremenom matične knjige proširuju upisivanjem karakteristika životinje, produktivnosti, konstitucije, reproduktivne sposobnosti i životnog vijeka. Prve matične knjige sadržavale su poprilično puno grešaka, no s vremenom su se unaprjeđivale i grešaka je bilo sve manje i manje. Uzgojna udruženja upisivala su jedinku po rođenju, dok su se za života i nakon smrti upisivali podaci o životnim performansama, osvojenim nagradama, ocjenama jedinke itd.

Primjena genomskih informacija u uzgoju

Do početka 21. stoljeća, odabir elitnih jedinki odnosno roditelja budućih generacija, rutinski se provodio primjenom podataka iz pedigrea te korištenjem podataka o proizvodnji. Razvojem NGS (engl. *Next generation sequencing*) tehnika sekvenciranja i genotipiziranja s polimorfizmima jednog nukleotida ili SNP (engl. *Single nucleotide polymorphisms*) 2007. godine, razvijen je novi pristup koji se naziva genomska selekcija. Samo dvije godine poslije, 2009. u nekim se zemljama ovaj pristup počinje koristiti kod pojedinih pasmina. U odnosu na klasični, ovim pristupom postiže se znatno veći učinak na povećanje stope genetskog napretka (Garcia-Ruiz i sur., 2016.) te značajno smanjenje generacijskih intervala (Grafikon 1.). Također, omogućava točnije procjene uzgojnih vrijednosti u odnosu na ranije korištene uzgojne modele te omogućuje veću pouzdanost u selekciji.



Grafikon 1: Genetski napredak za mliječnost prije i poslije primjene genomske selekcije (Garcia-Ruiz i sur., 2016.)

U programima genomske selekcije, potrebno je razviti tzv. referentnu populaciju, koju zapravo čini populacija s mnogo (najčešće i tisućama) životinja s prikupljenim fenotipovima i SNP genotipovima (Van der Werf, 2013.). Zatim se s ovim informacijama razvije prediktivna jednadžba, pomoću kojih se procjenjuju performanse životinja ili budućih elitnih roditelja samo na temelju genotipova. Na ovaj se način za neka svojstva genetski napredak uvećao za čak 3-4 puta.Međutim, unaprjeđenje uzgoja životinja i dalje se vrlo brzo razvija. Veliki izazovi i dalje postoje u kontekstu udruživanja uzgojnih organizacija te razmjene genetskih i fenotipskih informacija, primjerice u slučaju mliječnog govedarstva. U uzgoju domaćih životinja se u vrlo skoroj budućnosti kao nadogradnju genomskoj selekciji očekuju i velike inovacije u području automatizacije i prikupljanja odlika fenotipova koristeći digitalne precizne tehnologije, koje pak uključuju bežične senzore (mjerenje otkucaja srca, temperature tijela, glasanje životinja itd.), strojno učenje i umjetnu inteligenciju (Neethirajan i Kemp, 2021.). Na ovaj način će se uzgoj domaćih pasmina još više automatizirati, što će u kombinaciji s genomskom selekcijom zasigurno predstavljati novo razdoblje ili novu eru u evoluciji vrsta i pasmina.

Zaključak

Domaće životinje potječu od svojih divljih ili ishodišnih predaka, od kojih su se postupno razvile pasmine kakve danas poznajemo. Zahvaljujući kontinuiranom radu uzgajivača te stručnjaka i znanstvenika, danas je njihov broj i dalje velik. Selekcijom su razvijene suvremene pasmine i hibridi, a samim time došlo je do unaprjeđenja njihovih proizvodnih svojstava. Razvojem selekcijskih programa ubrzan je genetski napredak brojnih pasmina, dok je umjetnim osjemenjivanjem i embrio transferima omogućeno širenje genetskog materijala značajno brže. Također, posljednjih 10-ak godina genomskom selekcijom napredak je u slučaju nekih svojstava uvećan višestruko, što je već produktivne pasmine učinilo još produktivnijima. Uslijed ovih promjena, uzgajati lokalne pasmine postao je još veći izazov. Usprkos daljnjem unaprjeđenju tehnologija uzgoja te posljedično tome i uzgajanim pasminama, neophodno je promijeniti pristup ali i svijest u uzgoju domaćih životinja, te očuvati genetsku raznolikost integracijom najsuvremenijih alata genomike i tehnologije uzgoja.

Literatura

FAO (2015): The Second Report on the State of the World's Animal Genetic Resources for Food and Agriculture. (ur.) Scherf, B.D. i Pilling, D. FAO Commision on Genetic Resources for Food and Agriculture Assessments, Rome. Dostupno na: (http://www.fao.org/3/a-i4787e/index.html.) Pristupljeno 01.02.2022.

Frantz L., Haile J., Lin A. T., Scheu A., Geörg C., Benecke N.... Larson G. (2019): Ancient pigs reveal a near-complete genomic turnover following their introduction to Europe. Proceedings of the National Academy of Sciences of the United States of America. 116(35): 17231–17238.

Garcia-Ruiz A., Cole J.B., VanRaden P.M., Wiggans G.R., Ruiz- Lopez F.J., Van Tassell C.P. (2016). Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. Texas A&M University Collage Station, 113: 3995- 4004

Gootwine E. (2020). Genetics and breeding of sheep and goats. U: Bazer, F., Lamb, C. i Wu, G. (ur.) Animal Agriculture. Academic Press. Cambrige. 183-198.

Hole F. (1984). A reassessment of the neolithic revolution. Paleorient, 10(2): 49-60.

Larson G. Fuller D.Q. (2014). The Evolution of Animal Domestication. Annual Review of Ecology, Evolution, and Systematics. 45:115-136.

Librado P., Fages A., Gaunitz C., Leonardi M., Wagner S., Khan N. Hanghøj K., Alquraishi SA., Alfarhan A.H., Al-Rasheid K.A., Sarkissian C.D., Schubert M., Orlando L. (2016). The evolutionary Origin and Genetic Makeup of Domestic Horses. Genetics. 204: 423-434.

Linnaeus C. (1758). Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. Holmiæ. Salvii L.

Loftus R.T., Machugh D.E., Bradley D.G., Sharp P.M., Cunningham P. (1994). Evidence of two independent domestications of cattle. Proceedings of the National Academy of Sciences of the United States of America. 91: 2757-2761.

Lush J.L. (1943). Animal Breeding Plans. Copy Right by Iowa State Collage Press, Iowa.

Neethirajan S. Kemp B. (2021): Digital livestock farming. Sensing and Bio-Sensing Research. 32: 100408.

Olenbroek K., van der Waaij L. (2015). Textbook of animal breeding and genetics for BSC students. Centre for genetic resources the Netherlands and Animal Breeding and Genomics Centre, Wageningen. Dostupno na: (https://wiki.groenkennisnet.nl/display/TAB/Chapter+1.3+Domestication+and+animal+breeding). Pristupljeno 02.09.2021.

Pitt D., Sevane N., Nicolazzi E.L., MacHugh D., Park D.E.S., Colli L., Martinez R., Bruford M.W., Wengel P.O. (2017): Domestication of cattle: Two or three events?. Evolutionary Applications. 12: 123-136.

Van der Werf J.H.J. (2013). Genomic selection in animal breeding programs. U: Gondro C., J.H.J. van der Werf and B.J. Hayes (ur.). Genome-Wide Association Studies and Genomic Prediction. Methods in Molecular Biology. 1019: 543- 561.

Transboundary breed summary. Domestic Animal Diversity Information System (DAD-IS). Food and Agriculture Organization of the United Nations. www.fao.org

From wild ancestors to domesticated species and modern breeds

Abstract

From the beginning of domestication to the develompment of local and modern breeds and hybrids, numerous and complex processes have taken place. This is nicely demonstrated by the change in animal species and the enormous genetic and phenotypic diversity. Natural selection is a process influenced by the laws of nature, where the environment encourages the selection of individuals. Unlike natural selection, in farm animal populations the breeder systematically selects elite individuals for future reproduction, which is especially important in the last 10 years, since genomic selection has been applied. As a result today, several thousand breeds are known worldwide, which is necessary to preserve due to negative human impacts and climate change.

Key words: domestication, breeds, genetic diversity, genomic markers, genomics

ORIGINAL SCIENTIFIC PAPER

The estimation of linkage disequilibrium decay in Croatian Holstein cattle: potential for genomic selection

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Abstract
The objective of this study was to estimate linkage disequilibrium (LD) in 101 Croatian Holstein cows based on Illumina Bovine50K SNP chip. The average autosomal whole-genome LD (r^2) was 0.16 and for SNPs 100 Kbp apart 0.30. The average within-chromosomal LD ranged from 0.12 for BTA28 to 0.23 for BTA20, with no straightforward relation between chromosome length and r^2. The decay of LD showed an exponential trend with physical distance showing a very steep decay of LD within the first 150 Kbp. The results implicate a sufficient density of the 50K SNP chip for successful implementation of genomic selection (GS) and genome wide association studies (GWAS) in the population of Holstein cattle in Croatia.

Key words: Holstein cattle, linkage disequilibrium, SNP, genomic selection

Introduction

The development of genotyping technology and reduced whole-genome (SNPs) genotyping costs in many livestock species strongly contributed to the interest in implementation of genomic selection (GS) (Meuwissen et al., 2001), genome-wide association studies (GWAS) (Meuwissen and Goddard, 2000; Hirschhorn and Daly, 2005) and understanding genomic architecture and historical population structure (Hayes et al., 2003). The success of these genomic techniques strongly depends on the extent of linkage disequilibrium (LD) and its rate of decline with distance between loci. LD refers to non-random association between alleles at different loci mostly due to physical proximity. Estimates of LD are affected by population history and evolutionary forces (Ardlie et al., 2002), sample size, marker type, marker density and distribution, and strictness of SNP filtering (Bohmanova et al., 2010). In livestock, the most common measures of LD are the squared correlation coefficient (r^2) (Hill and Robertson, 1968) which is equivalent to the covariance and the correlation between alleles at two different loci and the normalized D' (Lewontin, 1964). r^2 is less sensitive to sample (population) size than D', and D' tends to be inflated with small sample sizes and/or low allele frequencies (Bohmanova et al., 2010), making r^2 the preferred measure for LD.
Livestock populations have smaller effective population sizes than human and therefore LD is expected to extent larger genetic distances (Farnir et al., 2000). In cattle, LD in highly selected populations is extended over larger distances (Farnir et al., 2000) compared to moderately selected populations (Thevenon et al., 2007). Studies used SNPs for evaluating the extent of LD in intensively selected populations (such as Holstein) revealed moderate levels of LD (r^2≥0.2) between markers with inter-marker distance up to 100 Kbp (McKay et al., 2007; Marques et al., 2008; Sargolzaei et al., 2008). Khatkar et al. (2008) reported r^2≥0.2 between SNPs less than 60 Kbp apart in Australian Holstein. The objective of this study was to estimate the genome-wide level of LD in Croatian Holstein cattle in order to examine the potential of GWAS and GS.

Material and methods

The data included 101 Holstein females from 32 herds that were progenies of 45 sires. Females were genotyped using the Illumina BovineSNP50K BeadChip containing 52,445 SNPs. Only autosomal SNPs were included in the analysis. Markers with a minor allele frequency <5% (n=9,047), >10% missing genotypes (n=791), >10% missingness per animal (n=0), and strong deviation ($P<10^{-6}$) from Hardy-Weinberg equilibrium (n=11) were excluded from the analysis. This editing resulted in 40,763 SNPs that passed the above control and were used for further analysis. The PLINK (version 1.9: Purcell et al., 2007; Chang et al., 2015) was used to calculate LD between pairs of SNPs using the r^2 statistics. The dplyr (Wickham et al., 2021) and ggplot (Wickham, 2016) packages in the R programming environment (R Core Team, 2020) were used for post hoc statistical analysis of genome wide LD.

Results and discussion

This study provides an overview of LD in Croatian Holstein dairy cattle using a 50K SNP panel. We used r^2 value to estimate the extent of LD due to small sample size. According to Bohmanova et al. (2010), estimates of r^2 are not influenced by sample size when if at least 55 animals were used in the calculation. The overall average genome-wide LD was 0.16. To examine the decay of LD with physical distance, syntenic SNP pairs on autosomes were sorted into bins (intervals) based on their inter-marker distance. The average r^2 was calculated for each bin (Table 1). A moderate level of r^2 (0.30) was estimated for distances shorter than 100 Kbp. The average r^2 has been declined from 0.15 to 0.10 when moving from 100 to 800 Kbp. LD comparisons between different studies are difficult due to differences in sample size, LD measures, marker types and density of markers (Bohmanova et al., 2010). The results of this study revealed similar values at a distance of 100 Kbp as reported MacKay et al. (2007) for Holstein in their study of eight cattle breeds (Angus, Charolais, Dutch Black and White Dairy, Holstein, Japanese Black, Limousin, Brahman, and Nelore). Espigolan et al. (2013) genotyped Nellore cattle and found a mean LD (r^2) between adjacent markers of 0.17. Marques et al. (2008) studied BTA14 in Holstein cattle and found a moderate LD level (0.2) separated by 100 Kbp. An average r^2 estimate of 0.16 (in 60-100 Kbp bins) was reported for North American Holstein bulls (Bohmanova et al., 2010). In the study by El Hou et al. (2021) conducted on three French beef cattle breeds (Charolaise, Blonde d'Aquitaine, Limousine), the average r^2 varied from 0.50 (distances smaller than 15 Kbp) to less than 0.10 (distances greater than 120 Kbp). On the other hand, r^2 ranged between 0.231 (distances between 0 and 50 Kbp) and 0.065 (distances between 150 and 200 Kbp) in Korean Hanwoo cattle (Lee and Kong, 2021). LD values decrease as the distance between markers on the genome increases. Bovine studies using SNP data have shown that the average LD was close to zero for distances between markers greater than 500 Kbp (McKay et al., 2007).

Table 1. Genome wide linkage disequilibrium (LD) classified by inter-marker distance

Distance (Kbp)	N	avg (r^2)	sd (r^2)	Distance (Kbp)	N	avg (r^2)	sd (r^2)
[0,100]	77153	0.30	0.32	(500,600]	25619	0.11	0.14
(100,200]	65967	0.15	0.19	(600,700]	15121	0.11	0.14
(200,300]	62968	0.12	0.16	(700,800]	8818	0.10	0.13
(300,400]	55611	0.11	0.14	(800,900]	5229	0.10	0.13
(400,500]	40370	0.11	0.14	(900,1000]	2952	0.09	0.13

Graphical representation of LD decay was obtained by plotting the average LD (expressed as r^2) vs. average between-marker distance of bins spanning 100 Kbp (Figure 1). Decay of LD showed a clear exponential trend with physical distance. The changes of r^2 revealed a very steep decay of LD within the first 150 Kbp and intermediate decay from 150 to 250 Kbp. Decay after 250 Kbp was negligible.

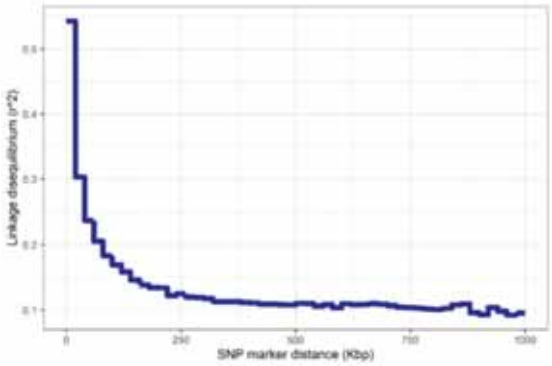

Figure 1. Decay of r^2 as a function of physical distance

Table 2 shows the mean r^2 of the 29 individual autosomes (BTA). Some chromosomes had higher LD than others, however there was no clear relationship between chromosome length and r^2. A linear relationship was observed between LD (r^2) and chromosome length in the study of Sahiwal cattle (Mustafa et al., 2018). On the other hand, Bohmanova et al. (2010) found no association between LD level and chromosome size. In this study, the average r^2 between adjacent SNP ranged from 0.12 (BTA28) to 0.23 (BTA20). The median for r^2 was 0.15 and was observed for BTA3, BTA5, BTA9, BTA17, BTA22, BTA23, BTA26, and BTA29. In the study conducted by Bohmanova et al. (2010), the average r^2 was slightly higher, ranging from 0.17 (BTA27, BTA28 and BTA 29) to 0.25 (BTA 7 and BTA14). The average LD between SNPs ranged from 0.002 to 0.19 for r^2 across all autosomes in the study conducted by Mustafa et al. (2018) in Sahiwal cattle. The extent of LD was considerably higher in this population than in many other populations of small ruminants, especially sheep (e.g., French Lacaune, Baloche et al., 2014; Chinese Merino, Liu et al., 2017), which is logical considering the history of the breeds under consideration (different selection intensities).

Table 2. Linkage disequilibrium (LD) classified by inter-marker distance of 100 Kbp on 29 autosomes (BTA)

Chr	avg (r^2)	sd (r^2)	Chr	avg (r^2)	sd (r^2)	Chr	avg (r^2)	sd (r^2)
1	0.16	0.22	11	0.14	0.19	21	0.18	0.26
2	0.17	0.22	12	0.13	0.18	22	0.15	0.21
3	0.15	0.20	13	0.20	0.26	23	0.15	0.21
4	0.14	0.20	14	0.18	0.22	24	0.14	0.18
5	0.15	0.20	15	0.13	0.17	25	0.13	0.17
6	0.17	0.23	16	0.19	0.24	26	0.15	0.20
7	0.16	0.21	17	0.15	0.20	27	0.14	0.22
8	0.16	0.22	18	0.13	0.18	28	0.12	0.17
9	0.15	0.21	19	0.21	0.28	29	0.15	0.21
10	0.17	0.21	20	0.23	0.28			

Evaluation of genome-wide LD and pattern of LD decay can be used to estimate SNP density required for GWAS studies and implementation of GS (Ardlie et al., 2002; Hayes et

al., 2008). The consensus is that roughly r^2>0.3 was considered useful LD for GWAS (Ardlie et al., 2002; Khatkar et al., 2008). For the effective GS with satisfied accuracy of 85%, the threshold of the suggested LD level (r^2) should be 0.20 (Meuwissen et al., 2001). The obtained values of LD in this population are very close to the proposed benchmarks and would probably reach them with the increased sample size. Therefore, the determined LD in this population implies a good potential for GS and GWAS, as has been confirmed in many other Holstein populations worldwide (Hayes et al., 2009).

Conclusions

This moderate extend of LD level determined in this population implies that the 50K SNP chip should be sufficient for the GS and GWAS in Croatian Holstein cattle. However, more samples should be used to get more information on this issue.

Literature

Ardlie K. G., Kruglyak L., Seielstad M. (2002). Patterns of linkage disequilibrium in the human genome. Nature Reviews Genetics. 3(4): 299-309.

Baloche G., Legarra A., Sallé G., Larroque H., Astruc J. M., *et al.* (2014). Assessment of accuracy of genomic prediction for French Lacaune dairy sheep. Journal of Dairy Science 97(2):1107-1116.

Bohmanova J., Sargolzaei M., Schenkel F. S. (2010). Characteristics of linkage disequilibrium in North American Holsteins. BMC Genomics. 11:421.

Chang C. C., Chow C. C., Tellier L. C., Vattikuti S., Purcell S. M., *et al.* (2015). Gigascience. 4(1): s13742-015-0047-8.

El Hou A., Rocha D., Venot E., Blanquet V., Philippe R. (2021). Long-range linkage disequilibrium in French beef cattle breeds. Genetics Selection Evolution. 53: 63.

Espigolan R., Fernando B., Arione A. B., Fabio R. P. S., Daniel G.M.G., Tonussi R.L., Cardoso D.F., Oliveira H.N., Tonhati H., Sargolzaei M., Schenkel F.S., Carvalheiro R., Ferro J.A., Albuquerque L.G. (2013). Study of whole genome linkage disequilibrium in Nellore cattle. BMC Genomics. 14: 305.

Farnir F., Coppieters W., Arranz J. J., Berzi P., Cambisano N., Grisart B., Karim L., Marcq F., Moreau L., Mni M., Nezer C., Simon P., Vanmanshoven P., Wagenaar D., Georges M. (2000). Extensive genome-wide linkage disequilibrium in cattle. Genome Research. 10: 220–227.

Hayes B. J., Visscher P. M., McPartlan H. C., Goddard M. E. (2003). Novel multilocus measure of linkage disequilibrium to estimate past effective population size. Genome Research. 13: 635–643.

Hayes B. J., Lien S., Nilsen H., Olsen H. G., Berg P., Maceachern S., Potter S., Meuwissen T. H. E. (2008). The origin of selection signatures on bovine chromosome 6. Animal Genetics. 39:105–111.

Hayes B. J., Bowman P. J., Chamberlain A. J., Goddard M. E. (2009). Invited review: Genomic selection in dairy cattle: Progress and challenges. Journal of Dairy Science. 92: 433-443.

Hill W. G., Robertson A. (1968). Linkage disequilibrium in finite populations. Theoretical and Applied Genetics. 38: 226–31.

Hirschhorn J. N., Daly M. J. (2005). Genome-wide association studies for common diseases and complex traits. Nature Reviews Genetics. 6(2):95-108.

Khatkar M. S., Nicholas F. W., Collins A. R., Zenger K. R., Cavanagh J. A., Barris W., Schnabel R.D., Taylor J.F. Raadsma H.W. (2008). Extent of genome-wide linkage disequilibrium in Australian Holstein-Friesian cattle based on a highdensity SNP panel. *BMC* Genomics. 9:187.

Liu S., He S., Chen L., Li W., Di J., Liu M. (2017). Estimates of linkage disequilibrium and effective population sizes in Chinese Merino (Xinjiang type) sheep by genome-wide SNPs. Genes Genomics. 39(7): 733-745.

Lee G. H., Kong H. S. (2021). Linkage Disequilibrium Analysis of Hanwoo in Gyeonggi Region using Hanwoo SNP Chip. Journal of Animal Breeding and Genomics. 5(4): 235-242.

Marques E., Schnabel R. D., Stothard P., Kolbehdari D., Wang Z., Taylor J.F., Moore S.S. (2008). High density linkage disequilibrium maps of chromosome 14 in Holstein and Angus cattle. BMC Genetics. 9: 45.

McKay S. D., Schnabel R. D., Murdoch B. M., Matukumalli L. K., Aerts J., Coppieters W., Crews D., Neto E.D., Gill C.A., Gao C., Mannen H., Stothard P., Wang Z., Tassell C.P.V., Williams J.L., Taylor J.F., Moore S.S. (2007).Whole genome linkage disequilibrium maps in cattle. BMC Genetics. 8: 74.

Meuwissen T. H. E., Hayes B. J., Goddard M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. Genetics. 157: 1819-1829.

Meuwissen T. H. E., Goddard M. E. (2000). Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. Genetics. 155: 421–430.

Mustafa H., Ahmad N., Heather H. J., Eui-soo K., Khan W. A., Pasha T.N., Ali A., Kim J.J., Sonstegard T.S. (2018). Whole genome study of linkage disequilibrium in Sahiwal cattle. South African Journal of Animal Science. 48: 353–360.

Purcell S. N. B., Neale B., Todd-Brown K., Thomas L., Ferreira M. A. R., Benderba D., Mallerba J., Sklarbaa P., Bakkerba P.I.W., Dalyba M.J., Sham P.C. (2007). PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American journal of human genetics. 81(3): 559-575.

R Core Team (2020). R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. Available at: https://www.R-project.org.

Sargolzaei M., Schenkel F. S., Jansen G. B., Schaeffer L. R. (2008). Extent of linkage disequilibrium in Holstein cattle in North America. Journal of Dairy Science. 91: 2106-2117.

Thevenon S., Dayo G. K., Sylla S., Sidibe I., Berthier D., Legros H., Boichard D., Eggen A., Gautier M. (2007). The extent of linkage disequilibrium in a large cattle population of western Africa and its consequences for association studies. Animal Genetics. 38: 277–286.

Wickham H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, N.York.

Wickham H., François R., Henry L., Müller K. (2021). dplyr: A Grammar of Data Manipulation. R package version 1.0.7.

Procjena neravnoteže povezanosti genomskih markera kod holstein goveda u Republici Hrvatskoj: potencijal za provedbu genomske selekcije

Cilj ovog rada je bio procijeniti neravnoteže povezanosti genomskih markera (LD) u populaciji Holstein krava (n=101) koristeći Illumina Bovine50K SNP čip. Prosječni r^2 je bio 0,16, a 0,30 za markere međusobno udaljene 100 Kbp. Prosječni kromosomalni r^2 je bio u rasponu od 0,12 (BTA28) do 0,23 (BTA20), bez jasne veze između r^2 i veličine kromosoma. Utvrđeno je eksponencijalno opadanje LD sa porastom fizičke udaljenosti markera, s najvećim padom unutar prvih 150 Kbp. Dobiveni rezultati upućuju na dostatnu gustoću 50K SNP čipa za uspješnu provedbu genomske selekcije (GS) i genomske asocijacijske studije (GWAS) u populaciji holstein pasmine goveda u Republici Hrvatskoj.

Ključne riječi: Holstein, neravnoteža povezanosti markera, SNP, genomska selekcija

PREGLEDNI RAD

Genomski tragovi selekcije kod domaćih životinja

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Sažetak
Zbog značajnog porasta broja stanovnika i sve većih potreba za hranom, porastao je i broj uzgajanih životinja. Porastom potreba za životinjskim proizvodima, s vremenom je opadao i broj uzgajanih pasmina jer se u uzgoju koriste isključivo visokoproduktivne pasmine. Kao rezultat ovih promjena, značajno je opala i njihova genetska raznolikost. Kako bi se spriječio gubitak genetske raznolikosti i omogućilo stvaranje uspješnih konzervacijskih programa, važno ju je planski istražiti te utvrditi populacijske parametre, kao i tragove selekcije genoma domaćih životinja. Cilj ovog rada je opisati genetsku raznolikost i tragove selekcije, te metode koje se koriste u utvrđivanju istih u konzervacijskim programima.

Ključne riječi: genetska raznolikost, tragovi selekcije, genetski drift, selekcija, mutacija

Uvod

Genetska je raznolikost definirana kao prisutnost genetskih razlika između i unutar vrsta ili pasmina. Mjerila genetske raznolikosti najčešće se izražavaju brojem alela prisutnih u populaciji (Oldenbroek i van der Waaij, 2014.). Kako bi se očuvala raznolikost pasmina, bitno je pratiti razinu alela u populaciji koja je u korelaciji s genetskom raznolikošću, odnosno što je veći broj alela u populaciji, to je veća i genetska raznolikost. U suprotnome, do gubitka raznolikosti može doći zbog različitih uzroka kao što su genetski drift, mutacije, migracije, te selekcija i inbriding. Očuvanje genetske raznolikosti u većini slučajeva postiže se povećavanjem efektivne veličine populacije proporcionalno smanjenjem razine inbridinga po generaciji, ali neovisno o ovome inbriding ipak može porasti i ostaviti negativne posljedice. Upravo je inbriding jedan od najadekvatnijih i najrobusnijih parametara koji upućuje na intenzivnu selekciju unutar pasmine te kao takav u genomu ostaje kao trag selekcije. Tragovi selekcije se definiraju kao genetski obrasci ili otisci ostavljeni na genomskim regijama koje su bile podvrgnute selekciji. Osim inbridinga, postoji veliki broj parametara i metoda koje se koriste za otkrivanje specifičnih tragova selekcije. Primjerice, za otkrivanje tragova selekcije između populacija koriste se parametri kao što su fiksacijski indeks (F_{ST}), produljena homozigotnost haplotipova (engl. *extended haplotype homozygosity*, EHH), i dr., dok se unutar populacije koristi opadanje neravnoteže povezanosti alela ili LD (engl. *Linkage disequilibrium*), Tajima D i dr. (Qanbari i Simianer, 2014.).

Genetska raznolikost

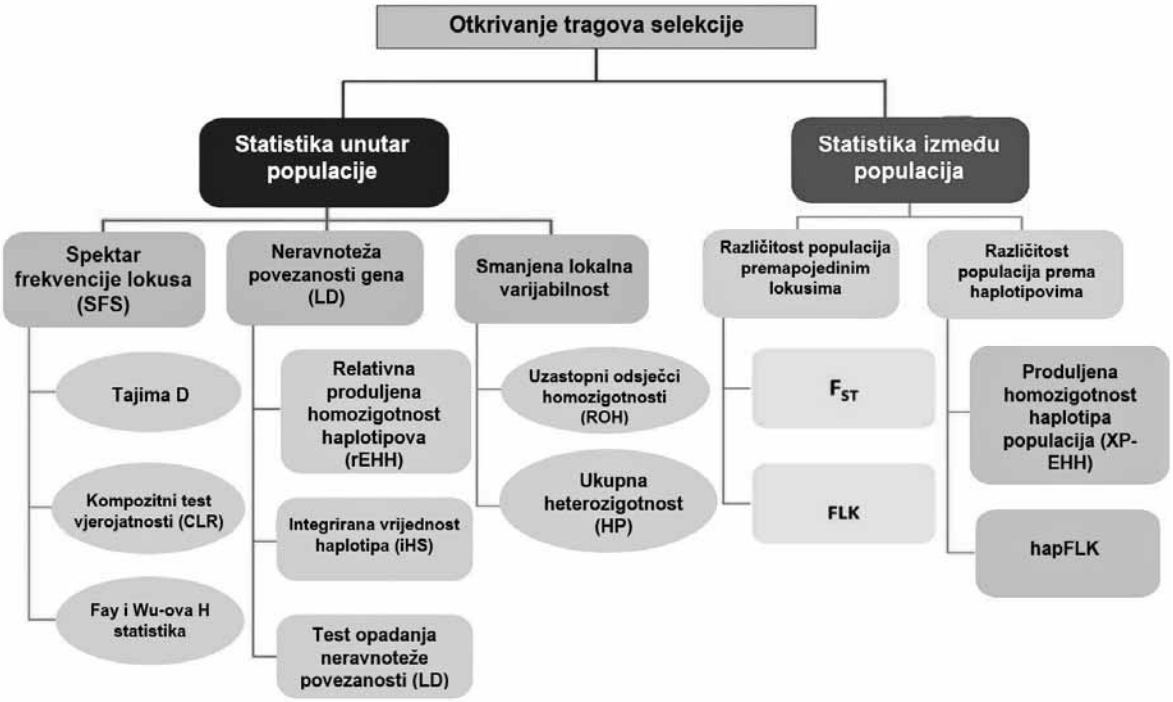
Analiza genetske raznolikosti razlikuje dvije kategorije: genetsku raznolikost između populacija i genetsku raznolikost unutar populacije (Kantanen i sur., 2000.). Genetska raznolikost uvelike ovisi o prisutnosti i brojnosti alela, ali i o njihovoj frekvenciji u populaciji. Različite pasmine na primjer, imaju različite genetske specifičnosti za određena svojstva, ovisno o svrsi i namjeni uzgoja određene pasmine. Također, raznolikost postoji i unutar populacije, a odnosi se na razlike između životinja u toj populaciji. Gotovo je nemoguće da ne postoji genetska varijabilnost u populaciji. Iznimno, može se dogoditi u

onoj populaciji koja ima visoku razinu inbridinga ili su genetski identične npr. klonovi. (Oldenbroek i van der Waaij, 2014.). Genetska raznolikost može se smatrati odrazom ravnoteže između pojave i nestanka alela, primjerice mutacijama. Tako se novi aleli pojavljuju u svakoj generaciji, spontano nastali mutacijom zbog pogreške u replikaciji DNK ili zbog oštećenja DNK. Do promjene genetske raznolikosti dolazi iz nekoliko razloga: mutacija, migracija, selekcije, te genetskog drifta. Mutacije nisu konstatne u genomu, nego su uvjetovane brzinom gubitka, odnosno fiksacijom alela (Ellegren i Galtier, 2016.). Isto tako i migracije mogu imati veliki učinak na genetsku raznolikost, posebno imigracija (kada nove životinje useljavaju u populaciju) i na taj način unosi nove alele, dok emigracija (kada životinja napušta populaciju) ima manji učinak, osim ako je populacija jako mala ili životinja ima posebne ili rijetke alele (Lacy, 1987.). Selekcija je tijekom posljednjih nekoliko desetljeća ubrzala genetsko poboljšanje brojnih pasmina. Također je uz razvitak transportnih i komunikacijskih sustava olakšano i širenje genetskog materijala. Ova je praksa posljedično dovela do razvoja učinkovitih uzgojnih programa, a što je imalo negativan učinak na lokalne pasmine širom svijeta, koje su zamijenjene visokoproduktivnim pasminama (Groeneveld i sur., 2010.). Genetski drift je proces slučajnih promjena u učestalosti alela i haplotipova u populacijama različitih veličina (Biwas i Akey, 2006.), te je posebno važan u malim populacijama. Kada je broj jedinki u populaciji mali, slučajnost može biti odlučujući faktor koji će odrediti koji će aleli u populaciji biti češći, a koji rjeđi (Lacy, 1987.). Pojava koja je usko povezana s genetskim driftom je pojava tzv. efekta uskog grla, koji je tako nazvan jer je populacija prošla kroz usko grlo koje smanjuje njezinu efektivnu veličinu. Do pojave uskog grla dolazi zbog neke nagle promjene u populaciji poput pojave bolesti i slično, a dovodi do drastičnog smanjenja broja jedinki u populaciji. Sasvim slučajno neki aleli mogu „preživjeti“, dok drugi također sasvim slučajno mogu nestati. Tada će genetski drift imati znatne učinke na genetsku strukturu populacije, dok populacija ne postane dovoljno velika da smanji učinke genetskog drifta. Iako, čak i ako se populacija koja je prošla usko grlo brojčano oporavi, može imati niske razine frekvencije alela tijekom dužeg vremena, zbog širenja genetskog drifta kada je populacija bila mala (Reece i sur., 2011.).

Tragovi selekcije

Kako bi se očuvala genetska raznolikost domaćih životinja, važno je pratiti tragove selekcije prisutne u genomu. Identifikacija tragova selekcije jedan je od ključnih interesa u istraživanjima raznolikosti životinja, jer se pomoću njih mogu identificirati geni i korisne mutacije koje imaju selektivnu prednost u određenim populacijama (Zhao i sur., 2015.). Metode za otkrivanje tragova selekcije temelje se na različitim parametrima i testovima (Shema 1.). Tako primjerice metoda za otkrivanje razlika između populacija najčešće uključuje fiksacijski indeks ili F_{ST}, dok se metode za analize tragova selekcije unutar populacija temelje na spektru frekvencije lokusa (SFS) (uključuje Tajimin D i dr.), neravnoteži povezanosti (LD) (uključuje EHH, XP-EHH i dr.) (Paima i sur., 2018.) ili smanjenoj varijabilnosti regija u odnosu na prosjek genoma. F_{ST} je parametar koji mjeri razinu razlika između populacija na temelju frekvencije alela, dok se unutar populacije vrlo često razmatra genomski inbriding (Biswas i Akey, 2006.). Vrijednost F_{ST} kreće se od 0 do 1, pri čemu niske vrijednosti upućuju na manje razlike između populacija i obrnuto (Zhao i sur., 2015.). Raspon F_{ST} vrijednosti u populacijama domaćih životinja najčešće se kreće od 0,05 do 0,4, ovisno o genetskoj udaljenosti istraživanih populacija (Walsh i Lynch, 2018.). Najčešće korišten test neutralnosti temeljen na spektru frekvencije lokusa je Tajima D. Tajima D je statistička metoda koja se računa razlikom između prosječnog broja razlika svih parova i ukupnog broja polimorfnih lokusa, a zapravo pokazuje da li se određena regija genoma u populaciji mijenja slučajno/neutralno ili pod utjecajem drugih sila kao što je selekcija (Tajima, 1989., Sabeti, 2002.). Osim prethodno opisanih mjera genetske

raznolikosti, Voight i sur. (2006.) predložili su test koji se proteklih godina puno primjenjuje, a temelji se na EHH (Extended haplotype homozigosity) fenomenu ili dugim homozigotnim haplotipovima. Ovaj je pristup koristan jer su unutar njegovog okvira razvijene metode za testiranje raznolikosti unutar i između populacija. Također, promjena u neravnoteži povezanih gena se može utvrditi prikazivanjem opadanja vrijednosti LD-a s porastom udaljenosti između promatranih lokusa, što u konačnici omogućuje otkrivanje tragova selekcije (de Simoni Gouveia i sur., 2014.). U praksi, to znači da će geni odnosno aleli koji se nalaze blizu jedan drugoga, biti u pozitivnoj korelaciji i da će se pojavljivati češće u kombinacijama u odnosu na druge alele koji se nalaze dalje od njih.



Shema 1. Podjela metoda za otkrivanje tragova selekcije (Saravanan i sur., 2020.)

Zaključak

Tragovi selekcije koristan su alat koji može omogućiti mnoštvo korisnih informacija o strukturi neke populacije ili pasmine, te pomoći u planskom sparivanju jedinki. Isto tako, tragovi selekcije pomažu u očuvanju genetske raznolikosti domaćih životinja, koja bilježi sve veći gubitak zbog pretjeranog korištenja malog broja suvremenih visokoproizvodnih pasmina. Genetska raznolikost temeljni je preduvjet za očuvanje i održavanje genetskih resursa domaćih životinja. Gubitak genetskih resursa predstavlja veliki izazov u uzgoju domaćih životinja, posebice lokalnih pasmina te se može spriječiti isključivo pravilnim upravljanjem i programima očuvanja.

Metode za utvrđivanje tragova selekcije mogu se podijeliti na analize unutar i između populacija, dok se najčešće zbog svoje robusnosti i jednostavnosti koriste genomski inbriding i F_{ST} ili fiksacijski indeks. Za postizanje točnijih rezultata u analizi tragova selekcije, preporučljivo je koristiti više različitih pristupa i metoda kako bi se ovisno o različitim utjecajima, „uhvatio“ trag selekcije.

Razvojem novih metoda i pristupa za otkrivanje tragova selekcije dolazi do daljnjeg razvoja genetike i genomike, što uvelike pomaže u kreiranju, ali i provođenju uzgojnih programa.

Literatura

Biswas S. and Akey J. M. (2006). Genomic insights into positive selection. Trends in Genetics. 22 (8): 437-446.

de Simoni Gouveia J. J., da Silva M. V., Paiva S. R., de Oliveira S. M. (2014). Identification of selection signatures in livestock species. Genetics and Molecular Biology. 37(2): 330-342.

Ellegren H., and Galtier N. (2016). Determinants of genetic diversity. Nature Reviws Genetics. 17: 422-433.

Groeneveld L. F., Lenstra J. A., Eding H., Toro M. A., Scherf B., Pililing D., Negrini R., Finalay E. K., Jianlin H., Groeneveld E., Weigend S. and The GLOBALDIC Consortium. (2010). Genetic diversity in far animals – a review. Animal Genetics. 41: 6-31.

Kantanen J., Olsaker I., Holm L-E, Lien S., Vilkki J., Brusgaard K., Eythorsdottir E., Danell B., Adalsteinsson S. (2000). Genetic diversity and population structure of 20 north European cattle breeds. Journal of Heredity. 91(6): 446-457.

Lacy R. C. (1987). Loss of Genetic Diversity from Managed Populations: Interacting Effects of Drift, Mutation, Immigration, Selection, and Population Subdivision. Conservation Biology. 1(2): 143-158.

Oldenbroek, K., and van der Waaij, L. (2014). Textbook animal breeding : animal breeding and genetics for BSc students. Centre for Genetic Resources and Animal Breeding and Genomics Group, Wageningen University and Research Centre. 311. Raspoloživo: https://edepot.wur.nl/365431

Paim T., Ianella P., Paiva S. R., Caetano A., Mcmanus C. (2018). Detection and evaluation of selection signatures in sheep. Pesquisa Agropecuária Brasileira. 53(5): 527-539.

Reece J. B., Urry L. A., Cain M. I., Wasserman S. A., Minorsky P. V., Jackson R. B. (2011). Natural selection, genetic drift, and gene flow can alter allele frequencies in a population. Campbell Biology (9th Edition). 476-480. Raspoloživo: https://archive.org/details/isbn_9781256158769

Qanbari S. and Simianer H. (2014). Mapping signatures of positive selection in the genome of livestock. Livestock Science. 166: 133-143.

Sabeti P. C., Reich D. E., Higgins J. M., Levine H. Z. P., Richter D. J., Schaffner S. F., Gabriel S. B., Platko J. V., Patterson N. J., McDonald G. J., Ackerman H. C., Campbell S. J., Altshuler D., Cooper R., Kwiatkowski D., Ward R., Lander E. S. (2002). Detecting recent positive selection in the human genome from haplotype structure. Nature. 419(6909): 832-837.

Saravanan K. A., Panigrahi M., Kumar H., Bhushan B., Dutt T., Mishra B. P. (2020). Selection signatures in livestock genome: A review of concepts, approaches and applications. Livestock Science. 241: 1-11.

Tajima F. (1989). Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. Genetics. 123 (3): 585-595.

Voight, B. F., Kudaravalli, S., Wen, X., Pritchard, J. K. (2006). A Map of Recent Positive Selection in the Human Genome. PLoS Biology. 4(3): e72.

Walsh B. and Lynch M. (2018). Evolution and selection of quantitative traits. Oxford University Press.

Zhao F., McParland S., Kearney F., Lixin D., Berry D. P. (2015). Detection of selection signatures in dairy and beef cattle using high-density genomic information. Genetics Selection Evolution. 47(1): 49.

Selection signatures in farm animals

Abstract
Due to human population expansion and growing demand for food, the number of farm animals has increased. For that reason, only modern, highly productive breeds/hybrids are used in breeding, while the number of local breeds has decreased. Because of these changes, genetic diversity has significantly decreased over time. In order to prevent the loss of genetic diversity and create sustainable conservation programs, it is important to perform detailed analyses of population parameters and selection signatures of farm animals. The aim of this paper is to describe genetic diversity and selection signatures and to review the available methodology in conservation programs.

Key words: genetic diversity, selection signatures, genetic drift, selection, mutation

ORIGINAL SCIENTIFIC PAPER

The effect of partial replacement of maize with dried whey on N bilance from a diet based on alfalfa haylage in wether sheep

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Abstract
The aim of this study was to determine the effect of partial replacement of ground maize grain (GM) with dried whey (DW), as an supplement to the alfalfa haylage (AH), on the nitrogen (N) bilance in wether sheep. Four feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, were investigated: only GM (treatment A) or GM replaced with DW in the quantity of 10% (treatment B), 20% (treatment C) or 30% (treatment D). According to results obratined a DW has a potential to replace a GM, as a supplement to AH of medium quality. It was concluded that the replacement of 10%, 20% and 30% has no negative affect on N intake, N excretion, nor the N balance in wether sheep. A decision of replacement and quantity of GM to be replaced by DW is dependent on the prodction economy and market prices of these feeds.

Key words: alfalfa haylage, maize grain, dried whey, N bilance

Introduction

The alfalfa haylage (AH) is rich in crude protein (CP) but is poor in energy (Miller et al. 2001). If the diet does not contain a sufficient amount of energy, significant losses of N occur with the excretion via urine and faeces from the organism (Miller et al. 2001). Previous studies have demonstrated that the energy supplements to diets based on haylage increases nitrogen utilization in wether sheep (Vranić et al., 2011; 2018). Dried whey (DW) is a by-product of the dairy industry. It is rich in lactose as an energy source (about 700 g kg^{-1} DM) (DeFrain et al., 2004) which is similar to corn (11.6 and 12.17 MJ kg^{-1} ME for ruminants, respectively) (Schingoethe and Beardsley, 1975). The final product of lactose degradation in rumen is butyric acid while starch propionic acid (Chamberlain et al., 1993), so the mechanism and rate of absorption of newly formed acids through the rumen wall is different and their impact on nutrient utilization (DeFrain et al., 2004). It has been reported that the total or partial replacement of maize with DW (50 or 100%) increases urine excretion, but does not affect the N bilance in dairy cows (Susmel et al., 1995). Furthermore, the addition of DW to forage increases the utilization of nitrogen components in diets (Calsamiglia et al., 2010), whereas the degradation and absorption of whey proteins (lactoglobulins) in the small intestine increases the retention of N (Susmel et al., 1995). The hypothesis of this study was that the replacement of maize with DW, as a supplement to alfalfa haylage, increases feed intake and N bilance in sheep. The aim of this study was to determine the effect of partial replacement of maize with DW on N intake and N bilance from a diet based on alfalfa haylage in wether sheep nutrition.

Materials and Methods

Alfalfa crop (*Medicago sativa* L.) was mown in the early flowering growth stage (around 30 % of the crop was in flowering growth stage), wilted during 24 h at 500 – 600 g (DM) kg^{-1} fresh crop, and baled in the roller bales wrapped with 6 layers of light blue plastic film. Alfalfa haylage (AH), for experimental purposes, was chopped to particle size 3 – 5 cm, compressed into plastic bags (around 10 kg bag^{-1}), and stored in the cold chamber at 4 °C until feeding.

The maize BC 566 were harvested after they reached physiological maturity, and stored in a dry chamber in 3 plastic bins, mechanically ground (GM) to the particle size of 3 mm and stored. DW was obtained by Dukat d.d., M. Čavić 9, 10 000 Zagreb, Croatia. The animals were fed twice a day (9 a.m. and 4 p.m.) with an equal amount of concentrate separated from the voluminous diet thus enabling the intake of the offered total daily meal. The total amount of concentrate intake was 30 g d^{-1} kg^{-1} $M^{0.75}$ (GM or GM + DW). Four wether sheep (Suffolk x Lake-Solčava sheep), aged about 2 years and body weight of 60 – 66 kg were selected. Four feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, were investigated: only GM (treatment A) or GM replaced with DW in the quantity of 10% (treatment B), 20% (treatment C), or 30% (treatment D).

Experimental design

A feeding trial was performed with 4 sheep and 4 feeding treatments over 4 periods using an incomplete change-over design. Each period consisted of 3 phases: acclimatization period, measurement period of *ad libitum* intake, and measurement period of N bilance parameters. During the acclimatization period, the animals were located in individual pens (1.5 x 2.2 m) followed by their translocation to individual crates (1.36 m x 1.53 m x 1.49 m) and a 2-days measurement of *ad libitum* intake. Afterwards, the N bilance measurement was performed during a period of 5 days. Each of the four experimental periods lasted 17 days.

The feed intake and produced amount of faeces and urine were recorded daily. Likewise, the samples of an offered feed, refused feed, faeces and urine were collected daily and stored at a temperature 4 °C. A 30 mL of 2M H_2SO_4 was added daily to each urine container to prevent the evaporation of N. By the end of each experimental phase, the samples collected were stored at -20 °C. The DM contents of samples were determined by oven drying to a constant weight at 60 °C in a fan-assisted oven (ELE International). The ash contents of the offered feed, refused feed and faeces were measured by igniting the samples in a microwave oven (Milestone PIYRO, Italy) at 550 °C for 3 h. The total N concentrations were determined by the Kjeldahl method (AOAC 1990, ID 954.01) using a Gerhardt nitrogen analyser. In addition, N concentration was expressed as crude protein (CP) (total N x 6.25) g kg^{-1} DM for the offered feed and refused feed. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the procedure described by Van Soest et al. (1991) utilising Ankom Filter bag technology (USA). The pH of the silage was determined in a water extract containing 10 g of fresh silage and 100 ml of distilled water using a pH meter 315i (WTW). The efficiency of N was determined using the following equations: (i) N intake = N offered - N in food residues and (ii) N utilisation = N intake – N excreted (via faeces + urine)

Statistical analysis of the results were performed using SAS statistical software (SAS Institute, 1999); GLM, and MIXED procedure. The statistical significance was defined as $P < 0.05$.

Results and Discussion

The chemical composition of the feeds used in this experiment is presented in Table 1. The average CP content of alfalfa haylage was from 77 to 167.5 g kg^{-1} DM determined in the grass silages produced on family farms in the northwest part of Croatia (Vranić et al., 2005).

Table 1. The chemical composition of the alfalfa haylage, maize grain and dried whey used in the experiment (in g kg^{-1} DM unless stated otherwise).

Chemical parameter	Alfalfa haylage	Maize grain	Dried whey
DM	549.39	918.30	950
OM	913.31	979.60	NA
CP	122.45	84.26	90
Starch	103.96	584.16	NA
Lactose	NA	NA	750
NDF	534.56	219.99	NA
ADF	397.42	31.17	NA
pH	6.02	NA	NA
NH_3-N (g N kg^{-1} total N)	38.31	NA	NA
Butyric acid	5.47	NA	NA
Acetic acid	5.13	NA	NA
Lactic acid	41.02	NA	NA

DM - dry matter; OM - organic matter; NH_3-N - ammonium nitrogen; CP - crude protein; NA - not analyzed

The high pH value of AH and the low concentration of lactic acid (41.02 g kg^{-1} DM) are consistent with previous studies (Hopkins, 2000) where a high pH value and low acetic acid content of wilted haylage at a high DM content was observed. According to Chamberlain and Wilkinson (1996), anaerobic conditions in the silo are difficult to achieve when ensiling a feed with a high DM content. The N intake, excretion and N bilance are presented in Table 2.

Table 2. Intake, excretion, and N balance of the investigated feeding treatments (in g kg^{-1} DM)

Feeding treatment	N intake			N excreted		
	haylage	maize/whey	total	urine	faeces	N Bilance
A	18.1	7.8	25.0	9.1	10.4	6.5
B	19.3	7.9	27.3	9.5	10.8	6.9
C	19.5	8.0	27.4	10.1	11.2	6.1
D	19.3	8.3	27.6	9.7	10.8	7.1
Significancy	NS	NS	NS	NS	NS	NS
SEM	0.56	0.23	0.51	0.47	0.36	0.58
0 vs. add	NS	NS	NS	NS	NS	NS
lin	NS	NS	NS	NS	NS	NS
Q	NS	NS	NS	NS	NS	NS

N, nitrogen; A, B, C, D, feeding treatments, with the addition of 30 g kg^{-1} $M^{0,75}$ of concentrate, which consisted of only ground maize (treatment A) or ground maize supplemented with dried whey in the quantity of 10 % (treatment B); 20% (treatment C) or 30% (treatment D); SEM, standard error of the mean; Lin, the linear effect of the ground maize supplementation; Q, the quadratic effect of the ground maize supplementation; values in the same column did not differ significantly $P>0.05$

The effect of energy supplements to forage, on the nutritive value and nutrients utilization in ruminants, is variable and depends not only on the type and amount of carbohydrate feed (Chamberlain et al., 1993) but also on the type and nutritive value of the basic forage (Orr et al. , 2001; Vranic et al., 2018). Sugar supplementation, in a haylage-based ruminant diet, is a more efficient energy source for microbial protein synthesis which reduces the NH_3 content in the rumen and increases the utilization of N (compared to a starch supplementation) (Chamberlain et al., 1993) if the basic forage is of high nutritive value (Kim et al., 2000). Otherwise, if the basic forage has a low CP content (62 g kg^{-1} ST), energy

supplements can lead to a negative N balance (Kim et al., 2000). In this study, alfalfa haylage was of average nutritive value (CP content 120 g kg^{-1} DM) and the replacement of GM with DW did not have a negative effect on the investigated N balance parameters in wether sheep (P>0.05). However, a tendency of an increased N intake from a diet increased secretion of N from the organism, and the increased N balance with an increase of DW ratio in a diet, were observed (Table 2). Furthermore, a maximum of 30% of GM was replaced by DW in this study, while in previous studies 100% maize replacement by DW resulted in better utilization of N components of a meal (Calsamiglia et al., 2010). In the studies where a higher replacement of maize with DW (50 or 100%), as a concentrate in a diet based on a haylage, did not affect the balance and utilization of N, whereas an almost double increased urinary excretion of N (with complete replacement of maize with DW) due to the diuretic effect of whey was observed (Susmel et al., 1995). Moreover, the addition of DW to AH resulted in lower *in vivo* digestibility of CP as a consequence of the faster passage of feed through the digestive system (Vranić et al., 2016).

Conclusion

Dried whey has a potential to replace a corn grain, as a supplement to alfalfa haylage of medium quality. The replacement of 10%, 20% and 30% has no negative affect on N intake, N excretion, nor the N balance in wether sheep. A decision of replacement and quantity of GM to be replaced by DW is dependent on the prodction economy and market prices of these feeds.

Literature

AOAC (1990). Official methods of the association of analytical chemists.Vol. 2, 15th Edition. AOAC, Arlington, Virginia, USA.

Calsamiglia S., Ferret A., Reynolds C.K., Kristensen N.B., van Vuuren A.M. (2010). Strategies for optimizing nitrogen use by ruminants. Animal. 4:1184-1196.

Chamberlain D.G., Robertson S., Choung J.J. (1993). Sugars Versus Starch as Supplements to Grass-Silage - Effects on Ruminal Fermentation and the Supply of Microbial Protein to the Small-Intestine, Estimated from the Urinary-Excretion of Purine Derivatives, in Sheep. Journal of the Science of Food and Agriculture. 63: 189-194.

Chamberlain A.T., Wilkinson J.M. (1996). Feeding the Dairy Cow. Chalcombe Publications, PainShall, Ln2 3LT, UK.

DeFrain J.M., Hippen A.R., Kalscheur K.F., Schingoethe D.J. (2004). Feeding lactose increases ruminal butyrate and plasma beta-hydroxybutyrate in lactating dairy cows. Journal of Dairy Science. 87: 2486-2494.

Hopkins A . (2000). Grass, its production and utilisation. British Grassland Society.

Kim K.H., Lee S.S., Jeon B.T., Kang C.W. (2000). Effects of the pattern of energy supply on the efficiency of nitrogen utilization for microbial protein synthesis in the non-lactating cows consuming grass silage. Asian-Australasian Journal of Animal Sciences. 13: 962-966.

Miller L.A., Moorby J.M., Davies D.R., Humphreys M.O., Scollan N.D., MacRae J.C., Theodorou M.K. (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (Lolium perenne L.): milk production from late-lactation dairy cows. Grass and Forage Science. 56: 383-394.

Orr R.J., Rutter S.M., Penning P.D., Rook A.J. (2001). Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science. 56: 352-361.

SAS (1999).SAS® Software, SAS Institute Inc., Cary, North Carolina, USA.

Schingoethe D.J., Beardsley G.L. (1975). Feeding Value of Corn-Silage Containing Added Urea and Dried Whey. Journal of Dairy Science. 58: 196-201.

Susmel P., Spanghero M., Mills C.R., Stefanon B. (1995). Rumen Fermentation Characteristics and Digestibility of Cattle Diets Containing Different Whey, Maize Ratios. Animal Feed Science and Technology. 53: 81-89.

Van Soest P.J., Robertson J.B., and Lewis B.A. (1991). Method for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.

Vranić M., Knežević M., Leto J., Perčulija G., Bošnjak K., Kutnjak H., Maslov L. (2005). Kvaliteta voluminozne krme na obiteljskim poljoprivrednim gospodarstvima u RH: Monitoring kvalitete travne silaže tijekom dvije sezone zimske hranidbe muznih krava. Mljekarstvo. (55)4: 283-296.

Vranić M., Perčulija G., Bošnjak K., Leto J., Kutnjak H., Vnučec I., Hajredini P. (2011). Dodatak kukuruzne silaže travnoj silaži povećava iskoristivost dušika u hranidbi kastriranih ovnova. Stočarstvo: časopis za unapređenje stočarstva. 3: 189-199.

Vranić M., Bošnjak K., Pintar J., Leto J., Čačić I., Stipić I., Protulipac M., Bukal M. (2016). Utjecaj dodatka sirutke sjenaži lucerne na konzumaciju i probavljivost obroka u hranidbi kastriranih ovnova. Stočarstvo : časopis za unapređenje stočarstva. 69: 17-24.

Vranić M., Grbeša D., Bošnjak K., Mašek T., Jareš D. (2018). Intake and digestibility of sheep-fed alfalfa haylage supplemented with corn. Canadian Journal of Animal Science. 98: 135-143.

Utjecaj djelomične zamjene kukuruza sirutkom u prahu, kao dodatka sjenaži lucerne, na bilancu dušika u hranidbi kastriranih ovnova

Sažetak

Cilj istraživanja bio je utvrditi utjecaj djelomične zamjene mljevenog zrna kukuruza sirutkom u prahu, kao dodatka sjenaži lucerne (SL), na bilancu dušika (N) u hranidbi kastriranih ovnova. Istraživana su četiri hranidbena tretmana hranidbe SL uz dodatak 30 g koncentrata kg^{-1} $M^{0,75}$ koji se sastojao od samo mljevenog zrna kukuruza (tretman A) ili mljvenog zrna kukuruza zamijenjenog sa sirutkom u prahu 10 % (tretman B), 20 % (tretman C) ili 30 % (tretman (D). Zaključeno je da sirutka u prahu ima potencijal zamijene zrna kukuruza, kao dodatak sjenaži lucerne srednje kvalitete. Zamjena 10 %, 20 % ili 30 % nema negativnog utjecaja na konzumaciju N, izlučivanje N, niti na bilancu N kod kastriranih ovnova. Odluka o zamjeni i količini krmiva istraživanih krmiva u obroku obuhvaća područje ekonomike i tržišnih cijena ovih krmiva.

Ključne riječi: sjenaža lucerne, zrno kukuruza, sirutka u prahu, bilanca N

IZVORNI ZNANSTVENI RAD

Utjecaj sustava hranidbe na svojstva polovica i kakvoću mesa crnih slavonskih svinja

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Sažetak
Istraživanje je provedeno na 60 crnih slavonskih svinja uzgajanih u ekstenzivnom sustavu te podijeljenih u dvije skupine s obzirom na sustav hranidbe. Prva skupina (n=30) hranjena je smjesom žitarica (50 % kukuruz, 30 % ječam, 10 % zob, 10 % soja) uz dodatak lucerne tijekom vegetacijskog razdoblja. Druga skupina (n=30) hranjena je žirom tijekom zadnje faze tovnog razdoblja (90 dana). Nakon postizanja završnih težina u tovu te starosti od 20 mjeseci svinje su zaklane u komercijalnoj klaonici te su im određena svojstva polovica te parametri kakvoće mesa. Svinje hranjenje žirom tijekom zadnjeg razdoblja tova imale su deblju leđnu slaninu i najduži leđni mišić (*m. longissimus dorsi* – MLD), te također duži trup i opseg buta. Suprotno tome, svinje hranjene standardnim obrokom imale su duži but od svinja hranjenih žirom. Statistički značajna razlika je utvrđena za većinu svojstava polovica osim za debljinu MLD-a. Obzirom na parametre kakvoće mesa, statistički značajne razlike su zabilježene za pH_{24} vrijednosti izmjerene u *m. semimembranosus*, CIE L* vrijednost te gubitak vode određen EZ metodom. Svinje hranjene žirom su imale veći pH_{24} i gubitak mesnog soka, dok je veća CIE L* vrijednost boje mesa zabilježena kod svinja koje su hranjene standardnim obrocima.

Ključne riječi: crne slavonske svinje, ekstenzivni sustav, žir, kakvoća mesa

Uvod

Lokalne pasmine svinja se tradicionalno uzgajaju u ekstenzivnom sustavu na pašnjacima i u šumama. Svinje s pristupom šumskim površinama imaju mogućnost konzumacije žira. Nadalje, konzumacijom žira koji sadrži visoke razine škroba (Irinislimane i Belhaneche-Bensemra, 2017.) ostvaruju se uvjeti za postizanjem kompenzacijskog rasta svinja koji se očituje u nakupljaju većeg sadržaja mišićnih lipida i intramuskularne masti (Lebert, 2008.). U Istočnim dijelovima Hrvatske žir hrasta lužnjaka (*Quercus robur, L.*) bio je dominantan te se koristio kao izvor hrane pri uzgoju crne slavonske pasmine svinja (Margeta, 2019.). Crna slavonska svinja je jedna od tri autohtone pasmine svinja s područja Republike Hrvatske i tradicionalno se uzgaja u ekstenzivnom ili poluintenzivnom sustavu (Margeta i sur., 2016.) gdje se dodatna hranidba žirom može provoditi tijekom zimskog razdoblja godine, odnosno od mjeseca studenog do veljače (Margeta i sur., 2017.). Hranidba žirom ima pozitivan utjecaj na kvalitetu mesa, ali i zdravstveni status crnih slavonskih svinja. Karolyi i sur. (2007.) navode da se crne slavonske svinje hranjene žirom razlikuju od onih koje su hranjene standardnom smjesom u pogledu profila masnih kiselina u mišiću. Cilj istraživanja bio je istražiti utjecaj hranidbe svinja standardnom smjesom na bazi žitarica te hranidbe žirom u zadnjem dijelu tovnog razdoblja na svojstva polovica te parametre kakvoće mesa crnih slavonskih svinja uzgajanih u ekstenzivnom sustavu držanja.

Materijal i metode

Istraživanje je provedeno na 60 svinja crne slavonske pasmine uzgajanih u ekstenzivnom sustavu uzgoja te hranjenih smjesom žitarica u količini od 2 kg/dan uz dodatak zelene lucerne ad libitum. Smjesa žitarica se sastojala od 50 % kukuruza, 30 % ječma, 10 % zobi, 10 % soje. U dobi od 17 mjeseci svinje su razdvojene u dvije skupine uz različit režim hranidbe. Prva skupina (n=30) hranjena je standardnom smjesom na bazi žitarica (2,0 kg/dan, 12 % s.b., 12,5 MJ/ME), dok je druga skupina (n=30) hranjena žirom (5,0 kg/dan, 4,1 % s.b.). Navedeni režim hranidbe provodio se tijekom zadnjeg dijela tovnog razdoblja u trajanju od 90 dana (od mjeseca studenog do siječnja). Nakon završetka tovnog razdoblja, u dobi od 20 mjeseci te završnim težinama od 123 kg do 135kg, svinje su transportirane u komercijalnu klaonicu gdje su omamljene s CO_2 te usmrćene.

Naklon usmrćivanja, na liniji klanja su utvrđena svojstva polovica: debljina leđne masnoće, debljina mišića, dužina trupa „a“ (izmjerena je od *os pubis* do 1. rebra) i dužina trupa „b“ (od *os pubis* do *atlasa*) te dužina i opseg buta. Od parametara kakvoće mesa utvrđene su pH vrijednosti u *musculus semimembranosus* (MS) i *longissimus lumborum* (LL), CIE L*, CIE a*, CIE b* boja mesa te otpuštanje mesnog soka.

Opisna statistika za svojstva polovica te parametre kakvoće mesa, uključujući značajan utjecaj sustava hranidbe, obrađeni su pomoću psych i multcopm paketa (Revelle, 2017.; Hothorn i sur., 2016.) dok je homogenost varijance testirana Levenovim testom pomoću car paketa (Fox i Weisber, 2014.) u programskom okruženju R (R Development Core Team, 2020.). Značajnost razlika između skupina testirana je Fisherovim LSD testom.

Rezultati i rasprava

Analiza svojstva polovica

Opisna statistika te utvrđene statistički značajne razlike između analiziranih skupina svinja za svojstva polovica prikazani su u tablici 1. Svinje koje su u zadnjoj fazi tova hranjene sa žirom imale su značajnije deblju leđnu masnoću, dužinu polovica “a” (P<0,001) te veći opseg buta (P<0,01) u odnosu na svinje koje su bile hranjene standardnom smjesom na bazi žitarica. Suprotno tome, značajnije duži but (P<0,001) utvrđen je kod svinja hranjenih standardnom smjesom.

Tablica 1. Opisna statistika za svojstva polovica istraživanih skupina svinja u različitim sustavima hranidbe

Svojstva	Sustav hranidbe						*p*
	Standardna smjesa			Zir			
	Mean	SD	SE	Mean	SD	SE	
Debljina leđne masnoće, mm	18,30	13,68	2,50	31,48	9,10	1,63	***
Debljina mišića, mm	57,57	7,38	1,35	60,61	7,39	1,33	n.s.
Dužina polovica “a”, cm	86,33	3,34	0,61	91,10	4,87	0,88	***
Dužina polovica “b”, cm	103,03	5,05	0,92	107,10	5,35	0,96	**
Dužina buta, cm	36,00	3,02	0,55	31,84	2,95	0,53	***
Opseg buta, cm	64,27	5,25	0,96	67,71	4,44	0,80	**

n.s. – nema statističke značajnosti; * P<0,05; ** P<0,01; *** P<0,001

Prema istraživanju Tejerina i sur. (2011.) hranidba žirom osigurava svinjama veći sadržaj energije i masnih kiselina te manji sadržaj proteina. Zbog toga je kod svinja koje u svojim obrocima konzumiraju i žir očekivano pronaći i veći sadržaj masti, posebice debljinu leđne

masnoće. Prema rezultatima našeg istraživanja svinje koje su hranjene žirom imale su debljinu leđne masnoće od 31,48 mm što je značajno veće (P<0,001) od svinja hranjenih standardnim smjesama koje su imale debljinu leđne slanine 18,30 mm. Nadalje, rezultati ukazuju da su svinje hranjene standardnim smjesama imale veću debljinu mišića od svinja hranjenih žirom no značajna razlika između dvije analizirane skupine nije utvrđena (p>0,05). Sukladno rezultatima našeg istraživanja, Margeta i sur. (2017.) navode veće vrijednosti debljine mišića i leđne slanine kod svinja hranjenih žirom u odnosu na svinje koje su bile hranjene standardnim smjesama. Autori navode da je debljina leđne slanine u skupini svinja hranjenih žirom iznosila 32,57 mm, dok je utvrđena vrijednost debljine mišića bila 55,12 mm. U skupini svinja koje su bile hranjene standardnim smjesama dužina polovica kretala se od 86,33 cm (mjera a) do 103,03 cm (mjera b), dok su svinje hranjene žirom ostvarile dužine polovica od 91,10 cm (mjera a) do 107,10 cm (mjera b). Dobiveni rezultati su sukladni rezultatima Margete i sur. (2017.) koji navode da su crne slavonske svinje hranjene žirom imale duže polovice od skupine hranjene standardnom smjesom, no nije utvrđena statistički značajna razlika između skupina. Nadalje, isti autori navode da su svinje hranjene žirom imale duže butove u odnosu na crne slavonske svinje hranjene standardnom smjesom dok je utvrđena vrijednost opsega butova bila veća kod svinja hranjenih standardnom smjesom te je iznosila 67,71 cm u odnosu na svinje druge skupine koje su imale vrijednost opsega buta 64,27 cm.

Analiza parametara kakvoće mesa

Opisna statistika za svojstva kakvoće mesa prikazana je u tablici 2. Statistička značajnost između analiziranih grupa utvrđena je za završnu pH vrijednosti izmjerenu u *m. semimembranosus* (P<0,01), CIE L* vrijednost boje mesa (P<0,05) te otpuštanje mesnog soka izmjereno EZ metodom u *longissimus lumborum* (P<0,05).

Tablica 2. Opisna statistika za svojstva kakvoće mesa istraživanih skupina svinja u različitim sustavima hranidbe

Svojstva	Sustav hranidbe						P
	Standardna smjesa			Žir			
	Mean	SD	SE	Mean	SD	SE	
pH_{45}, MS	6,56	0,16	0,03	6,50	0,18	0,03	n.s.
pH_{45}, LL	6,38	0,18	0,03	6,44	0,16	0,03	n.s.
pH_{24}, MS	5,68	0,11	0,02	5,76	0,10	0,02	**
pH_{24}, LL	5,76	0,18	0,03	5,80	0,12	0,02	n.s.
CIE L*	48,93	8,27	1,51	44,99	2,97	0,53	*
CIE a*	13,88	6,25	1,14	13,08	1,72	0,31	n.s.
CIE b*	5,04	3,20	0,58	4,29	1,52	0,27	n.s.
EZ_{24} drip, LL, %	1,61	0,99	0,18	2,49	1,73	0,31	*
EZ_{48} drip, LL, %	5,58	1,66	0,30	4,69	2,44	0,44	n.s.

MS - *m. semimembranosus*; LL - *longissimus lumborum*; n.s. – nema statističke značajnosti; * P<0,05; ** P<0,01; ***P<0,001

Prema Honikelu (1999.) početna pH vrijednost mesa ne smije biti veća od 6,0, dok se završna pH vrijednost treba kretati u rasponu od 5,4 to 5,85. Skupina svinja hranjenih žirom imala je veće vrijednosti početnog pH izmjerene u *longissimus lumborum* u odnosu na svinje hranjene standardnim smjesama te niže vrijednosti izmjerene u *m. semimembranosus*. Vrijednosti početne pH izmjerene kod obje analizirane skupine svinja bile su veće od preporučenih razina. Visoke početne pH vrijednosti povezane su sa svojstvima bolje sposobnosti zadržavanja vode te tamnijom bojom mesa (Tomović i sur., 2014.) koje je karakteristično za autohtone pasmine svinja (Lebret, 2008.), ali isto mogu ukazivati na neke neželjene promjene koje se mogu javiti u mesu (tvrdo, čvrsto i suho meso). Visoke početne

pH vrijednosti su zabilježili Alfonso i sur. (2005.) kod Basque pasmine (6,6) te Kasprzyk i sur. (2017.) kod Pulawska pasmine svinja (6,17). Iz rezultata našeg istraživanja se može uočiti da su se vrijednosti završnog pH kretale od 5,68 do 5,76 kod svinja hranjenih standardnim smjesama te od 5,76 do 5,80 kod svinja hranjenih žirom. Dobiveni rezultati su sukladni rezultatima istraživanja Margete i sur. (2017.) koji navode veće početne pH vrijednosti izmjerene u *longissimus lumborumu* i *m. semimembranosus* kod skupine svinja hranjenih žirom u odnosu na svinje koje su dobivale standardnu smjesu. U odnosu na druge autohtone pasmine svinja, završne pH vrijednosti iz našeg istraživanja su bile sukladne vrijednostima dobivenima kod Pulawska pasmine svinja (5,62) (Kasprzyk i sur., 2017.) te veće u odnosu na Alantejano (5,57) i Bisaro pasminu (5,44) (Martins i sur., 2020.). Svinje hranjene standardnom smjesom imale su više vrijednosti parametara boje mesa u odnosu na svinje hranjene žirom. Režim hranidbe nije imao značajan utjecaj na CIE a* i CIE b* parametre, dok je kod CIE L* utvrđena značajna razlika između skupina ($P<0,05$). Margeta i sur. (2017.) navode da crne slavonske svinje hranjene žirom imaju tamniju boju mesa, te značajnu razliku u pogledu CIE L* parametra u odnosu na svinja koje su konzumirale standardnu smjesu. Suprotno rezultatima našeg istraživanja, Tejeda i sur. (2020.) su u svom istraživanju zabilježili više CIE L* vrijednosti kod Iberijske pasmine svinja uzgajanih u ekstenzivnim uvjetima te hranjenima žirom u odnosu na svinje koje su bile hranjenje standardnim smjesama. Autori navode da je utvrđena razlika u boji posljedica utjecaja različitog sustava hranidbe, a ne samo sustava držanja kako navodi López-Bote i sur. (2008.). Naime, u ekstenzivnim uvjetima držanja svinjama je omogućeno više kretanja i veća tjelesna aktivnost nego što je to slučaj u intenzivnom sustavu uzgoja. Veća tjelesna aktivnost dovodi do veće sinteze mioglobina.

Zaključak

U istraživanju su prikazane razlike svojstava polovica i parametara kakvoće mesa svinja crne slavonske pasmine uzgajane u ekstenzivnom sustavu te hranjene u dva različita režima, standardnom smjesom i žirom. Dobiveni rezultati ukazuju na to da hranidba žirom tijekom zadnja tri mjeseca tovnog razdoblja utječe na parametre kakvoće mesa što upućuje na mogućnost poboljšavanja određenih svojstava promjenom hranidbe. Potrebna su daljnja istraživanja u pogledu utjecaja hranidbe žirom na masno-kiselinski profil mesa crne slavonske pasmine.

Literatura

Alfonso L., Mourot J., Insausti K., Mendizabal J. A., Arana A. (2005). Comparative description of growth, fat deposition, carcass and meat quality characteristics of Basque and Large White pigs. Animal Research. 54(1): 33-42.

European Commission (2019): Communication from the commission to the european parliament, the european council, the council, the european economic and social committee and the committee of the regions. The European Green Deal.

Fox J., Weisberg S., Adler D., Bates D., Baud-Bovy G., Ellison S., Heiberger R. (2014): Package 'car. 'Version 2.0-21.

Honikel, K.O. (1999). Biochemical and physico-chemical characteristics of meat quality. Tehnologija mesa. 40(3/5): 105-123.

Hothorn T., Bretz F., Westfall P., Heiberger R. M., Schuetzenmeister A., Scheibe S., Hothorn M. T. (2016). Package 'multcomp'. Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.

Irinislimane H., Belhaneche-Bensemra N. (2017). Extraction and characterization of starch from oak acorn, sorghum, and potato and adsorption application for removal of maxilon red GRL from wastewater. Chemical Engineering Communications. 204(8): 897-906.

Lebret B. (2008). Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. Animal. 2(10): 1548-1558.

Karolyi D., Salajpal K., Kiš G., Đikić M., Jurić I. (2007). Influence of finishing diet on fatty acid profile of longissimus muscle of Black Slavonian pigs. Poljoprivreda. 13(1): 176-179.
Kasprzyk A., Tyra M., Babicz M. (2017). Fatty acid profile of pork from a local and a commercial breed. Archiv fuer Tierzucht. 58(2): 379.
Lebret B. (2008). Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. Animal. 2(10): 1548-1558.
López-Bote C.J., Toldrá F., Daza A., Ferrer J.M., Menoyo D., Silió L., Rodríguez M.C. (2008). Effect of exercise on skeletal muscle proteolytic enzyme activity and meat quality characteristics in Iberian pigs. Meat Science. 79: 71–76.
Margeta V., Gvozdanović, K., Margeta P., Kušec I. D., Radišić Ž., Galović D., Kušec G. (2016). Low input production system suitable for Black Slavonian pig breeding. Acta argiculturae Slovenica. 5: 122.
Margeta V., Gvozdanović K., Kušec I. D., Margeta P., Kušec G., Radišić Ž. (2017). The effect of the acorn in feeding on the production and slaughter traits of crna slavonska pig. *In 11th International Symposium Modern Trends in Livestock Production*. 11.-13.10.2017., Beograd, Srbija. pp: 327-334.
Martins J. M., Fialho R., Albuquerque A., Neves J., Freitas A., Nunes J. T., Charneca R. (2020). Growth, blood, carcass and meat quality traits from local pig breeds and their crosses. Animal. 14(3): 636-647.
R Development Core Team (2020): R: A language and environment for statistical computing, Version 4.0.2. Computer software]. Vienna, Austria. (dostupno na: https://www. R-project. org.)
Revelle W. R. (2017). psych: Procedures for personality and psychological research.
Smol M., Marcinek P., Duda J., Szołdrowska D. (2020). Importance of sustainable mineral resource Management in Implementing the circular economy (CE) model and the European green Deal strategy. Resources. 9(5): 55.
Tejeda J. F., Hernández-Matamoros A., Paniagua M., González E. (2020). Effect of Free-Range and Low-Protein Concentrated Diets on Growth Performance, Carcass Traits, and Meat Composition of Iberian Pig. Animals. 10(2): 273.
Tejerina D., García-Torres S., de Vaca M. C., Vázquez F. M., Cava R. (2011): Acorns (*Quercus rotundifolia Lam.*) and grass as natural sources of antioxidants and fatty acids in the "montanera" feeding of Iberian pig: Intra-and inter-annual variations. Food chemistry. 124(3): 997-1004.

Effect of feeding system on the carcass and meat quality traits of black slavonian pigs

Abstract

The study was conducted on 60 black slavonian pigs bred in an extensive system and divided into two groups according to the feeding system. The first group (n = 30) was fed a mixture of cereals (50% corn, 30% barley, 10% oats, 10% soybeans) with the addition of alfalfa during the growing season. The second group (n = 30) was fed acorns during the last phase of the fattening period (90 days). After reaching the final weight in fattening at the age of 20 months, the pigs were slaughtered in a commercial slaughterhouse and their characteristics of halves and meat quality parameters were determined. Pigs fed acorns during the last fattening period had thicker back bacon and the longest back muscle (*m. longissimus dorsi* - MLD), as well as a longer carcass and thigh circumference. In contrast, pigs fed a standard meal had a longer leg than pigs fed acorns. A statistically significant difference was found for most properties of the halves except for the thickness of the MLD. Considering the meat quality parameters, statistically, significant differences were recorded for pH_{24} values measured in semimembranosus, CIE L * value, and water loss determined by the EC method. Pigs fed acorns had higher pH_{24} and loss of meat juice, while a higher CIE L * value of meat color was recorded in pigs fed standard meals.

Key words: black slavonian pigs, extensive system, acorn, meat quality

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08

Viticulture, Enology and Pomology

IZVORNI ZNANSTVENI RAD

Mogućnosti kontrole ocjenjivača u senzornom ispitivanju vina

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Sažetak
Senzorna ispitivanja opterećena su mogućnošću pojave pogreške čiji izvor nije samo okolišni, nego i fiziološki; mjerni instrument sadrži izvore pogrešaka uvjetovane svim dimenzijama koje određuju čovjeka. Stoga je znanje i profesionalnost ocjenjivača ključna u osiguranju kakvoće rezultata svih subjektivnih metoda. Osim osiguranja edukacije i selekcije, u laboratorijskom radu potrebna je kontrola rada i rezultata ocjenjivača radi osiguranja pouzdanosti rezultata. Senzorna ispitivanja dio su analitičkih metoda provjere i utvrđivanja kakvoće za potrebe stavljanja vina na tržište u Hrvatskoj. Standardi laboratorijskog rada sukladni su međunarodnoj normi HRN EN ISO/IEC 17025, zahvaljujući kojoj su kreirani i u primjeni različiti interni alati osiguranja uvjeta za profesionalan i pouzdan rad. Primjena kontrolnih karata i drugih analiza rezultata je baza za nadogradnju u podizanju ljesvice kakvoće ovih specifičnih analiza. Cilj ovog rada bio je ispitati mogućnost analize negativnih rezultata i korištenih atributa loše kakvoće vina, te korelacije i χ^2 testa u kontroli rada ocjenjivača. Vina s manama i bolestima, te drugim nedostacima u senzornim analizama imaju negativan rezultat koji ocjenjivač mora obrazložiti. U obrazloženju može koristiti jedan ili više atributa loše kakvoće iz izbornika u internom programu kojeg se koristi za senzorna ispitivanja. U radu su analizirani rezultati dva ocjenjivača, proizvedeni tijekom dvije godine. Pokazano je da χ^2 test može biti dobar alat u analizi konzistentnosti rada ocjenjivača kroz vrijeme, a korišteni atributi podloga za dublje analize osjetljivosti i percepcije kakvoće ocjenjivača, temeljene na dužem vremenskom razdoblju.

Ključne riječi: senzorno ocjenjivanje, ocjenjivači, pouzdanost rezultata, vino

Uvod

Područje subjektivnih analitičkih metoda opterećeno je dodatnim zahtjevima osiguranja kakvoće provedbi ispitivanja zbog izvora pogreški koje počivaju u samom mjernom instrumentu. Navedene pogreške opisuje se kao fiziološke; nije ih moguće eliminirati i nije ih poželjno zanemarivati. Najvažnije grupe fizioloških pogreški (Stone, 2012) su: pogreška centralne tendencije (izbjegavanje ekstrema i pozicioniranje rezultata u prosječnom rasponu skale), pogreška očekivanja (vrednovanje uzorka obzirom na očekivanja temeljena na informacijama koje ocjenjivač ima o uzorku), „halo efekt“ (pogreška uzrokovana razlikom načina ocjenjivanja; uzorci u seriji i uzorci pojedinačno), te pogreška popustljivosti (kada ocjenjivači ocjenjuju višim rezultatima od realnih, što je najčešće u slučaju kada interni ocjenjivači analiziraju uzorke za koje su interesno vezani). Razumljivo je stoga zašto su ocjenjivači ključan element osiguranja pouzdanosti rezultata i zašto je njihova edukacija i selekcija predmet brojnih međunarodnih standarda (ISO, 2012.; ASTM, 1981.). Bez obzira na reference znanja i osjetilnih sposobnosti ocjenjivača koje mogu biti na najvišoj razini, rezultate i rad ocjenjivača potrebno je kontrolirati, osobito zbog činjenice da u većini senzornih ispitivanja u području kontrole kakvoće hrane nema ponavljanja (Jackson, 2002).

Vina i voćna vina u Hrvatskoj podliježu obveznoj senzornoj analizi prije stavljanja na tržište, osim u slučaju vina bez zaštićene oznake izvornosti od 2019. (NN 32/2019). Svrha senzorne analize je provjera proizvoda u odnosu na mane i bolesti, provjera sukladnosti proizvoda s pripadajućom specifikacijom, te određivanje kakvoće za tradicionalne izraze "Vrhunsko vino KZP" i "Kvalitetno vino KZP". Senzorna ispitivanja rade certificirani ocjenjivači; profesionalci u području vinarstva, s akademskom razinom obrazovanja i najmanje 4 godine iskustva u struci prije stjecanja prava na certificiranje. Tehnički standardi rada sukladni su ISO 8589. Protokol i rad komisije akreditirani su prema normi HRN EN ISO/IEC 17025 što je omogućilo i uvođenje dodatnih alata osiguranja pouzdanosti rezultata. Od posebnog značaja su sudjelovanje ocjenjivača u usporednim analizama, kontrolne karte komisije i ocjenjivača, te definiranje pouzdanosti rezultata obzirom na pojedinačne rezultate pet ocjenjivača. U senzornim analizama se koristi deskriptivnu metodu "DA/NE" i brojčanu metodu "100 bodova" i ocjenjivanje je apsolutno (uzorak po uzorak). Kontrolne karte prikazuju odstupanja ocjenjivača u odnosu na raspone definirane kvalitativnim značajkama rezultata. Rasponi metode „100 bodova" za pojedine vrijednosne grupe su: 1 – 59, 60 – 71, 72 – 81 i 82 – 100. Odstupanja rezultata ocjenjivača od rezultata komisije mogu biti: 1, 2, 3, -1, -2, i -3. Odstupanja kod metode „Da/ne mogu biti -1 i 1. Navedenim metodama je zajedničko objasniti negativan rezultat, tj. navesti atribute (napomene) nezadovoljavajuće kakvoće. Ocjenjivači mogu navesti jednu ili više napomena iz izbornika koji je dio programa kojeg koriste u radu. Unatoč svim uvjetima u odnosu na tehničke standarde rada i rada komisije, subjektivnost ocjenjivača je vazdaprisutna kao opterećenje u analitičkom radu; stoga je iznalaženje boljih i novih čimbenika osiguranja kakvoće rezultata stalna obveza.

Cilj ovog rada bio je prikazati mogućnost korištenja napomena loše kakvoće u praćenju rada ocjenjivača, te χ^2 testa u analizi konzistentnosti ocjenjivača kroz vrijeme, a time i mogućnosti utjecaja na izbor ocjenjivača i razinu pouzdanosti rezultata.

Materijal i metode

Za potrebe ove analize korišteni su rezultati dva ocjenjivača, proizvedeni tijekom dvije godine kontinuiranog rada (2017.-2018.). Ocjenjivači, određeni slučajnim izborom, označeni su s „D" i „I". U analizi rezultata ocjenjivača deskriptivnom analizom su obrađeni neki atributi loše kakvoće navedeni uz negativne rezultate. Rezultati ocjenjivača uspoređeni su s rezultatima komisija, pri čemu je za analizu konzistentnosti i odstupanja određena korelacija i proveden χ^2 (Chi sq) test. Chi sq je neparametarski test kojim se testira razliku izmedu opaženih i očekivanih frekvencija, te nezavisnost ili homogenost dvije varijable ili faktora.

Podaci su analizirani u statističkom programu Minitab, 2019. (Minitab, LLC, Pennsylvania, USA).

Rezultati i rasprava

U tablici 1. prikazan je broj rezultata s odstupanjima svakog ocjenjivača. Vrijednosti u stupcu 0 predstavljaju broj rezultata u bodovnom rasponu u kojem je rezultat komisija. Ocjenjivač D imao je 13,7 % odstupanja u 2017. i 11,8 % u 2018., a ocjenjivač I 18,1 % u 2017. i 21, 5% u 2018. Najveći udio odstupanja je u intervalu -1, što znači rezultat u nižem bodovnom rasponu u odnosu na rezultat komisije.

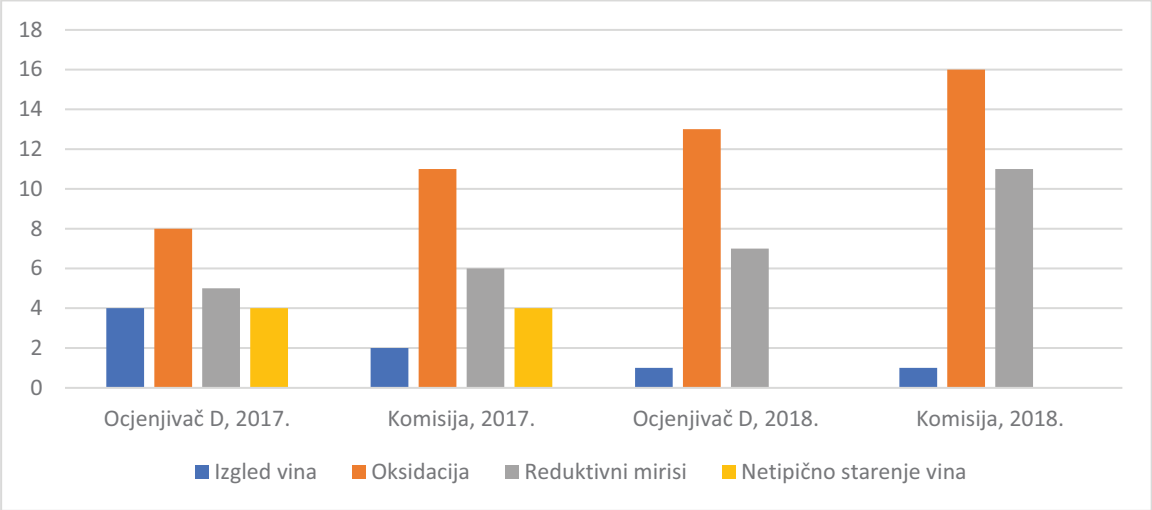
Analiza negativnih rezultata i korištenih atributa loše kakvoće

U analizi vrste i intenziteta korištenja atributa loše kakvoće korišeni su svi negativni rezultati. Ocjenjivač I imao je u 2017. 16,0 % negativnih rezultata, što je značajno više u odnosu na 11,3 % koliko su imale komisije.

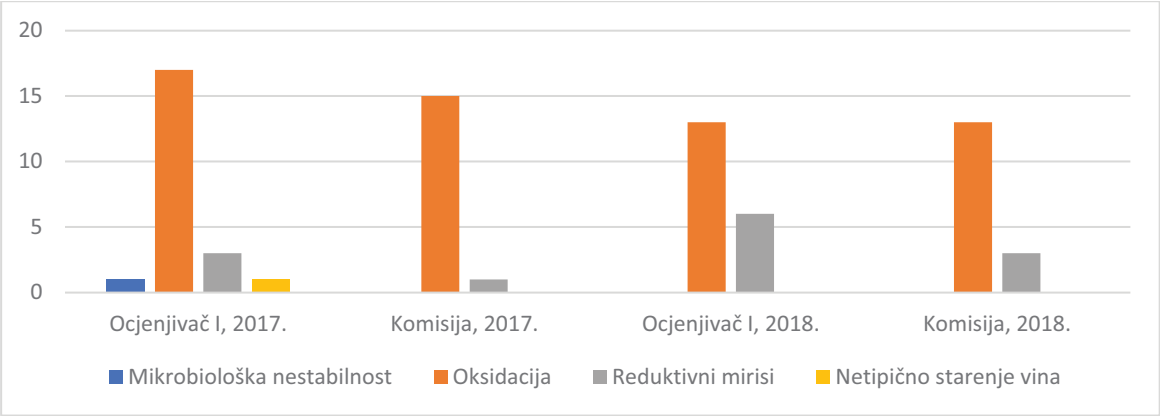
Tablica 1. Podaci senzornih analiza i rezultata

Ocjenjivač	Godina	Odstupanja u intervalima						
		-3	-2	-1	0	1	2	3
D	2017.	1	8	26	409	21	7	2
	2018.	0	4	22	382	18	7	0
I	2017.	0	7	29	199	8	0	0
	2018.	1	4	52	494	75	3	0

Slijedeće godine ovaj je ocjenjivač bio značajno usklađeniji s komisijama; imao je 6,3 % negativnih rezultata, a komisije 5,2. Ocjenjivač D imao je manje razlike u odnosu na komisije; u 2017. imao je 11,%, a komisije 9,7 % loših uzoraka, a u 2018. isti udio kao komisije. Ovi podaci su opći pokazatelji; ne govore o usklađenosti ocjenjivača i komisija u odnosu na slaganje oko negativnih rezultata i konkretnih uzoraka. U daljnjoj obradi podataka nedostaci su grupirani kao: mikrobiološki nedostaci, oksidacija, reduktivni mirisi, netipično starenje vina i loš izgled vina (boja i/ili bistroća) (Grafikon 1 i Grafikon 2).



Grafikon 1. Neki korišteni atributi loše kakvoće, ocjenjivač D



Grafikon 2. Neki korišteni atributi loše kakvoće, ocjenjivač I

Iako je broj rezultata ograničen i nedostatan za analizu koja bi omogućila donošenje zaključaka, ipak je vidljiva razlika u brojnosti i vrsti korištenih deskriptora. Obzirom na izraženu razliku između komisije i ocjenjivača I (ukupno više loših uzoraka i pojedinačno u

analiziranim problemima kakvoće), može se raspravljati o općem pristupu i percepciji kakvoće, te većoj osjetljivosti prema manjkavostima. U 2018. ocjenjivač I i komisija nisu se razlikovali u izboru atributa loše kakvoće, ali je ocjenjivač imao veći broj negativnih rezultata nego komisije. Ocjenjivač D usklađeniji je s komisijama; u percepciji loše kakvoće, korištenim atributima i njihovoj učestalosti. U percepciji najčešćih mana; oksidacije i reduktivnim mirisima ova su dva ocjenjivača pokazala različite pristupe; ocjenjivač I je bio osjetljiviji na ove mane u odnosu na komisije, a ocjenjivač D pokazao je manju osjetljivost na obe mane i imao je manje rezultata s ovim deskriptorima u odnosu na konačne rezultate.

Analiza konzistentnosti ocjenjivača

Za provođenje χ^2 testa korišteni su podaci intervalnih odstupanja -2, -1,0 i 1, a ostali intervali nisu korišteni jer χ^2 test ne dopušta provođenje testa kada je vrijednost pojavljivanja 0.

Iz prikaza broja odstupanja (Tablica 1) vidljivo je kako najveći broj rezultata ocjenjivača D pada u isti interval u kojem je i rezultat komisija. Zamjetno je smanjenje broja odstupanja u 2018. u odnosu na godinu prije, pri čemu treba naglasiti da ocjenjivač nije imao odstupanja u ekstremima 3 i -3. Rezultati ocjenjivača I bili su visoko usklađeni s konačnim rezultatima; Pearsonov koeficijent korelacije bio je 0,79 u 2017. i 0,90 u 2018. Usklađenost rezultata ocjenjivača D bila je srednje snage; uz koeficijent korelacije 0,57 u 2017. i 0,52 u 2018.

Za analizu konzistentnosti primjenom neparametarskog χ^2 testa postavljene su hipoteze: H_0: Ne postoji statistički značajna razlika između rezultata ocjenjivača D kroz dvije godine. H_1: Postoji statistički značajna razlika između rezultata ocjenjivačaa D kroz dvije godine. Odbacivanje ili prihvaćanje nulte hipoteze H_0 ovisi o p-vrijednosti. Ako je p-vrijednost manja od 0,05, nultu hipotezu se odbacuje, dok za p-vrijednosti veće od 0,05 nedostaje dovoljno dokaza za odbacivanje nulte hipoteze, tj. ne postoji statistički značajna razlika rezultata mjerenja ili ocjenjivača u odnosu na promatrano vrijeme. U slučaju ocjenjivača D (Tablica 2), prema rezultatima testa ne postoji statistički značajna razlika između analiziranih varijabli. Naime, χ^2 iznosi 3,1775, broj stupnjeva slobode (df) je 3, a p-vrijednost veća od 0,05, što znači nedovoljno dokaza za odbacivanje nulte hipoteze, tj. ne postoji statistički značajna razlika rezultata mjeritelja D kroz analizirane dvije godine. Može biti zaključeno kako je ocjenjivač D bio konzistentan u promatranom vremenu. I u slučaju ocjenjivača I postavljene hipoteze bile su iste, međutim u ovom slučaju χ^2 iznosi 162,306, uz broj stupnjeva slobode 3 (df), p-vrijednost je manja od 0,05 (Tablica 3). To znači odbacivanje nulte hipoteze, tj. postoji razlika između rezultata ocjenjivača I tijekom dvije analizirane godine, što znači da ocjenjivač I nije pokazao konzistentnost kroz promatrano vrijeme. Korelacijski koeficijenti pokazali su kako je ocjenjivač I u drugoj godini imao značajno bolju usklađenost s konačnim rezultatima pa se izostanak konzistentnosti može povezati s pozitivnim pomakom u kvaliteti rada ovog ocjenjivača. Međutim, u zaključivanju o konzistentnosti potrebno je analizirati dulje razdoblje kako bi se vidjelo je li ocjenjivač varira kroz vrijeme, što može biti lošije u odnosu na izostanak konzistentnosti kada je vezan uz pozitivne pomake u kvaliteti rada.

Tablica 2. Rezultati χ^2 testa, ocjenjivač D

Kategorija	Broj slučaja	Test proporcija	Očekivanje	Doprinos Chi-Sq testu
-2	8	0,03	13,92	2,5177
-1	26	0,05	23,20	0,3379
0	409	0,88	408,32	0,0011
1	21	0,04	18,56	0,3208
Stupnjevi slobode (df)=3	**Uzorak=464**	**p-vrijednost=0,365**		**Chi-Sq=3,17754**

Tablica 3. Rezultati χ^2 testa, ocjenjivač I

Kategorija	Broj slučaja	Test proporcija	Očekivanje	Doprinos Chi-Sq testu
-2	4	0,028807	18,004	10,893
-1	52	0,119342	74,588	6,841
0	494	0,818930	511,831	0,621
1	75	0,032922	20,576	143,951
Stupnjevi slobode (df)=3	**Uzorak=625**	**p-vrijednost=0,000**		**Chi-Sq=162,306**

Monitoring ocjenjivača može biti dio rutinskog laboratorijskog rada kada analize određenih proizvoda definiranih svojstava provode stalni ocjenjivači i kontinuirano (Bi i Kuesten, 2012; Sipos i sur., 2021; Pinto, 2021). Međutim, problem senzornih ispitivanja proizvoda može biti kada ih provodi veliki broj ocjenjivača, a pritom proizvodi nemaju jasne sastavnice senzorne kakvoće kao što može biti slučaj sa zaštićenim oznakama izvornosti vina i velikim brojem različitih proizvoda unutar zaštićene oznake. Jedan od zahtjeva prema ocjenjivačima je i slaganje s komisijom u izboru atributa kakvoće u opisivanju uzorka, i vrstom i intenzitetom (Gawel i Godden, 2008). Dostupnih referenci o korištenju atributa loše kakvoće u kontroli ocjenjivanja u operativnom laboratorijskom radu nema. Prema nekim autorima ocjenjivači su općenito usklađeniji kada je riječ o lošim vinima nego o dobrim (Hodgson, 2008). Potrebno je navesti kako u ovoj analizi nisu uključeni svi atributi koji se pojavljuju u analizama što ograničava mogućnost donošenja pouzdanih zaključaka. Na sigurnost zaključivanja utječe i činjenica da su u analizi korišteni svi rezultati i izvor pogreške može biti rezultat ispitivanja koji nije pouzdan. Za daljnju i kvalitetniju obradu podataka potrebno je filtrirati rezultate i sve koji nisu pouzdani isključiti. Nadalje, prvi uzorak uvijek je kontrolni uzorak i ocjenjivači to znaju, što također može uzrokovati pojavu tzv. pogreške prvog uzorka ili utjecaja redoslijeda (Stone, 2012). Stoga je i prve uzorke poželjno ne uključivati u analizu. U ovom radu korišten je ograničen broj podataka; i negativnih rezultata s atributima i vremenskog razdoblja. S ciljem provedbe kvalitetnije analize i sigurnijeg zaključivanja potrebno je imati veći broj podataka i dulje razdoblje.

Zaključak

U ovom radu prikazana je mogućnost korištenja analize deskriptora loše kakvoće i neparametarskog Chi-Square testa ($\chi 2$) s korelacijom radi provjere kakvoće rada ocjenjivača; usklađenosti s komisijama i konzistentnosti kroz vrijeme. Iako je uzorak malen u odnosu na promatrano vrijeme, iz dobivenih rezultata moguće je planirati daljnje smjernice detaljnijih analiza. Rezultati analize ponašanja ocjenjivača kroz vrijeme (konzistentnost), kao i analize osjetljivosti na pojedine nedostatke kakvoće mogu biti korisni u seleciji ocjenjivača za ciljane analize kao i komunikaciji na relaciji ocjenjivač – usmjerena edukacija.

Literatura

ASTM (1981). Guidelines for the Selection and Training of Sensory Panel Members. [ASTM STP 758. 1981(E)]. Pennsylvania, USA. American Society for Testing and Materials.

Gawel, R., and Godden, P. W. (2008). Evaluation of the consistency of wine quality assessments from expert wine tasters. Australian Journal of Grape and Wine Research, 14, 1–8. Raspoloživo: https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0238.2008.00001.x

Hodgson, R. T. (2008). Evaluation of wine judges at a major U.S. wine competition. Journal of Wine Economics, 3(2), 105–113.

HRN EN ISO/IEC 17025 (2017). Opći zahtjevi za osposobljenost ispitnih i umjernih laboratorija. [ISO/IEC 17025:2017; EN ISO/IEC 17025:2017]. Hrvatski zavod za norme.

ISO (2007). Sensory analysis - General guidance for the design of test rooms. [ISO 8589. 2007-12-15 (E)]. Geneva, Switzerland: International Organization for Standardization.

ISO (2012). Sensory analysis - General guidelines for the selection, training and monitoring of selected assessors and expert sensory assessors. [ISO 8586. 2012(E)]. Geneva, Switzerland: International Organization for Standardization.

Jackson, R. S. (2002). *Wine Tasting. A Professional Handbook.* Chapter 5 – Quantitative (technical) wine assessment. 113-182., San Diego, California: Academic Press. A Division of Harcourt, Inc.

Kuesten, C., and Bi, J. (2012). Application of a panel performance reliability versus product effect size (PR-ES) framework: A protein powder case study. Food Quality and Preference, 89, 104152. Raspoloživo: https://doi.org/10.1016/j.foodqual.2020.104152.

Pinto, M. M. (2021). Evaluating uncertainty in sensory analysis. A case study of the panel of tasters of the Dão Regional Wine Commission. Ciência e Técnica Vitivinícola, 36(1), 22-31.

Sipos, L., Nyitrai, Á., Hitk G., Friedrich, L. F., and Kókai, Z. (2021). Sensory Panel Performance Evaluation—Comprehensive Review of Practical Approaches. Appl. Sci.11, 11977. Raspoloživo: https://doi.org%2F10.3390%2Fapp112411977

Stone, H., Bleibaum, R. N., Thomas, H. A., and Sidel, J. L. (2012). *Sensory Evaluation Practices.* 4rd edition. San Diego, California: Academic Press and Elsevier Inc.

Possibilities of assessors' monitoring in sensory evaluation of wine

Abstract

Sensory tests are burdened with the possibility of the error occurrence whose source is not only environmental but also physiological; the measuring instrument contains sources of errors conditioned by the all human dimensions. Therefore, the knowledge and professionalism of the assessor are crucial in ensuring the quality of the results in all subjective testing. In addition to education and selection, laboratory work requires control of the work and results of assessors to ensure the reliability of the results. Sensory testing is part of analytical methods of control and quality clasification in Croatia before placing wine on the market. Laboratory standards are organised according to HRN EN ISO/IEC 17025, and various internal tools have been created and applied to ensure conditions for professional and reliable work. Control charts and other existing forms of results monitoring are the basis for upgrading and creating new tools in quality assurance in laboratory work. The aim of this paper was to test the possibility of using the attributes of wine faults and $\chi 2$ test in the assessors' monitoring. In case of negative results and wines with faults, the assessor have to explain it with one or more appropriate descriptors. He may choose one or more attributes of poor quality from the menu in the internal program used for sensory testing. The results of two assessors, produced during two years were analyzed. It has been shown that the $\chi 2$ test could be a good tool to monitor the assessor's consistency over time and that the analysis of attributes based on a longer period can be the basis for deeper testing of assessors' sensitivity and perception of specific quality parameters.

Key words: sensory evaluation, assessor, result reliability, wine

IZVORNI ZNANSTVENI RAD

Utjecaj defolijacije na kemijski sastav grožđa sorte 'Graševina' u uvjetima vinogorja Srijem

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Sažetak
Defolijacija je ampelotehnički zahvat kojim se uklanjaju listovi vinove loze. Ovaj zahvat može se koristiti u različitim fenofazama razvoja vinove loze. Ovisno o periodu obavljanja, defolijacija može biti rana ili kasna. Rana defolijacija podrazumijeva period uklanjanje lišća prije cvatnje do zametanja bobica, dok se kasna defolijacija obavlja početkom šare bobica. U ovom radu su istraživana tri termina defolijacije te njihov utjecaj na kemijski sastav grožđa sorte Graševina. Defolijacija nakon cvatnje rezultirala je najvišim sadržajem ukupnih polifenola, kod defolijacije u zoni grožđa početkom šare utvrđen je najviši sadržaj šećera i ukupnih kiselina, dok je defolijacija iznad zone grožđa početkom šare sveobuhvatno imala statistički najmanje značajan utjecaj na kemijski sastav grožđa

Ključne riječi: defolijacija, vinova loza, Graševina

Uvod

Graševina je gospodarski najvažnija sorta vinove loze koja se uzgaja na gotovo jednoj četvrtini ukupnih vinogradarskih površina u Republici Hrvatskoj (Maletić i sur., 2015., Vinogradarski registar – APPRRR). Regiju Slavonija i Hrvatsko Podunavlje čine dvije vinogradarske podregije Slavonija i Hrvatsko Podunavlje. Podregiju Hrvatsko Podunavlje čine tri vinogorja: Srijem, Erdut i Baranja. Ampelotehnički zahvati izvode se tijekom vegetacije na zelenim dijelovima vinove loze, a mogu obuhvaćati pljevljenje, prorjeđivanje grozdova, defolijaciju i vršikanje (Mirošević, 2008.). Defolijacija je ampelotehnički zahvat kojim se odstranjuju bazalne ili vršni listove u različitim fenofazama. Defolijacija se može primijeniti u razdoblju od prije početka cvatnje do početka šare s različitim efektima na grožđe. Ovim zahvatom značajno utječemo na promjenu mikorklimata trsa (Sabbatini i Howell, 2010.). Fotosintetska aktivnost listova u zoni grožđa u fenofazi šare je niža nego u zoni iznad grozdova i zato provedba defolijacija u ovoj fazi ima intenzivan utjecaj na mikroklimat trsa, a manji na kemijski sastav grožđa. Suprotno od navedenog, uklanjanjem listova u zoni grožđa prije početka cvatnje postiže se veći utjecaj na fenologiju vinove loze, mikoklimat trsa i kemijski sastav grožđa (Pastore i sur., 2013.). Osrečak i sur. (2011.) su istraživali utjecaj djelomične defolijacije na sortama Traminac i Manzoni bijeli. Primjena zahvata utjecala je na povećanje udjela fenolnih spojeva, dok na ostale parametre nije imala značajniji utjecaj. U posljednjih nekoliko godina, značajna se pažnje posvećuje defolijaciji iznad zone grozda nakon šare. Uslijed klimatskih promjena dolazi do ranijeg početka vegetacije kod vinove loze kao i ranijeg dozrijevanja grožđa koje se odvija pri visokim temperaturama. Spomenuto značajno utječe na povećanje sadržaja šećera, smanjenje sadržaja organskih kiselina i na degradaciju hlapljivih spojeva u grožđu (Palliotti i sur., 2014.). U posljednje se vrijeme istražuje utjecaj kasne djelomične defolijacije iznad zone grozda početkom šare kojom se može odgoditi dozrijevanje grožđa. Primjena defolijacije iznad zone grožđa odgodila dozrijevanje sorte Rizling rajnski (Palliotti i sur., 2013.). U

istraživanjima Buesa i sur. (2019.) prikazano je kako je kasna defolijacija iznad zone grožđa uspješan zahvat u odgađanju dozrijevanja grožđa. Navedene tvrdnje usuglašene su s istraživanjima Palliotti i sur. (2013.) u kojim je primjenom defolijacijom iznad zone grožđa nakon šare odgođeno dozrijevanje sorte Sangiovese.

Cilj istraživanja je utvrditi utjecaj različitih rokova defolijacije na kemijski sastav grožđa i gospodarske karakteristike sorte Graševina u vinogorju Srijem.

Materijal i metode

Pokusni vinograd smješten je na obroncima Fruške gore južno od lokaliteta Principovac u blizini mjesta Ilok (vinogorje Srijem). Površina predmetnog vinograda je 0,5 ha. Ekspozicija nasada je sjeverozapad-jugoistok na nadmorskoj visini od 204 m. Razmak sadnje je 0,8 × 2,0 m. Uzgojni oblik je dvostruki Guyot. Prosječno opterećenje trsa je 2,0 kg. Nasad je podignut na podlozi *Vitis berlandieri x Vitis riparia* Kober 5BB.

Pokus je postavljen slučajnim bloknim rasporedom s četiri pokusne varijante 2020. godine. Svaka varijanta ima tri ponavljanja s pet trsova. Jedna varijanta predstavlja bazalnu defolijaciju po završetku cvatnje (Dfc), druga bazalnu početkom šare (Dfs), treća defolijaciju iznad zone grozda također početkom šare (Dfsa) te je četvrta varijanta kontrola (K). Tijekom bazalne defolijacije uklonjena u četiri bazalna lista, dok su se u defolijaciji iznad zone grozda uklonili svi listovi iznad zone grozda.

U tehnološkoj zrelosti je utvrđen prirod po trsu. Na reprezentativnima uzorcima grožđa utvrđen je sadržaj šećera (Oe^0), ukupna kiselost (g L^{-1}), pH vrijednost, ukupnih polifenola i pojedinačnih organskih kiselina.

Sadržaj šećera utvrđen je refraktometrom. Sadržaj ukupnih kiselina utvrđen je direktnom titracijom. U tikvicu je ispipetirano 10 mL uzorka te se dodalo nekoliko kapi bromtimolplavog koji služi kao indikator. Titriralo se s 0,1M natrijevom lužinom (NaOH) do pojave plavo-maslinasto zelene boje te se na osnovi njezinog utroška izračunavala ukupna kiselost koja se izražava kao vinska kiselina u g. pH vrijednost se utvrdila pomoću pH metra. Sadržaj pojedinačnih organskih kiselina (vinske, jabučne i limunske) u moštu određen je pomoću HPLC-a (High-Performance Liquid Chromatography) iz prosječnog uzorka svježe iscijeđenog, centrifugiranog (13000 x g, centrifuga VWR Mega Star 600R) i pročišćenog mošta (Zoecklein i sur., 1995.).

Postupak ekstrakcije polifenolnih spojeve iz kožice bobice: uzorci liofiliziranih (lifilizator Christ) i usitnjenih kožica grožđa odvagani su u tri ponavljanja od 500 mg. U svaki uzorak dodano je 10 mL 20 % otopine acetonitrila. Tako pripremljeni uzorci ekstrahirani su na magnetnoj miješalici u trajanju od sat vremena uz zagrijavanje na 50 °C. Dobiveni ekstrakti fenola profiltrirani su pomoću PTFE filtera promjera pora 0,22 µm i takvi su korišteni u daljnjoj analizi.

Određivanje ukupnih fenola spektrofotometrijski Folin-Ciocalteu metodom: U odmjernu tikvicu od 10 mL otpipetirano je 100 µL ekstrakta fenola te je dodano 6 mL destilirane vode i 50 µL Folin-Ciocalteu-ovog reagensa. Reakcijska smjesa miješana je 8 minuta i 30 sekundi te je nakon toga dodano 2 mL 20 % otopine natrijeva karbonata. Odmjerne tikvice zatim su nadopunjene destiliranom vodom do oznake. Tako pripremljene otopine stajale su 16 minuta u vodenoj kupelji na 50 °C. Apsorbancija je mjerena pri valnoj duljini od 765 nm. Slijepa proba je ovako pripremljena otopina te je samo, umjesto ekstrakta, dodan 1 mL destilirane vode. Rezultati su izraženi u mg kg^{-1} suhe kožice.

Značajnost razlika između pokusnih varijanata utvrđena je primjenom jednosmjerne analize varijance (engl. One - Way ANOVA). Usporedba srednjih vrijednosti provedena je pomoću Duncan Multiple Range testa. Za statističku obradu podataka upotrijebljen je SAS v 9.3 statistički softvera (2012, SAS Institute Inc., Cary, NC, SAD).

Rezultati i rasprava

Iz dobivenih rezultata (Tablica 1.) je vidljivo kako ne postoji značajna razlika između pokusnih varijanta u prirodu po trsu. Najveći prirod je zabilježen kod kontrolne varijante (3,70 kg), a najmanji (3,31 kg) ima varijanta defolijacije u fenofazi šare iznad zone grozdova (Dfsa). Dobiveni rezultati su u skladu s istraživanjima Palliotti i sur. (2013.) koji su utvrdili da ne postoji značajna razlika u prirodu po trsu, masi grozda i masi bobe između defolijacije u fenofazi šare iznad zone grozdova i kontrole. Suprotno od navedenog su pak utvrdili Pastore i sur. (2013.) u svom istraživanju u kojem su utvrđene značajne razlike između defolijacije prije cvatnje i defolijacije nakon šare u prirodu po trsu i masi grozda. U njihovom istraživanju dokazano je kako s ranom defolijacijom u vegetaciji možemo očekivati značajno smanjenje priroda.

Tablica 1. Usporedba srednjih vrijednosti promtranih gospodarskih svojstava

Pokusna varijanta	Prirod po trsu (kg trs -1)	Sadržaj šećera (Oe^0)	Sadržaj ukupnih kiselina (g L^{-1})	pH vrijednost
K	3,70 a	82,00 c	6,40 b	3,32 c
Dfc	3,63 a	84,00 b	5,64 d	3,32 bc
Dfs	3,51 a	86,00 a	6,57 a	3,38 ab
Dfsa	3,31 a	75,00 d	5,96 c	3,41 a

*srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini $p<0.05$ korištenjem Duncan's multiple-range testa

Provedenim istraživanjem utvrđene su značajne razlike u sadržaju šećera (Oe^0) između pokusnih varijanata. Prosječno najmanji sadržaj šećera (75,00 Oe^0) utvrđen je kod defolijacija u fenofazi šare iznad zone grozdova (Dfsa), dok je najviši (86,00 Oe^0) utvrđen kod varijanta defolijacija u zoni grožđa početkom fenofaze šare grozda (Dfs). Varijanata defolijacija nakon cvatnje (Dfc) ima utvrđen sadržaj šećera od 84,00 Oe^0, dok kontrola (K) sadrži 82,00 Oe^0 šećera. Sadržaj ukupnih kiselina mošta značajno se razlikuje među varijantama. Prosječni najniži sadržaj (5,64 g L^{-1}) utvrđen je kod varijante defolijacije nakon cvatnje (Dfc), dok je najviši sadržaj ukupnih kiselina (6,57 g L^{-1}) utvrđen kod defolijacije u zoni grožđa početkom fenofaze šare grozda (Dfs). Kod kontrolne varijante (K) je utvrđen viši sadržaj (6,4 g L^{-1}) u odnosu na varijantu defolijacije u fenofazi šare iznad zone grozdova (5,96 g L^{-1}). Značajne razlike su utvrđene i kod pH vrijednosti mošta. Kod varijante defolijacija u fenofazi šare iznad zone grozdova (Dfsa) utvrđena je najviša vrijednost te se ona značajno razlikuje od ostalih pokusnih varijanta. Najniža vrijednost je utvrđena kod kontrolne varijante. Sabbatini i Howell (2010.) u svom istraživanju utvrdili su kako se, ovisno o intenzitetu defolijacije prije cvatnje, razlikovao sadržaj šećera, dok se pH vrijednost i sadržaj ukupnih kiselina nisu statsitički značajno razlikovali između pokusnih varijanata. Kod defolijacija prije cvatnje u usporedbi je zabilježen viši sadržaj šećera u odnosu na defolijaciju nakon šare prema istraživanjima Pastore i sur. (2013.). Razlike nisu utvrđene kod sadržaja ukupnih kiselina i pH vrijednosti. Kod defolijacija iznad zone grožđa utvrđen je značajno niži sadržaj šećera u odnosu na kontrolnu varijnatu prema istraživanjima Palliotti i sur. (2013.) zbog odgode dozrijevanja grožđa. Statistički značajne razlike utvrđene su u sadržaju organskih kiselina između pokusnih varijanata (Tablica 2.).

Tablica 2. Usporedba srednjih vrijednosti sadržaja pojedinačnih organskih kiselina (g L^{-1})

Pokusna varijanta	Limunska kiselina (g L^{-1})	Vinska kiselina (g L^{-1})	Jabučna kiselina (g L^{-1})
K	0,14 a	4,54 a	0,91 b
Dfc	0,11 b	4,36 b	0,88 b
Dfs	0,13 a	4,35 b	0,87 b
Dfsa	0,11 b	4,37 b	0,97 a

*srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini $p<0.05$ korištenjem Duncan's multiple-range testa

Najviši sadržaj vinske kiseline (4,54 g L^{-1}) utvrđen je kod kontrola (K). Između ostalih pokusnih varijanata nije bilo statistički značajne razlike. Kod varijante defolijacije u fenofazi šare iznad zone grozdova (Dfsa) utvrđen je najviši sadržaj jabučne kiseline (0,97 g L^{-1}). Između ostalih pokusnih varinata nije bilo statistički značajne razlike. Kod sadržaja limunske kiseline kod kontrole i defolijacije u fenofazi šare iznad zone grozdova (Dfsa) utvrđena je statistički viša vrijednost u odnosu na preostale dvije pokusne varijante (Dfc i Dfs).

Tablica 3. Usporedba srednjih vrijednosti sadržaja ukupnih polifenola (mg kg^{-1} suhe kožice)

Pokusna varijanta	Sadržaj ukupnih polifenola (mg kg^{-1})
K	7940,00 b
Dfc	9006,67 a
Dfs	7793,33 c
Dfsa	6500,00 d

*srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini $p<0.05$ korištenjem Duncan's multiple-range testa

Utvrđene su značajne razlika u sadržaju ukupnih polifenola između pokusnih varijanti (Tablica 3.). Kod varijante defolijacije nakon cvatnje (Dfc) utvrđen je najviši ukupni sadržaj polifenola (9006,67 mg kg^{-1}). Najmanji sadržaj ukupnih polifenola (6500 mg kg^{-1}) utvrđen je kod varijanta defolijacije u fenofazi šare iznad zone grozdova (Dfsa). Varijanta defolijacija u zoni grožđa početkom fenofaze šare grozda (Dfs) imala je vrijednost sadržaj ukupnih polifenola od 7793,33 mg kg^{-1}, dok je kod kontrole (K) utvrđena vrijednost ukupnih polifenola u iznosu od 7940,00 mg kg^{-1}. Kemp i sur. (2011.) su u svome istraživanju utvrdili kako rana defolijacija značajno utječe na povećanje sadržaja ukupnih polifenola u odnosu na kontrolu. Pastorea i sur. (2013.) utvrdili su viši sadržaj ukupnih polifenola u obje varijante defolijacije u odnosu na kontrolu. Viši sadržaj ukupnih polifenola kod provođenja defolijacije utvrdili su u svom radu Osrečak i sur. (2011.). na sortama Manzoni bijeli i Traminac, dok na sorti Graševina nije utvrđena značajna razlika u odnosu na kontrolu. Suprotno od navedenog je utvrđeno u istraživanju Palliotti i sur. (2013.).

Zaključak

Na osnovi provedenog istraživanja 2020. godine na sorti Graševina vidljivo je kako ne postoje statistički značajne razlike u prirodu po trsu. Defolijacija koja je provedena nakon cvatnje imala je najviši sadržaj ukupnih polifenola. Defolijacija u zoni grožđa početkom fenofaze šare grozda rezultirala je s najvišim sadržajem šećera i najvišom ukupnom kiselosti. Defolijacija u fenofazi šare iznad zone grozdova imala je očekivano najniži sadržaja šećera te najniži sadržaj ukupnih polifenola. Spomenuta varijanta defolijacije imala je najviši sadržaj jabučne kiseline koji nam ukazuje na usporen proces dozrijevanja. Ovisno o željenom učinku svaka varijanta defolijacije može se primjenjivati u vinogradarskoj proizvodnji. Ovo istraživanje je bazirano na jednogodišnjem pokusu koji bi trebalo primijeniti u višegodišnjem istraživanju kako bi se dobili što relevantniji rezultati.

Literatura

Buesa I., Basile B., Caccavello G., Merli M.K. (2019). Delaying berry ripening of Bobal and Tempranillo grapevines by late leaf removal in a semi-arid and temperate-warm climate under different water regimes. Australian Journal od Grape and Wine Research. 25(1):70 – 82. Available from: http://redivia.gva.es/handle/20.500.11939/6321

Kemp B., Harrison R., Creasy, G. (2011). Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in *Vitis vinifera* L. cv. Pinot Noir wine. Australian Journal of Grape and Wine Research. 17 (2): 270 - 279. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0238.2011.00150.x

Maletić E., Karoglan Kontić J., Pejić I., Preiner D., Zdunić G., Bubola M., Stupić D., Andabaka Ž., Marković Z., Šimon S., Žulj Mihaljević M., Ilijaš I., Marković D. (2015). Zelena knjiga: Hrvatske izvorne sorte vinove loze. Zagreb, Hrvatska: Državni zavod za zaštitu prirode.

Mirošević N., Karoglan Kontić J. (2008). Vinogradarstvo. Nakladni zavod Globus, Zagreb.

Osrečak M., Kozina B., Maslov L., Karloglan M. (2011). Utjecaj djelomične defolijacije na koncentraciju polifenola u vinima Graševine, Traminca i Manzonija bijelog (*Vitis vinifera* L.). Objavljeno u Zborniku radova 46th Croatian and 6th International Symposium on Agriculture. Milan Pospišil (ur.), 972 – 975. Opatija, Hrvatska

Palliotti A., Panara F., Silvestroni O., Lanari,V., Sabbatini P., Howell G.S., Gatti M., Poni S. (2013). Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (Vitis vinifera L.) grapevines. Australian Journal od Grape and Wine Research. 19: 369 - 377.

Palliotti A., Tombesi S., Silvestroni O., Lanari V., Gatti M., Poni S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripeninf: A review. Scientia Horticulturae 178: 43–54.

Pastore C., Zenoni S., Fasoli M., Pezzotti M., Tornielli G.B., Filippetti I. (2013). Selective defoliation affects plant growth, fruit transcriprional ripening program and flavonoid metabolism in grapevine. BMC Plant Biology. 13 (30):1 – 16. Available from: https://bmcplantbiol.biomedcentral.com/articles/10.1186/1471-2229-13-30

Sabbatini P., Howell G.S. (2010). Effects of early defoliation on yield, fruit composition and harvest season cluster rot complex od grape vines. HortScience. 45 (12): 1804 - 1808.

SAS System Software, v. 9.3. (2012). SAS Institute Inc., Cary, NC, USA.

Vinogradarski registar - APPRRR. https://www.apprrr.hr/registri/

Zoecklein B. K. (1995). Wine Analysis and Production. Chapman & Hall. New York. SAD.

Influence of a partial defoliation on the chemical composition of grapes of the grapevine variety Grasevina in conditions of the wine growing area Srijem

Abstract

Defoliation is a canopy management technique of leaf removal from vine. This operation can be used during various phenophases of grapevine development. Depending on the period, defoliation can be early or late. Early defoliation is performed in the period before blooming to berry development, while late defoliation is preformed at begining the verasion. In this research, defoliation was performed at three different stages and the impact on chemical composition of grape was evaluated. Defoliation after berry development resulted in the highest amount of total polyphenols, defoliation in the grape zone at the beginning of verasion resulted in the highest amount of sugar and total acids, while the apical defoliation did not significantly affect chemical composition of grape.

Key words: defoliation, grapevine, Grasevina

IZVORNI ZNANSTVENI RAD

Bioaktivne komponente i mineralni sastav lješnjaka 'Istarski duguljasti' i 'Rimski'

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Sažetak
Cilj ovog istraživanja je bio utvrditi sadržaj ukupnih fenola i flavonoida, antioksidacijsku aktivnost te mineralni sastav dviju najviše uzgajanih sorata lijeske u Hrvatskoj 'Rimski i 'Istarski duguljasti' . U plodovima sorata 'Rimski' i 'Istarski duguljasti' utvrđene su sljedeće vrijednosti bioaktivnih tvari: ukupni fenoli 1,572 ± 0,12 i 1,912 ± 0,21 mg GAE g^{-1} uzorka, ukupni flavonoidi 5,9 ± 0,57 i 5,9 ± 0,03 mg ekvivalenti kvercetina g^{-1} uzorka te antioksidacijska aktivnost 3,25 ± 0,08 i 4,43 ± 0,24 mM Trolox g^{-1} uzorka (respektivno). Kod plodova sorte 'Istarski duguljasti' utvrđen je veći sadržaj fenola kao i antiooksidacijska aktivnost u odnosu na plodove sorte 'Rimski'. Najviši sadržaj ukupnih flavonoida utvrđen je kod ekstrakcije maceracijom u trajanju od 24 h. Plodovi obje sorte imali su sadržaj kalcija, magnezija, željeza, bakra i cinka srazmjeran sadržaju navedenih minerala utvrđenih i u drugim znanstvenim publikacijama za druge sorte lijeske. Na temelju dobivenih rezultata može se zaključiti da su plodovi sorata 'Rimski' i 'Istarski duguljasti' uzgajani u agroekološkim uvjetima zapadne Slavonije vrijedan izvor bioaktivnih komponenata.

Ključne riječi: antioksidacijsaka aktivnost, lješnjak, ukupni fenoli, ukupni flavonoidi

Uvod

Lijeska je jedna od najvažnijih lupinastih vrsta, a prema podacima FAOSTAT-a (2021) najveći proizvođači su Turska (776046 t), zatim Italija (98530 t), Azerbajdžan (53793 t), SAD (39920 t) te Čile (35000 t). U Hrvatskoj lijeska se uzgaja na površini od 6449 ha, s ukupnim prirodom od 3154 t (DZS, 2021). Većina proizvodnje lijeske je smještena u kontinentalnom dijelu, a u nasadima su najzastupljenije sorte 'Rimski i 'Istarski duguljasti' (Vujević i sur., 2017). Zbog sve veće percepcije orašastih plodova kao prirodnih izvora određenih esencijalnih elemenata u ljudskoj prehrani, istraživanja koncentracije esencijalnih elemenata i elemenata u tragovima (B, Se, Cu, Zn, Fe i Mn) u plodovima lupinastih vrsta postaju sve važnija (Celik i sur., 2014; Altun i sur., 2013; Pelvan i sur., 2012). Cilj ovog istraživanja bio je utvrditi sadržaj ukupnih fenola i flavonoida, antioksidacijsku aktivnosti te mineralni sastav dviju najviše uzgajanih sorata lijeske u Hrvatskoj, 'Rimski' i 'Istarski duguljasti' u agroekološkim uvjetima zapadne Slavonije.

Materijal i metode

Istraživanje je provedeno na plodovima lijeske sorata ‘Istarski duguljasti’ i ‘Rimski’. Nasad lijeske smješten je u mjestu Rešetari, okolica Nove Gradiške (ZŠ 45° 14' 34.4286", ZD 17° 25' 10.7466"). U voćnjaku ukupne površine 1,2 ha u jesen 2015. godine posađene su dvogodišnje sadnice lijeske na vlastitom korijenu, s razmakom između redova 5 m, a u redu 4 m. Lijeska je uzgajana kao stablo u uzgojnom obliku vaza. Odnos površine na kojoj je

zasađena sorta 'Rimski' kao glavna te 'Istarski duguljasti' kao sorta oprašivač iznosi 80:20. Voćnjak nije navodnjavan niti zaštićen zaštitnom mrežom, a zaštita od bolesti i štetočina provodila se sukladno integriranoj proizvodnji. Berba plodova obje sorte provedena je u punoj zrelosti, u prvoj dekadi mjeseca rujna 2020. godine. Prirod po stablu iznosio je 8,5 kg. Nakon berbe plodovi su sušeni tradicionalnom metodom - na suncu, do postizanja sadržaja vlage ispod 10 %. Za uzorke su odabrane tri repeticije sa po 50 plodova svake sorte. Prije analiza plodovima je uklonjena lupina pomoću drobilice, a zatim su usitnjeni i homogenizirani u mlinu GM200 (Retsch GmbH, Hann, Njemačka). Određivanje sadržaja ukupnih fenola napravljeno je po modificiranoj metodi Fernandes i sur. (2012). Uzorci su pomiješani sa 0,1 mL Folin- Ciocalteu reagensa, 0,250 mL 20% otopine Na_2CO_3 i 4,4 mL vode. Reakcija se provodila 60 minuta nakon čega je izmjerena absorbanca pri 725 nm UV-Vis spectrophotometers Cary 100 (Agilent, Technologies, Santa Clara, SAD). Rezultati su izraženi kao miligram ekvivalenata galne kiseline po 1 g uzorka (mg GAE g^{-1}). Antioksidacijska aktivnost određena je prema DPPH metodi Oyvind (1989) modificiranoj po metodi Bartoszek i Polak (2016). UV-vis spektrofotometrijska mjerenja provedena su pri 515 nm pomoću Cary 100 spektrometra. Antoksidacijska aktivnost izražena u mM mL^{-1} Troloxa po 1 gramu uzorka izračunava se prema formuli:

$$(1)\ Antioksidacijska\ aktivnost = c \cdot \frac{V}{m}$$

gdje je: c - koncentracija Troloxa izračunata iz kalibracijskog pravca (mM mL^{-1});V - volumen etanola korišten za ekstrakciju (mL); m - masa uzorka (g)

Za određivanje ukupnih flavonoida korištena je modificirana kolorimetrijska metoda s $AlCl_3$ prema Khlifi i sur. (2011). Ekstrakcija flavonoida iz uzorka provedena je na sljedeća tri načina: i) ultrazvučnom ekstrakcijom; ii) maceracijom; iii) kiselom maceracijom.

Ultrazvučnom ekstrakcijom 0,5 g uzorka pomješano je sa 10 mL metanola te je uzorak stavljen 30 minuta na sobnu temperatu na UZV kupelj (Falc Intruments srl., Treviglio, Italija). Ekstrakti su centrifugirani pri 5000 rpm 5 minuta. Supernanatant je bio pomješan s 5 mL metanola te ponovno ekstrahiran i centrifugiran. Supernatant je pomiješan s metanolom u omjeru 1:20. Maceracijom je 0,5 g uzorka pomiješano s 10 mL metanola na 24 h pri sobnoj temperaturi. Uzorci su centrifugirani 5 minuta pri 5 000 rpm, nakon čega se supernatant razrijedio s metanolom u omjeru 1:20. Kiselom maceracijom 0,5 g uzorka pomješano je s 10 mL metanola i 2 mL 37 % m m^{-1} HCl na 24 h pri sobnoj temperaturi. Uzorci su centrifugirani 5 minuta pri 5 000 rpm, nakon čega se supernatant razrijedio s metanolom u omjeru 1:20. U pripremljene ekstrakte dodano je 1, 0 mL 2% m V^{-1} otopine $AlCl_3$. Reakcija se odvijala 15 minuta pri sobnoj temperaturi, nakon čega je izmjerena apsorbancija uzoraka pri 415 nm. Rezultat izražen kao mg ekvivalenta kvercetina g^{-1} biljnog materijala izračunat je prema slijedećoj formuli:

$$(2)\ mg\ ekvivalent\ kvercetina = c'uz \cdot V \cdot \frac{0{,}001}{m}$$

gdje je: V - volumen otopine uzorka (10 mL); m - masa ispitivanog uzorka (0,5 g)

Za određivanje ukupnog dušika (organskog i anorganskog) koristila se Dumasova metoda (Dumas, 1831) korištenjem analizatora dušika i proteina NA2100 Nitrogen and Protein Analyzer (ThermoQuest-CE Instruments, Rodeno, Italija) prema odobrenoj metodi AOAC International (1995). Koncentracija P, K, Ca, Mg, S, Na, Fe, Mn, B, Cu i Zn za sve uzorke određene su induktivno spregnutom atomskom emisijskom spektroskopijom (ICP-AES) upotrebom Optima 2100 DV (PerkinElmer Inc., Wellesley, SAD). Prilagodba instrumentalnih parametara bila je bitna za simultano određivanje osam proučavanih elemenata koji su pokrivali raspon valnih duljina od 206,200 nm do 327,393 nm.

Podatci su statistički analizirani analizom varijance (ANOVA) te su nakon signifikantnog F-testa srednje vrijednosti uspoređene s odgovarajućim testovima na razini signifikantnosti $P \leq 0{,}05$ pomoću računalnog programa StatView (SAS Institute Inc. Version 5.0.1).

Rezultati i rasprava

Sadržaj ukupnih fenola i antioksidacijska aktivnost u plodovima lijeske 'Istarski duguljasti' i 'Rimski' prikazani su u Tablici 1. Plodovi sorte 'Rimski' imali su sadržaj ukupnih fenola 1,572 ± 0,12 mg GAE g^{-1} uzorka, a plodovi sorte 'Istarski duguljasti' 1,912 ± 0,21 mg GAE g^{-1} uzorka. Lješnjak, s rasponom od 430 do 1169 mg GAE 100 g^{-1} ima manju zastupljenost ukupnih fenola od pekan oraha, oraha i pistacija (Wu i sur., 2004). Dobivene vrijednosti ukupnih fenola su u suglasju s vrijednostima koje su dobili Pelvan i sur. (2012). U navedenom istraživanju raspon ukupnih fenola u plodovima sedam sorata lijeske uzgajanih u Turskoj se kretao od 178 ± 8 mg GAE 100 g^{-1} do 486 ± 18 mg GAE 100 g^{-1} (Pelvan i sur., 2012). Dobivene vrijednosti antioksidacijske aktivnosti u plodovima sorata 'Rimski' i 'Istarski duguljasti' sukladne su s onima koje su utvrdili Altun i sur. (2013). Antioksidacijska aktivnost kod plodova 'Istarski duguljasti' iznosila je 4,43 ± 0,24 mM Trolox g^{-1} uzorka, a kod plodova sorte 'Rimski' 3,25 ± 0,08 mM Trolox g^{-1} uzorka. Utvrđeno je da su plodovi sorte 'Rimski' imali manji sadržaj fenola i antioksidacijsku aktivnost od sorte 'Istarski duguljasti'. Pelvan i sur. (2012) navode da sadržaj ukupnih fenola ovisi o sorti kao i agroekološkim čimbenicima.

Tablica 1. Sadržaj ukupnih fenola i antioksidacijska aktivnost u plodovima lijeske sorata 'Rimski' i 'Istarski duguljasti'.

Sorta	Sadržaj ukupnih fenola (mg GAE g^{-1} uzorka)	Antioksidcijska aktivnost (mM mL^{-1} Trolox g^{-1} uzorka)
'Rimski'	1,57 ± 0,12 b	3,25 ± 0,08 b
'Istarski duguljasti'	1,91 ± 0,21 a	4,43 ± 0,24 a

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$.

Kao što je prikazano u tablici 2. maceracijom koja je trajala 24 h dobiven je najveći sadržaj ukupnih flavonoida što ukazuje da se prilikom ekstrakcije ultrazvukom, kao i kiselom maceracijom utjecalo na degradaciju flavonoida zbog njihove osjetljivosti na toplinu i izlaganje ultrazvuku. Sadržaj ukupnih flavonoida bio je najviši kod ekstrakcije maceracijom u trajanju od 24 h te je kod plodva 'Rimski' iznosio 5,9 ± 0,57 mg ekvivalenata kvercetina g^{-1} uzorka, a kod plodova 'Istarski duguljasti' 5,9 ± 0,03 mg ekvivalenata kvercetina g^{-1} uzorka. Dobivene vrijednosti sadržaja ukupnih flavonoida u suglasju su s istraživanjem Rusu i sur. (2019) na plodovima lijeske. Sadržaj esencijalnih i neesencijalnih minerala kod obje sorte lijeske dobiven korištenjem metode ICP-AES prikazan je u tablicama 3. i 4. Plodovi obje sorte imale su visok sadržaj esencijalnih i neesencijalnih minerala. U plodovima sorte 'Rimski' zabilježen je veći sadržaj dušika, kalija, kalcija i bakra u odnosu na plodove sorte 'Istarski duguljasti' koji je imao veći sadržaj fosfora, natrija, željeza, mangana, cika i bora. Kod plodova obje sorte zabilježen je sličan sadržaj magnezija i sumpora.
Müller i sur. (2020) su utvrdili sličan raspon sadržaja esencijalnih i neesencijalnih minerala u plodovima 15 sorata lijeske u Njemačkoj. Sličan raspon sadržaj minerala u plodovima sorte 'Tombul' u Turskoj utvrdili su Alasalvar i sur. (2003).

Tablica 2. Sadržaj ukupnih flavonoida (UF) u plodovima lijeske sorata 'Rimski' i 'Istarski duguljasti': postupak A-ultrazvučna ekstrakcija; postupak B – 24 h maceracija; postupak C – 24 h kisela maceracija .

Sorta	UF -postupak A (mg ekvivalenti kvercetina g^{-1} uzorka)	UF -postupak B (mg ekvivalenti kvercetina g^{-1} uzorka)	UF -postupak C (mg ekvivalenti kvercetina g^{-1} uzorka)
'Rimski'	1,1 ± 0,02 b	5,9 ± 0,57 a	2,4 ± 0,23 a
'Istarski duguljasti'	1,5 ± 0,13 a	5,9 ± 0,03 a	0,7 ± 0,04 b

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod $P \leq 0,05$.

Tablica 3. Sadržaj esencijalnih i neesencijalnih minerala (N, P, K, Ca, Mg, Na i S) u plodovima lijeske sorata 'Rimski' i 'Istarski duguljasti'.

Sorte	N	P	K	Ca	Mg	Na	S
	g 100 g^{-1}	mg 100 g^{-1}					
'Rimski'	2,20 ± 0,11a	299,0 ± 20 b	781,0 ± 45 a	190,0 ± 14 a	167,0 ± 12 a	< 0,20 b	160,0 ±12 a
'Istarski duguljasti'	1,91 ± 0,97 b	331,0 ± 22 a	165,0 ± 12 b	156,0 ± 12 b	163,0 ± 12 a	),37 ± 0,07 a	155,0 ±12 a

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod P ≤ 0,05.

Tablica 4. Sadržaj esencijalnih i neesencijalnih minerala (Fe, Mn, Cu, Zn i B) u plodovima lijeske sorata 'Rimski' i 'Istarski duguljasti'.

Sorte	Fe	Mn	Cu	Zn	B
	mg 100 g^{-1}				
'Rimski'	2,06 ± 0,29 b	0,54 ± 0,09 b	1,56 ± 0,25 a	2,02 ± 0,26 b	1,75± 0,25 b
'Istarski duguljasti'	2,33 ± 0,32 a	0,69 ± 0,12 a	0,71 ± 0,28 b	2,29 ± 0,29 a	2,13 ± 0,30 a

Vrijednosti obilježene različitim slovom su značajno različite pri LSD testu kod P ≤ 0,05.

4. Zaključak

Kod plodova sorte 'Istarski duguljasti' utvrđen je veći sadržaj fenola kao i antiooksidacijska aktivnost u odnosu na plodove sorte 'Rimski'. Visok sadržaj esencijalnih i neesencijalnih minerala utvrđen je kod plodova obje sorte. Plodovi sorata 'Rimski' i 'Istarski duguljasti' vrijedan su izvor bioaktivnih komponenata te se time potvrđuje status lješnjaka kao zdrastveno i nutritivno vrijednih plodova.

5. Literatura

Alasalvar C., Shahidi F., Liyanapathirana C.M., Ohshima T. (2003). Turkish Tombul Hazelnut (*Corylus avellana* L.) 1. Compositional Characteristics. J. Agric. Food Chem 51, 3790–3796. doi:10.1021/jf0212385.

Altun M, Çelik S.E., Güçlü K., Özyürek M., Erçag˘ E, Apak R. (2013). Total antioxidant capacity and phenolic contents of turkish hazelnut (*Corylus avellana* l.) kernels and oils. Journal of Food Biochemistry 37: 53–61. doi:10.1111/j.1745-4514.2011.00599.x.

AOAC International. 1995. Official Methods of Analysis. 16th ed. AOAC International, Arlington, VA.

Bartoszek M., Polak, J. (2016). A comparison of antioxidative capacities of fruit juices, drinks and nectars, as determined by EPR and UV–vis spectroscopies. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 153: 546–549. doi:10.1016/j.saa.2015.09.022.

Celik K.S., Aydinb F., Duzb M.Z., Aydinc I., Sait Erdoganb S., Akbab O., Hamamcib C. (2014). Simultaneous Determination of Transition Metals in Hazelnuts (*Corylus avellana* L.) by ICP-OES. Atomic Spectroscopy 35 (5):200-2004. doi: 10.46770/AS.2014.05.003.

Državni zavod za statistiku (2021). Proizvodnja povrća, voća i grožđa u 2020. –privremeni podaci. https://www.dzs.hr/Hrv_Eng/publication/2020/01-01-28_01_2020.htm

Dumas, J. B. A. (1831). Procedes de I'analyse organique. Ann. Chim. Phys, 47: 198–205.

FAOSTAT (2021) Food and agriculture organization statistics. Hazelnut production statistics. http://www.fao.org/faostat/en/#data/QC.

Fernandes V.C., Domingues V.F., de Freitas V., Delerue-Matos C., Mateus N. (2012). Strawberries from integrated pest management and organic farming: Phenolic composition and antioxidant properties. Food Chemistry 134 (4): 1926–1931.

Khlifi D., Hamdi M., Hayouni A. E., Cazaux S., Souchard J.P., Couderc F., Bouajila J. (2011). Global Chemical Composition and Antioxidant and Anti-Tuberculosis Activities of Various Extracts of Globularia alypum L. (*Globulariaceae*) Leaves. Molecules, 16 (12): 10592–10603. doi:10.3390/molecules161210592

Müller A.K., Helms U., Rohrer C., Möhler M., Hellwig F., Glei M., Schwerdtle T., Lorkowski S., Dawczynsk C. (2020). Nutrient Composition of Different Hazelnut Cultivars Grown in Germany. Foods 9, 1596. doi:10.3390/foods9111596

Oyvind M.A. (1989). Anthocyanins in Fruits of *Vaccinium oxycoccus* L. (Small Cranberry). Journal of food science 54 (2): 383 – 387.

Pelvan E., Cesarettin Alasalvar C, Uzman S. (2012). Effects of Roasting on the Antioxidant Status and Phenolic Profiles of Commercial Turkish Hazelnut Varieties (*Corylus avellana* L.). J. Agric.Food Chem. 60:1218−1223. doi.org/10.1021/jf204893x.

Rusu M.E., Fizes, I., Pop A., Gheldiu A.M., Mocan A. , Cris,G. Vlase L., Loghin F., Popa D.S., Tomuta I. (2019). Enhanced Recovery of Antioxidant Compounds from Hazelnut (*Corylus avellana* L.) Involucre Based on Extraction Optimization: Phytochemical Profile and Biological Activities. Antioxidants 8, 460. doi:10.3390/antiox8100460.

Vujević P., Milinović B., Jelačić T., Halapija Kazija D., Čiček D., Medved M. (2017). Stanje i važnost uzgoja lijeske u Republici Hrvatskoj. Pomologia croatica 21(1-2):207 – 215.

Wu X., Beecher G.R., Holden J.M., Haytowitz D.B., Gebhardt S.E., Prio, R.L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. Agric. Food Chem. 52, 4026−4037. doi: 10.1021/jf049696w.

Bioactive components and mineral composition of 'Istarski duguljasti' and 'Rimski' hazelnuts

Abstract

The aim of the study was to determine the total phenolic and flavonoid content, antioxidant activity and mineral composition of the two most commonly grown hazelnut cultivars in Croatia, 'Rimski' and 'Istarski duguljasti'. The following values of bioactive analysis were determined in the kernels of 'Rimski' and 'Istarski duguljasti': Total phenols 1.572 ± 0.12 and 1.912 ± 0.21 mg GAE g^{-1} of sample, total flavonoids 5.9 ± 0.57 and 5.9 ± 0.03 mg quercetin equivalents g^{-1} of sample, antioxidant activity 3.25 ± 0.08 and 4.43 ± 0.24 mM Trolox g^{-1} of sample (respectively). The fruits of the cultivar 'Istarski duguljasti' have a higher phenol content as well as antioxidant activity compared to the fruits of the cultivar 'Rimski'. The content of total flavonoids was highest in the extracts of the kernels macerated for 24 hours. The kernels of both cultivars had similar levels of calcium, magnesium, iron, copper, and zinc had similar levels of calcium, magnesium, iron, copper, and zinc as the levels of these minerals reported in other varieties. The results suggest that 'Rimski' and 'Istarski duguljasti' hazelnuts grown in the agro-ecological conditions of western Slavonia are a rich source of bioactive components.

Key words: antioxidant activity, hazelnut, total phenols, total flavonoids

IZVORNI ZNANSTVENI RAD

Maceration time effect on the mineral composition of Malvazija istarska (*Vitis vinifera* L.) wines

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Abstract
To investigate the effect of different maceration techniques on the mineral composition of Malvazija istarska wines, five different maceration treatments were carried out. Maceration techniques of two days at 8 °C (CRYO), and seven (M7), 14 (M14), 21 (M21), and 42 days (M42) at 16 °C, were studied and compared to control treatment without maceration (C). Analysis of macro- and microelements was conducted by inductively coupled plasma-optical emission spectrometry (ICP-OES). According to the obtained results, the longest maceration treatments (M21 and M42) resulted with the highest concentration of almost all macroelements. A proportional increase with maceration time was observed in the concentration of all analyzed microelements, with M42 treatment wine resulting in significantly highest concentrations.

Key words: Malvazija istarska, white wine, maceration, mineral composition, ICP-OES

Introduction

The overall wine quality depends on many factors, including its metal composition (Tariba, 2011). Metals affect wine flavor, freshness, aroma, color and taste (Pohl, 2007). Some macroelements (K, Ca, Mg and Na) are responsible for regulating yeast cellular metabolism by maintaining adequate pH and ionic balance (Pohl, 2007). Moreover, precipitation of K and Ca salts of tartaric acid influences taste and mouthfeel properties by altering pH which enhances the oxidation of Cu and Fe and the formation of clouding (Pohl, 2007). The information about wine mineral composition is essential for several reasons. Elements such as Al, Cu, Fe and Mn, may induce haze formation and result in undesirable changes in wine aroma and taste (Tariba, 2011), hence metal analysis is an important tool in wine quality control (Grindlay et al., 2011). Mineral content may also serve for characterization of the wines by their authenticity and geographical origin (Banović et al., 2009). Additionally, moderate daily wine consumption contributes to the requirements of the human organism for essential elements, such as Ca, Cr, Co, K, Se, Zn (Vrček et al., 2011), while other metals (Cu, Zn, As, Cd and Pb) can be potentially toxic when consumed in excess (Tariba, 2011). The concentration of metals in wine must be controlled according to health-protection regulations (Pohl, 2007). The legal threshold limit values for metals in wine are established by the International Organization of Vine and Wine (OIV, 2015) and Croatian Regulation on wine production (Official Gazette, Regulation on wine production, NN 2/2005), ensuring the wine metal content is within the toxicological safety limits. There are two main sources of metals in wine: primary source – soil on which vines are grown, and secondary source – external impurities that are in contact with grapes during growth or that occur in the process of winemaking (Pohl, 2007). To affect the sensory quality and increase the bioactive properties of white wines, many producers apply the grape maceration process. Since metals

are mainly extracted from the solid parts of the grape berry, their content may increase during maceration (Soto Vázquez et al., 2013; Shimizu et al., 2020). Several authors reported an increase in mineral content with a longer maceration duration (Shimizu et al., 2020, Rossi et al., 2022). While a lower temperature causes precipitation of K and Ca tartrates (Pohl, 2007), long macerations at high temperatures cause a greater extraction of metals from the grape berry (Soto Vázquez et al., 2013). The aim of this study was to investigate the effect of applying different maceration techniques on the mineral composition of wines produced from the Malvazija istarska grape variety (*Vitis vinifera* L.), an autochthonous white variety originating from the Istria region of Croatia.

Materials and methods

The grapes of *cv.* Malvazija istarska used for this experiment were produced in 2019, in the experimental vineyard of the Institute of Agriculture and Tourism in Poreč located in the viticultural region Croatian Istria and Kvarner. Grapes were harvested, destemmed, and crushed at the experimental cellar of the Institute of Agriculture and Tourism Poreč. Five out of six grapes must treatments were homogeneously transferred into 220 L stainless steel fermenters, each reproduced in three replicates (n = 3). Control treatment (C) was pressed immediately after crushing, while other treatments proceeded with the maceration processes of different duration periods as follows: pre-fermentative two days maceration at 8 °C (CRYO), seven days maceration at 16 °C (M7), and prolonged post-fermentative maceration at 16 °C for 14 days (M14), 21 days (M21), and 42 days (M42). The pectolytic enzyme of 4 g/hL (Endozym Aromatic, AEB, Brescia, Italy) was added to the juice of C treatment before it was cold settled for 24 hours, and to the mashes of the maceration treatments immediately after crushing. Each of the six treatments was inoculated with 30 g hL^{-1} of selected dry yeast (Fermol Arome Plus, *Saccharomyces cerevisiae*, AEB) and yeast supplement (Fermoplus floral, AEB), and the maceration/fermentation temperature was set to 16 °C. Before yeast inoculation, CRYO treatment was subjected to a temperature of 8 °C during the two days maceration process. During the maceration period, the caps of all maceration treatments were punched down three times a day. After the end of the maceration process, each treatment was pressed, racked off the sediment, and transferred into a clean tank and infused with nitrogen gas. The level of free and bound SO_2 was monitored throughout the whole process and corrected accordingly. Approximately 6 months after bottling, wines were subjected to analysis of macro- and microelements. Determination of macro- and microelements (Al, Cu, Fe, Mn, K, Ca, Mg, Na) was conducted using Optima DV 2000 inductively coupled plasma – optical emission spectrometer (PerkinElmer, Shelton, Connecticut, USA) equipped with a Meinhard spray chamber, nebulizer, and peristaltic sample delivery system, with the working conditions previously described by Rossi et al. (2022). Analyzed elements were identified in line with ICP-OES using the WinLab 1.35 PerkinElmer software and quantified by a direct calibration method. Statistical analysis was performed using Statistica 10.0. Software (Stat-Soft Inc. Tulsa, OK). Fischer's least significant difference test (LSD) was performed using a one-way analysis of variance (ANOVA).

Results and discussion

The mineral compositions of different Malvazija istarska treatment wines are presented in Figure 1 and Table 1. Total macroelements content ranged from 881.5 ± 3.66 to 1023.6 ± 2.02 mg L^{-1} (Figure 1). Significantly the highest concentration was found in the maceration treatments (M42, M21, M14 and M7) with no significant differences between those treatments, while a lower concentration was found in CRYO treatment wine. Control wine (C) obtained the lowest concentration of total macroelements: potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na). According to Grindlay et al. (2011), among all

macroelements, K usually reaches the highest concentration levels in wines (500-1500 mg L^{-1}), which is in agreement with the concentrations found in this study. Potassium in wine, especially in higher concentration, has a great nutritional value (Karataş et al., 2015). The concentration of Ca, Mg and Na in wine are usually ranging from 10 to 200 mg L^{-1} (Grindlay et al., 2011), which is accordant with our results. Magnesium (Mg) concentration increased with longer maceration treatments, being the highest in M21 and M42 treatment wines and the lowest in C wine. Prolonged post-fermentation maceration treatments, M21 and M42, showed the highest concentration of almost all analyzed macroelements, K, Mg and Ca, respectively. Such results correspond to those of Rossi et al., (2022), who also noted an increase in K, Mg and Ca concentrations resulting from the prolonged maceration process in Teran red wine. In an earlier study (Shimizu et al., 2020), K and Ca concentration decreased after 2nd day of maceration, while a proportional increase with maceration length was observed in our study. Sodium (Na), the extracellular cation, helps to maintain the acid-base balance and osmotic regulation (Karataş et al., 2015). The highest sodium concentration in our study was observed in M7 treatment wine, while the lowest concentration was found in M42 treatment wine, meaning a longer maceration duration promotes sodium decrease.

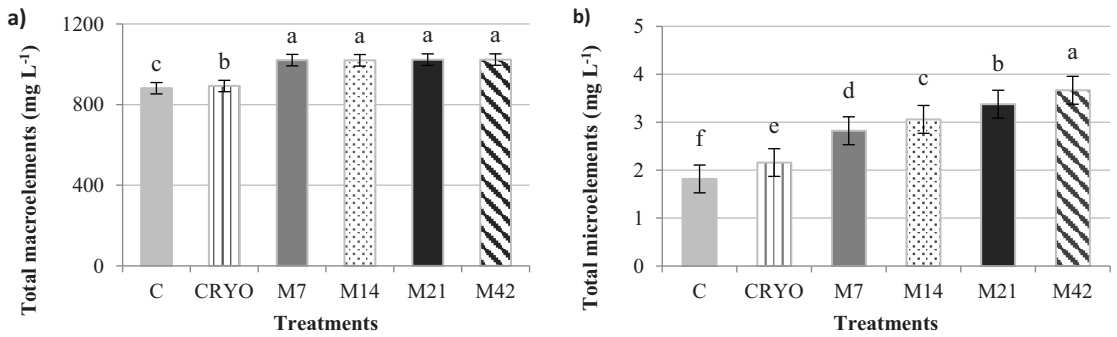

Figure 1 **a)** total concentration (mg L^{-1}) of macroelements in different Malvazija istarska treatments, **b)** total concentration of microelements (mg L^{-1}) in different Malvazija istarska treatments; C - control treatment, CRYO - pre-fermentative two days cryomaceration treatment, M7 - seven days maceration treatment, M14 - prolonged post-fermentative 14 day maceration treatment; M21 - prolonged post-fermentative 21 day maceration treatment, M42 - prolonged post-fermentative 42 day maceration treatment. Different lowercase superscript letters represent statistically significant differences between treatments at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

The concentration of total microelements (aluminium (Al), copper (Cu), iron (Fe) and manganese (Mn)), ranged from 1.82 ± 0.02 to 3.67 ± 0.03 mg L^{-1} and was significantly the highest in M42 treatment wine, containing 2-fold higher total microelements concentration than C treatment wine. Those elements are usually present in concentrations ranging from 0.1 to 10 mg L^{-1} (i.e. trace elements) (Grindlay et al., 2011). The concentration of Fe was the highest of all microelements and the values increased proportionally with maceration time, being 3-fold higher in M42 than in C treatment wine. Such results are in concordance with Rossi et al., (2022), who reported a correlation between Fe concentration and maceration duration. Other microelements showed a similar trend, meaning their concentration increased with longer maceration duration but were contained below the maximum acceptable levels in wine according to the national Official Gazette (Regulation on wine production, NN 2/2005). Seeds contain three times as much manganese as skins and thirty times as much as grape flesh (Ribéreau-Gayon et al., 2006), which can explain a proportional increase in manganese with the progression of maceration time, being the highest in M42 treatment wine. Cu, Zn, and Fe are essential metals, important for various physiological processes and have certain nutritional benefits (Tariba, 2011). Prolonged maceration with grape skins may also increase the concentration of Cr, Cu, Fe and Zn in

wine (Shimizu et al., 2020), which agrees with our results and the concentrations of Fe and Cu found in prolonged maceration treatments.

Table 1. Concentration (mg L^{-1}) of macro- and microelements of different Malvazija istarska wine treatments

	Treatments					
	C	**CRYO**	**M7**	**M14**	**M21**	**M42**
Macroelements						
K	716.57 ± 1.1^{d}	720.57 ± 1.01^{c}	817.63 ± 0.83^{b}	818.63 ± 0.83^{b}	820.47 ± 1.25^{a}	821.83 ± 1.07^{a}
Ca	32.77 ± 1.84^{d}	36.2 ± 0.95^{c}	56.83 ± 0.67^{b}	57.17 ± 0.67^{b}	59.47 ± 0.95^{a}	59.47 ± 0.95^{a}
Mg	97.83 ± 0.57^{e}	100.53 ± 0.42^{d}	105.63 ± 0.21^{c}	106.87 ± 0.15^{b}	108.47 ± 0.21^{a}	109.03 ± 0.21^{a}
Na	34.33 ± 0.21^{d}	35.37 ± 0.31^{c}	40.53 ± 0.40^{a}	37.57 ± 0.35^{b}	34.9 ± 0.10^{c}	33.27 ± 0.35^{e}
Microelements						
Al	0.653 ± 0.03^{d}	0.833 ± 0.03^{c}	0.906 ± 0.01^{b}	0.916 ± 0.01^{b}	0.926 ± 0.01^{ab}	0.956 ± 0.01^{a}
Cu	0.016 ± 0.00^{f}	0.026 ± 0.00^{e}	0.035 ± 0.00^{d}	0.042 ± 0.00^{c}	0.049 ± 0.00^{b}	0.063 ± 0.00^{a}
Fe	0.52 ± 0.01^{f}	0.65 ± 0.01^{e}	1.1 ± 0.02^{d}	1.3 ± 0.03^{c}	1.54 ± 0.01^{b}	1.76 ± 0.02^{a}
Mn	0.629 ± 0.00^{f}	0.650 ± 0.00^{e}	0.777 ± 0.00^{d}	0.797 ± 0.00^{c}	0.869 ± 0.00^{b}	0.886 ± 0.00^{a}

Results of macro- and microelements are expressed in mg L^{-1} as means ± standard deviations; C - control treatment, CRYO - pre-fermentative two-days cryomaceration treatment, M7 - seven days maceration treatment, M14 - prolonged post-fermentative 14 day maceration treatment; M21 - prolonged post-fermentative 21 day maceration treatment, M42 - prolonged post-fermentative 42 day maceration treatment. Different lowercase superscript letters represent statistically significant differences between treatments at $p < 0.05$ obtained by one-way ANOVA and least significant difference (LSD) test.

Conclusion

The composition of both macro- and microelements in different Malvazija istarska wines was highly affected by the maceration treatment. Total macroelements concentration was the highest in maceration treatment wines, particularly those with longer maceration time and higher maceration temperature. Total microelement concentration increased proportionally with maceration time, being significantly the highest in the longest post-fermentative maceration treatment. When observing the individual mineral concentration, almost all minerals increased with maceration time, especially when maceration time increased to 21 and 42 days. According to the increase in mineral composition of several maceration treatments, obtained results may serve wine producers choose the appropriate winemaking technology for improving the quality of their products.

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References

Banović, M., Kirin, J., Ćurko, N., Kovačević Ganić, K. (2009). Influence of vintage on Cu, Fe, Zn and Pb content in some Croatian red wines. Czech Journal of Food Sciences, 27(Special Issue 1), S401–S403.

Grindlay, G., Mora, J., Gras, L., de Loos-Vollebregt, M. T. C. (2011). Atomic spectrometry methods for wine analysis: A critical evaluation and discussion of recent applications. Analytica Chimica Acta, 691(1): 18–32.

Karataş D., AydinF , Aydin I., Karataş H. (2015): Elemental composition of red wines in Southeast Turkey. Czech Journal of Food Sciences, 33: 228-236.

OIV (2015). International Code of Oenological Practices - Annex: Maximum Acceptable Limits, Issue 2015/01. Paris, France: International Organisation of vine and wine.
Pohl, P. (2007). What do metals tell us about wine? Trends in Analytical Chemistry, 26(9): 941–949.
Regulation on wine production, NN 2/2005. Official Gazette, Zagreb (Croatia).
Ribéreau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D. (2006). Dry Extract and Minerals. In *Handbook of Enology*, Volume 2: The Chemistry of Wine - Stabilization and Treatments, 91–108. Chichester, England: John Wiley & Sons, Ltd.
Rossi, S., Bestulić, E., Horvat, I., Plavša, T., Lukić, I., Bubola, M., Ganić, K. K., Ćurko, N., Jagatić Korenika, A.-M., Radeka, S. (2022). Comparison of different winemaking processes for improvement of phenolic composition, macro- and microelemental content, and taste sensory attributes of Teran (*Vitis vinifera* L.) red wines. LWT, 154 (112619).
Shimizu, H., Akamatsu, F., Kamada, A., Koyama, K., Iwashita, K., Goto-Yamamoto, N. (2020). Variation in the mineral composition of wine produced using different winemaking techniques. Journal of Bioscience and Bioengineering, 130(2): 166–172.
Soto Vázquez, E., Rio Segade, S., Fernández Gomez, E. (2013). Incidence of the Winemaking Technique on Metal Content and Phenolic Composition of Red Wines. International Journal of Food Properties, 16(3): 622–633.
Tariba, B. (2011). Metals in Wine—Impact on Wine Quality and Health Outcomes. Biological Trace Element Research, 144(1): 143–156.
Vrček, I. V., Bojić, M., Žuntar, I., Mendaš, G., Medić-Šarić, M. (2011). Phenol content, antioxidant activity and metal composition of Croatian wines deriving from organically and conventionally grown grapes. Food Chemistry, 124(1): 354–361.

Utjecaj duljine maceracije na mineralni sastav vina ´Malvazije istarske´ (*Vitis vinifera* L.)

Sažetak
U svrhu ispitivanja utjecaja različitih tretmana maceracije masulja na mineralni sastav vina sorte ´Malvazija istarska´, postavljeno je pet različitih tretmana maceracije. Tretmani maceracije u trajanju od dva dana na 8°C (CRYO), te sedam (M7), 14 (M14), 21 (M21) i 42 dana (M42) na 16 °C uspoređivani su s kontrolnim tretmanom (C) bez maceracije. Analiza makro- i mikroelemenata provedena je tehnikom optičko-emisijske spektroskopije induktivno spregnute plazme (ICP-OES). Prema dobivenim rezultatima, tretmani produljene maceracije (M21 i M42) rezultirali su najvišim koncentracijama gotovo svih makroelemenata. Uočen je porast koncentracije svih mikroelemenata proporcionalno s duljinom trajanja maceracije, a kod tretmana M42 signifikantno najviše vrijednosti.

Ključne riječi: Malvazija istarska, bijelo vino, maceracija, mineralni sastav, ICP-OES

PREGLEDNI RAD

Potencijal uzgoja kivija u Zadarskoj županiji

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Sažetak
Kivi postaje sve popularnija voćna vrsta zbog svoje nutritivne vrijednosti, visokog sadržaja vitamina C te zbog svoje široke klimatske prilagodljivosti. Zbog sve veće potražnje i dobre otkupne cijene i dalje raste interes za njegov uzgoj. Tome pridonosi i činjenica njegovog kasnog dozrijevanja, od sredine rujna do početka listopada, što se dobro uklapa u organizaciju i strukturu voćarske proizvodnje. Svjetska proizvodnja kivijia je oko 4,4 milijuna tona godišnje, no za Republiku Hrvatsku statistički podaci ne postoje. Na tržištu postoji preko 70 sorata i dodatnih 130 selekcija stoga pravilan odabir sortimenta je ključan za uspješnu proizvodnju. Zadarska županija ima dugu tradiciju uzgoja voća te povoljne pedoklimatske uvjete za uzgoj kivija. Cilj rada je opisati potrebne agroekološke uvijete i sortiment za uzgoj kivija u cilju postizanja maksimalnih priroda kao ključni čimbenici profitabilne intenzivne proizvodnje.

Ključne riječi: kivi, sortiment, agroekološki uvijeti, Zadarska županija

Uvod

Kivi je najpoznatija voćna vrsta iz roda *Actinidia* koji obuhvaća više od 70 vrsta, a tri su od najvećeg komercijalnog značaja: zlatni (*A. chinensis*), zeleni (*A. deliciosa*) i otporni (*A. arguta*, poznata kao mini kivi) (Chamorro i sur., 2022.). Podrijetlom je iz Kine i zbog svoje klimatske prilagodljivosti ima širok areal uzgoja. Popularnost kivija među voćarima raste zbog visokih priroda po jedinici površine (Çelik i sur., 2006.) te zbog nutritivnih, organoleptičkih svojstava i zdravstvenih prednosti (Chamorro i sur., 2022.). Prema najnovijim podacima svjetska proizvodnja kivija je oko 4,4 milijuna tona godišnje, pri čemu su najveći proizvođači Kina s 50,59 % i Novi Zeland s 14,17% svjetske proizvodnje (FAO, 2021.). Novi Zeland ovisi o izvozu dok je izvoz u Kini manji od 1% (Ferguson, 2015.). Površine pod kivijem 2020. godine zauzimale su 270 457 ha širom svijeta, a od toga 44 830 ha otpada na Europu koja je 2020. godine proizvela 952 210 t kivija što čini 21,60 % svjetske proizvodnje (FAO, 2021.). Najveći proizvođači kivija u Europi su Italija s 11,83 % i Grčka s 6,9 % svjetske proizvodnje koje izvoze znatne količine (Ferguson, 2015.). Od zemalja u okruženju prema podacima vidljivo je da Bosna i Hercegovina ima proizvodnju od 17 t (Agencija za statistiku Bosne i Hercegovine) dok za Republiku Hrvatsku statistički podaci iz 2020 godine bilježe proizvodnju 375 t limuna (Državni zavod za statistiku). Plod ima veliku varijabilnost po morfološkim karakteristikama, vremenu dozrijevanja, prisutnosti ili odsutnosti dlakavosti i okusa (Chamorro i sur., 2022.). *Actinidia deliciosa* ima dlakavu smeđu kožicu, dok je pulpa svijetlo zelena, prozirna u kontrastu s bijelom bojom jezgre i crnim sjemenkama. S druge strane, kožica plodova *A. chinensis* je gola, a unutrašnjost joj je u većini slučajeva žuta. Osim toga, na jednom od svojih krajeva imaju ispupčenje, što im daje karakterističan oblik (Nishiyama, 2007.). *A. chinensis* i *A. deliciosa* dozrijevaju između 180 i 210 dana (Chamorro i sur., 2022.). Proizvodnja kivija rasla je zahvaljujući uspjehu sorte 'Hayward' vrste *A. chinensis var. Deliciosa* koja je najvažnija sorta u Kini s 33% ukupne proizvodnje (Ferguson, 2015.) kojoj se kasnije pridružila sorta 'Hort16A', koju je na tržište lansirao 'ZESPRITM GOLD Kiwifruit' i bio je prvi kivi žutog mesa u proizvodnji

izvan Kine te najvažnija sorta žutog mesa u međunarodnoj trgovini. (Chamorro i sur., 2022.). Ostale nove sorte zelenog, žutog i crvenog mesa uzgajaju se u različitim zemljama, ali još uvijek imaju mali doprinos međunarodnoj trgovini (Ferguson, 2015.). Zadarska županija ima dugu tradiciju proizvodnje voća, a spada u područje mediteranske klime koju obilježavaju blage zime i topla ljeta te zadovoljava ekološke uvijete za intenzivnu proizvodnju zlatnog kivija. Zbog toga se javlja veliki interes proizvođača za ovu sortu. Zlatni kivi je nutritivno bogatiji, ima više vitamina C i više šećera, ali nije pogodan za daljnju obradu nakon berbe, jer meso ploda u obradi postaje smeđe boje. Gubi se i njegov karakterističan okus. Zbog toga se od zelenog kivija ne rade sokovi, pekmezi i „ready to eat" proizvodi (Cassano i Drioli, 2007.). Interes za sortama zlatnog kivija u prvom redu je komercijalnog karaktera zbog visoke cijene koju postiže na svjetskom tržištu, a ujedno i zbog njegovog kasnog dozrijevanja od sredine rujna do početka listopada što bi se dobro uklopilo u organizaciju i strukturu voćarske proizvodnje. Intenzivni nasadi kivija su malobrojni, a proizvodnja plodova na nacionalnoj razini gotovo je nezamjetna. Potencijal ove isplative i tražene voćne vrste nije iskorišten, naročito ako se ima u vidu da su neka područja Hrvatske vrlo pogodna za njezin uzgoj. U posljednje vrijeme zbog visoke otkupne cijene zlatnog kivija sve se više govori o podizanju intenzivnih nasada kivija na području srednje Dalmacije i doline rijeke Neretve. Primjer je intenzivan nasad Zlatnog kivija u Zadarskom zaleđu kod sela Murvica s površinom od 11 ha.

Agroekološki uvjeti za uzgoj kivija

Kivi može uspješno rasti u mediteranskim i blagim klimatskim uvjetima. Najviše mu odgovaraju topla i vlažna ljeta, a blage zime. Središnja godišnja temperatura treba biti od 13,5 °C do 16 °C (Huang i Ferguson, 2001.). Temperature zraka u mnogim dijelovima Dalmacije pogodne su za uzgoj ove voćne vrste. Divlji kultivari kivija mogu podnijeti temperature i do -25°C u periodu mirovanja. Plemenite sorte kivija su više osjetljive na niske temperature jer su stvorene u uvjetima blaže klime (Blanchet i Chartier, 1998.). One su bujnije, imaju duži period vegetacije, veću produktivnost i rodnost što značajno utječe na njihovu smanjenu otpornost prema mrazovima (Ferguson, 1999.). Ovu tezu potvrđuju podaci istraživanja na području uzgoja zlatnog kivija u submediteranskim i mediteranskim područjima Italije gdje su pri temperaturama od -17°C u periodu mirovanja utvrđena mala oštećenja jednogodišnjih grana što je utjecalo na rodnost iduće godine (Ferguson, 1999.). Prema Blanchet i Chartier (1998.) vrlo mala oštećenja pojavila su se na -12 °C, a vrlo teška oštećenja višegodišnjih i jednogodišnjih organa na -23 °C do -30 °C. Niske temperature pred početak vegetacije krajem veljače i početkom ožujka mogu izazvati još veća oštećenja nego u periodu fiziološkog mirovanja kivija. U ožujku, kada krenu sokovi za samo par dana naglog zahlađenja kada se temperatura spusti na -2 °C do -3 °C, proizvodnja može biti kompletno uništena (Ferguson, 1999.). Visoke temperature ubrzavaju sazrijevanje plodova što negativno utječe na kvalitetu i kvantitetu uroda u tekućoj i idućoj godini (Blanchet i Chartier, 1998.). Kiviju je potrebno 800 - 900 mm/m^2 kiše dobro raspoređenih kroz cijelu godinu (Huang i Ferguson, 2001.). I nedostatak i višak padalina ima nepovoljan utjecaj na životne funkcije kivija, a posebno na visinu priroda i kvalitetu plodova. Ukoliko u tlu nedostaje vlage, kiviju je aktivnost jako smanjena kao i visina uroda, a plodovi su sitni i deformirani. U ljetnim mjesecima u fazi sazrijevanja plodova velike opasnosti za zlatni kivi predstavljaju velike količine kiše nakon dužih sušnih razdoblja što gotovo sigurno dovodi do pucanja plodova (Huang i Ferguson, 2001.). Iz tog razloga potrebno je osigurati sustav za navodnjavanje. Atmosferska pojava koje se također treba bojati je tuča (Montanaro i sur., 2007.). Zahtjevi zlatnog kivija prema tlu veći su od ostalih vrsta jagodastog voća. Tlo mora biti neutralne do slabo kisele reakcije sa pH od 5,5 do maksimalo 7,5 (Huang i Ferguson 2001.). Kiviju najbolje odgovaraju lagana, rahla, duboka tla, a može se prilagoditi laganim i

pjeskovitim terenima, gdje je uvijek moguće intervenirati navodnjavanjem i dodavanjem organskih i mineralnih gnojiva (Montanaro i sur., 2007.). Iskustva stečena u praksi pokazuju da se kivi u mediteranskom području može uzgajati na nadmorskoj visini do 250 m, a najpovoljniji položaji za uzgoj su do 50 m nadmorske visine. Za uzgoj su najpogodniji blago nagnuti tereni do 5 % i ravni tereni (Montanaro i sur., 2007.). Izbor strane svijeta ovisi o lokalitetu i tipu klime. Uz more gdje često pušu topli vjetrovi prednost imaju sjeverne strane, a u hladnijim područjima južne ekspozicije. Kivi se ne smije saditi na mjestima gdje su česti jaki i suhi vjetrovi, jer mu vjetar lomi grane i isušuje cvjetove i listove. Mladi nježni izbojci osobito su osjetljivi na vjetar, zato se kivi treba saditi na položajima zaštićenim od vjetra ili se podižu vjetrozaštitni pojasevi (Montanaro i sur., 2007.). Kivi je biljka koja traži mnogo svijetla. Za kvalitetan uzgoj potrebno je 1800 do 2500 sunčanih sati s temperaturom većom od 10 °C. Kivi dobro podnosi svjetlost dok ima dovoljno vlage u zraku (Huang i Ferguson, 2001.). Nedostatak svijetla utječe na sporo dozrijevanje plodova, a rezultat su mali plodovi i mali urod (Xiloyannis i sur., 2008.). Zlatni kivi je dvodomna biljka te je kod sadnje potrebno voditi računa o rasporedu oprašivača kako bi se kasnije omogućila dobra oplodnja. Problem rasporeda muških biljaka je od velike važnosti, jer nedovoljno oprašivanje smanjuje urod, oblik i veličinu plodova. U Italiji je najčešće korišten omjer muških i ženskih biljaka od 1:5 i 1:7. Muške biljke se u cilju boljeg oprašivanja sade tako da prva dođe na početno mjesto u redu i to polazeći od pravca puhanja dominantnog vjetra. Povećani broj oprašivača koristi oplodnji, ali istodobno stvara značajan gubitak prostora i direktno smanjuje rodnost po hektaru. U Italiji se počinju koristiti sustavi uzgoja koji imaju manji broj oprašivača pa omjer bude 1:9, a oprašivanje se pospješuje umjetnim putem sa posebnim atomizerima koji raspršuju suhi polen. Polen se uvozi sa Novog Zelanda, ili drugih zemalja na južnoj polutci i skladišti se do uporabe (Marangoni i sur., 2003).

Odabir i opis sortimenta

Kivi je dvodomna biljka, pa treba izabrati sorte ženskog cvijeta koje daju plod i prikladne muške sorte koje služe za oprašivanje (Huang i Ferguson, 2003.). Dvije sorte zlatnog kivija se najviše sade u Europi, a to su 'Hort16 A' i 'Jintao' (Brigati i Donati, 2003.). Postoji i manji broj voćnjaka gdje je zasađena nova talijanska sorta 'Soreli' te sorta 'Kuimi' koja je introducirana iz Kine (Buci i Costa, 2006.). Oko 70 sorata i dodatnih 130 selekcija *A. Chinensis* i *A. deliciosa* su selektirane u Kini te od tamo dolazi većina modernih sorata zlatnog kivija (Huang i Ferguson, 2001.).

Sorta 'Hort16A': Nastala je 1987. godine na Novom Zelandu. Prvi urod je bio 1990. godina i od tad su rađena daljnja istraživanja. Službeno se na tržištu pojavila 2000. godine pod tržišnim imenom ZESPRI GOLD. 'Hort16A' je bujna sorta koja cvate mjesec dana ranije od sorte 'Hayward'. Jako je rodna i može podnijeti puno veće opterećenje od 'Haywarda'. Plodovi su prepoznatljivog izgleda, ovalni i izduženi sa zaobljenim „kljunom“ na kraju (Testolin i Ferguson, 2010.). Kožicaje svjetlo do tamno smeđa prekrivena glatkim dlačicama koje su slične onima na breskvi. Meso gubi zelenu boju tokom sazrijevanja i ako su plodovi pobrani dovoljno kasno u sezoni meso će biti čisto i zlatnožute boje. Vrijeme berbe treba biti dovoljno kasno da meso bude zlatnožute boje, a da plodovi ne izgube tvrdoću te budu pogodni za skladištenje (Brigati i Donati, 2003.). Uobičajeno je da se bere isto kad i 'Hayward'. Kao oprašivači za ovu sortu se koriste se sorte 'Sparkler' i 'Meteor'. Obje sorte oprašivača su selekcionirane od populacija koje su nastale od divljeg sjemena u Kini (Brigati i Donati 2003.). Treća sorta oprašivač 'Bruce' selekcionirana je 2007. na Novom Zelandu.

Sorta 'Soreli': Otkrivena je na Sveučilištu u Udinama 2007. godine. Ova sorta ulazi u vegetaciju otprilike tjedan dana prije nego 'Hayward', ovisno o mjestu sadnje, plodovi dozrijevaju i do mjesec dana ranije. Plodovi su veliki i izduženi teži su od 100 g. Meso ploda je svijetložute boje, a okus je sladak (Buci i Costa, 2006.). Plodovi ove sorte nisu pogodni

za skladištenje. Vrlo brzo propadaju i gube kvalitetu (Beltramo i sur., 2008.). Kao oprašivač za ovu sortu koristi se sorta 'Belen'.

Sorta 'Kuimi': Selekcionirana je 1979. godina na Institutu za Hortikulturu pokrajine Jiangxi u Kini. Službeno je registrirana 1985. godine. Vrlo je bujna i rodna s puno cvjetova na kratkim izdancima (Huang i Ferguson, 2001.). Plodovi su prepoznatljivi po svom obliku, jer jako sliče na jabuku (Blanchet, 1998.). Plodovi mogu biti mase od 90 do 100 g. Kožica ploda je smeđe do tamno smeđe boje, a boja mesa je zeleno žuta i žuta (Huang i Ferguson, 2001.). Plodovi su vrlo sočni i dobrog okusa. Plodovi dozrijevaju sredinom rujna. Kao oprašivači za ovu sortu se koriste sorte 'Belen' i 'Tomuri' (Huang i Ferguson, 2007.).

Sorta 'Jintao': Ova sorta zlatnog kivija nastala je selekcijom iz divljih sorata *A. chinensis* na Botaničkom institutu Wuhan u Kini 1982. godine (Cipriani i Testolin, 2007.). Poznata je pod tržišnim imenom Jingold. 'Jintao' je manje bujna, ali rodnija sorta nego 'Hayward' (Huang i sur., 2002.). U uobičajenim godinama daje u prosijeku duplo veći urod nego 'Hayward' (Cipriani i Testolin, 2007.). U pokrajini Jiangxi u Kini zabilježeni su urodi veći i od 50 t/ha. Prvi urod daje već u drugoj godini nakon sadnje, a u punu rodnost ulazi već u četvrtoj godini (Huang i sur., 2002.). Počinje s cvatnjom tjedan dana ranije nego 'Hayward'. Rok berbe je od sredine do kraja rujna. Plodovi su izduženi i okruglasti, mogu biti teški od 65 g do 120 g. Kožica ploda je gotovo bez dlačica i svijetlo smeđe boje. Meso ploda je sjajne zlatno žute boje (Beltramo i sur., 2008). Glavna prednost ove sorte nad ostalim sortama zlatnog kivija je ta što se može dugo skladištiti (Huang i sur., 2002). Preporučeni oprašivači za ovu sortu su sorta 'Belen' i sorta 'Tomuri'. Tvrtka Consorzio kiwi gold je jedina licencirana za proizvodnju i razmnožavanje sadnica ove sorte u Europskoj uniji (Testolin i Ferguson, 2010).

Zaključak

Odabir pogodnog sortimenta važan je kod uspješne proizvodnje. Korištenjem novih sorata visoke kakvoće te primjenom suvremenih zahvata osiguravamo redovitu i obilnu rodnost u nasadu. Pomološke karakteristike ploda genetski su uvjetovane, međutim značajan utjecaj na genotip imaju okolišni uvjeti kao i tehnološki zahvati u uzgoju. Stoga je kod odabira sortimenta važno je proučiti klimatske uvjete područja uzgoja kako bi biološki potencijal određene sorte došao do punog izražaja. Obzirom na agroekološke zahtjeve kivija Zadarska županija zadovoljava uvijete koji su prikladni za uzgoj kivija.

Literatura

Beltramo C., Sartor C., Cavanna M., Beccaro G.L., Mellano M.G., Botta R. (2008). Valutazione dei parametri qualitativi durante il post-raccolta di frutti di actinidia proveniente da diverse regioni italiane. Proceedings National Meeting: Actinidia 2007. Societa Orticola Italiana. Cuneo-Torino, 27-29 November. Pp. 424-430.

Blanchet P., Chartier J. (1998). Sélection de kiwis chinois pour les zones chaudes: Chinabelle® et Pollichina®. L'Arboriculture Fruitière 513:37–40.

Brigati S., Donati L. (2003). Actinidia: risultati della ricercae le loro applicazioni nel settore della conservazione e distribuzione commerciale. Proceedings National Meeting, Actinidia: la novità frutticola del XX secolo. Societa Orticola Italiana. Verona, 21 November. Pp. 277-290.

Cassano A., Drioli E. (2007). Concentration of clarified kiwifruit juice by osmotic destilation. Journal of Food Engineering 79: 1397-1404.

Çelik H., Zenginbal H., Özcan M. (2006). Enhancing germination of kiwifruit seeds with temperature, medium and gibberellic acid. Horticultural Science. Volumen (33): 39-45.

Chamorro F., Carpena M., Fraga-Corral M., Echave J., Muhammad Shahid Riaz Rajoka, Barba Francisco J., Hui Cao., Jianbo Xiao., Prieto M.A., Simal-Gandara J. (2022).

Valorization of kiwi agricultural waste and industry by-products by recovering bioactive compounds and applications as food additives: A circular economy model. Food Chemistry. 370: 131315

Cipriani G., Testolin R. (2007). ' Jintao': a Chinese kiwifruit selection grown in Italy. Acta Horticulturae 753: 247-252.

FAO (2021). Faostat – Crops and livestock production. Available from: https://www.fao.org/faostat/en/#data/QCL

Ferguson A.R. (1999). New temperate fruits: Actinidia chinensis and Actinidia deliciosa. Objavljeno u *Perspectives on new crops and new uses*, (ed.), Janick J. ASHS Press, Alexandria VA, Usa, 342–347.

Ferguson A.R. (2015). Kiwifruit in the world. Hongwen Huang (ed.), Dujiangyan (Chengdu) (China), International Society for Horticultural Science. Acta horticulturae, 1096: 33-46.

Huang H., Ferguson A. R. (2001). Review: Kiwifruit in China. New Zealand Journal of Crop and Horticultural Science, 29:1, 1-14. Raspoloživo: https://www.tandfonline.com/

Huang H., Wang S., Renhung H., Zhang Z. (2002). Jintao a Novel, Hairless Yellow-fleshed Kiwifruit. Hortscience 37:1135-1136.

Huang H., Ferguson A. R. (2003). Kiwifruit (Actinidia chinesis and A. deliciosa) plantings and production in China, 2002. New Zealand Journal of Crop and Horticultural Science, 31:3, 197-202. Raspoloživo: https://www.tandfonline.com/

Huang H.-W., Ferguson A.R. (2007). Actinidia in China: natural diversity, phylogeographical evolution, interspecific gene flow and kiwifruit cultivar improvement. Acta Horticultureae 753, 31-40.

Marangoni B., Rombolà A.D., Toselli M., Feralli S. (2003). La pratica della fertilizzazione dell'actinidia. Proceedings National Meeting, Actinidia: la novita frutticola del XX secolo. Societa Orticola Italiana, Verona, 21 November. Pp. 163-197.

Montanaro G., Dichio B., Celano G., Xiloyannis C. (2007). Sustainable kiwifruit orchard management in semi-arid environments. Acta Horticulturae 753: 591-598.

Nishiyama I. (2007). Fruits of the Actinidia Genus. Advances in Food and Nutrition Research, (52): 293-324. ISSN 1043-4526, ISBN 9780123737113.

Testolin R., Ferguson A.R. (2009). Kiwifruit (Actinidia spp.) production and marketing in Italy. New Zealand Journal of Crop and Horticultural Science, 37:1, 1-32.

Xiloyannis C., Dichio B., Montanaro G., Lang A., Celano G., Mazzeo M. (2008). Fruit morphological and physiological traits influence calcium transport and accumulation in kiwifruit. Acta Horticulturae 767: 369-378.

Potential of growing kiwi in Zadar County

Abstract

Kiwi is becoming an increasingly popular fruit species due to its nutritional value, high content of vitamin C and due to its wide climatic adaptability. Due to the growing demand and good purchase price, interest in its cultivation continues to grow. The fact of its late ripening, from mid-September to early October, which fits well into the organization and structure of fruit production, also contributes to this. World production of kiwifruit is about 4.4 million tons per year, but there are no statistical data for the Republic of Croatia. There are over 70 varieties and an additional 130 selections on the market, so the correct choice of cultivars is key to successful production. Zadar County has a long tradition of fruit growing and favorable pedoclimatic conditions for growing kiwi. The aim of this paper is to describe the necessary agroecological conditions and assortment for kiwi cultivation in order to achieve maximum yields as key factors of profitable intensive production.

Key words: kiwi, cultivar, agroecological conditions, Zadar County

IZVORNI ZNANSTVENI RAD

Utjecaj kvasaca na sastav antocijana u vinu ´Trnjak´

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Sažetak
Saccharomyces cerevisiae najčešće je korištena vrsta kvasca u vinarskoj proizvodnji dok je vrsta *Lachancea thermotolerans* noviji pripadnik selekcioniranih ne-*Saccharomyces* kvasaca. Ističe se sposobnošću zakiseljavanja moštova niže ukupne kiselosti, iz toplih vinogradarskih regija, najčešće u sekvencijalnoj inokulaciji sa *S. cerevisiae*. Formiranje boje vina i sastav antocijana tijekom vinifikacije pod utjecajem su kvasaca, odnosno adsorpcijskih karakteristika stanične stjenke kvasca. Cilj ovog rada bio je utvrditi razlike u učinku sekvencijalne inokulacije komercijalnim sojevima *L. thermotolerans x S. cerevisiae* u usporedbi s tretmanima sa *S. cerevisiae* i epifitnim kvascima, na sastav antocijana u vinu ´Trnjak´. Rezultati analiza ukazali su na značajno niže koncentracije antocijana u tretmanu *L. thermotolerans x S. cerevisiae*.

Ključne riječi: antocijani, *Lachancea thermotolerans* ne-*Saccharomyces*, *Saccharomyces cerevisiae,*, sekvencijalna fermentacija

Uvod

Saccharomyces cerevisiae (Sc.) tradicionalno se najčešće koristi u proizvodnji vina zbog iznimnih fermentabilnih sposobnosti u širokom rasponu uvjeta provođenja alkoholne fermentacije. Posljednjih godina, selekcija kvasaca uključuje razvoj tehnika za selekciju sojeva koji bi mogli poboljšati vina u smislu njihove boje, strukture, mirisa i određenih zdravstvenih svojstava (Suárez-Lepe i Morata, 2012.). Među novim selekcijama ne-*Saccharomyces* kvasaca, *Lachancea thermotolerans (Lt.)* ističe se sposobnošću zakiseljavanja moštova iz toplih vinogradarskih regija, s višom pH vrijednosti. Sekvencijalna fermentacija s *Lt. x Sc.* rezultira snižavanjem pH vrijednosti i povećanjem ukupne kiselosti temeljem sinteze L-mliječne kiseline što doprinosi proizvodnji uravnoteženih vina. *Lt* osigurava alkoholnu jakost u rasponu od 5–9 % vol., a prema novijim podacima i do 10 % vol. alkohola (Hranilović i sur. 2018.), pa se 24-72 h kasnije sekvencijalno inokulira *S. cerevisiae* zbog potpune fermentacije šećera (Morata i sur., 2019.). *Lt.* može preživjeti nekoliko dana pri 9 % vol. alkohola (Kapsopoulou, 2007.), a postojana je i kad fermentacijom dominira *Sc.* (Mills i sur., 2002.). Općenito, boja mladih crnih vina uglavnom se oslanja na koncentraciju monomernih antocijana i srodnih spojeva, koji se ekstrahiraju iz kožice bobe grožđa tijekom proces maceracije (Ribereau-Gayon i sur., 2006.) Uloga koju imaju stanične stijenke kvasca u formiranju antocijana je njihova adsorpcijska sposobnost koja je posljedica kemijske interakcije između kvasca i metabolita koje kvasci proizvode tijekom alkoholnog vrenja (Morata i sur., 2003.). Zadržavanje pigmenata u staničnoj stijenci kvasca predstavlja izravan gubitak boje (Morata i sur., 2016.).

Kvasci mogu utjecati na boju vina smanjenjem pH vrijednosti, pogodujući stvaranju stabilnih pigmenata kao što su piranoantocijanini (Morata i sur., 2006.; Morata i sur., 2007.) ili polimerni pigmenti (Escott, 2018.), smanjujući adsorpciju antocijana u staničnim stjenkama (Suárez-Lepe, 2012.) ili štiteći antocijane od oksidativnog oštećenja oslobađajući reduktivne spojeve poput glutationa tijekom fermentacije i dozrijevanja na talogu (Morata i sur., 2016.). Mliječna kiselina koju sintetizira *Lt.* tijekom fermentacije može snažno utjecati na kiselost uz smanjenje od 0,3-0,5 pH jedinica u nekim slučajevima, pa s time može značajno utjecati na boju vina (Morata i sur., 2019.). Apsorpcija antocijana u staničnim stijenkama kvasca može biti između 3 i 6 % ukupne koncentracije u vinu (Morata i sur., 2006.). Kad je u pitanju vrsta *Sc.* apsorpcija ovisi o soju (Morata i sur., 2003.), a kod ne-*Saccharomyces* kvasaca ovisi o vrsti. U usporedbi s drugim vrstama, *Lt.* je pokazala srednje visok kapacitet apsorpcije (Morata i sur., 2016.). Neovisno o njihovom apsorpcijskom ponašanju tijekom fermentacije vina, oštećene stanice kvasca pokazale su istu sposobnost apsorpcije antocijana, što ukazuje na to da se sve veće razlike između apsorpcijskih sposobnosti određenih sojeva kvasaca, utvrđuju prema njihovoj sposobnosti da održe vitalnost stanice, kao i integritet stjenke i membrane tijekom fermentacije vina (Echeverrigaray i sur., 2020.).

´Trnjak crni´ je sorta s još uvijek nedovoljno istraženim podrijetlom. Smatra se autohtonom sortom Imotske krajine i zapadne Hercegovine. Ističe se manjim prinosom i izrazito kvalitetnim sastavom mošta i vina. Uz visoku koncentraciju šećera od 20-26 % i nižu do srednju koncentraciju ukupnih kiselina (4,5-6 gL^{-1}) ovisno o godini i terminu berbe, karakterizira ga i visoka koncentracija polifenola. Vina su stoga puna, ekstraktna, s koncentracijom alkohola od 12-15 % vol, intenzivno rubinske boje i vrlo ugodne arome (Mirošević i Turković, 2003., Sokolić, 2006., Maletić i sur., 2015.), ponekad neharmonična zbog niske ukupne kiselosti.

Cilj ovog rada bio je utvrditi razlike u učinku sekvencijalne inokulacije komercijalnih sojeva *L. thermotolerans* i *S. cerevisiae* te tretmana sa sojem *S. cerevisiae* u odnosu na epifitne kvasce u spontanoj fermentaciji na pH vrijednost i sastav antocijana u vinu ´Trnjak´.

Materijali i metode

Kvasci korišteni za pokretanje alkoholne fermentacije masulja sorte ´Trnjak´ u kontrolnom tretmanu *(i) Spont.* bili su epifitni kvasci (spontana fermentacija), u *(ii) Lt.xSc.* primijenjena je sekvencijalna inokulacija s *Lt.* (Laktia, Lallemand) x *Sc.* (BDX, Lallemand), a *(iii) Sc.* inokuliran je sa *Sc.* sojem (Fermol Premier Cru, AEB).

Grožđe sorte ´Trnjak´ s položaja Potpolje (Mostarsko vinogorje, BiH) berbe 2021., korišteno je u istraživanju. Ukupno 150 kg grožđa je prerađeno, homogenizirano i podijeljeno u 3 plastične posude zapremine 60 L te sulfitirano s 5%-tnom H_2SO_3. Proces maceracije masulja trajao je 8 dana uz redovitu remontažu masulja svakih 12 sati. Osnovni parametri u moštu bili su 89 °Oe, ukupna kiselost 6,3 gL^{-1} (kao vinska), a pH vrijednost 3,45. Tretman *(i) Spont.* kao kontrolna varijanta sulfitirana je s 50 mL 5%-tne H_2SO_3; tretman *(ii) Lt. x Sc.* sulfitiran je s 10 mL 5%-tne H_2SO_3 te inokuliran s kvascem *L. thermotolerans* (Laktia, Lallemand; 15g $50kg^{-1}$), a nakon 24 h uslijedila je inokulacija sa *S. cerevisiae* (BDX, Lallemand; 15g $50kg^{-1}$). Tretman *(iii) Sc.* sulfitiran je s 50 mL 5%-tne H_2SO_3 te inokuliran sa *S. cerevisiae* kvascem (Fermol Premier Cru, AEB; 15g 50 kg^{-1}). Nakon 8 dana maceracije i alkoholne fermentacije, masulji sva tri tretmana prešana su hidrauličnom prešom te je mošt u fermentaciji svakog pojedinog tretmana raspodijeljen u tri ponavljanja, otakanjem u staklene posude volumena 10 L s vrenjačom, u kojima je nastavljena alkoholna fermentacija. U prvom pretoku, nakon završene fermentacije, izvršena je korekcija sulfita s 10 mL 5%-tne H_2SO_3.

Analiza pojedinačnih polifenolnih spojeva provedena je pomoću tekućinsko-kromatografskog sustava (Agilent 1100) na fenil-heksilnoj koloni (Phenomenex, SAD)

Kromatogrami su istovremeno bilježeni detektorom s nizom dioda i fluorescencijskim detektorom. Identifikacija sastavnica vina provedena je usporedbom njihovih vremena zadržavanja na kromatografskoj koloni te pripadnih UV-Vis spektara i fluorescencije na karakterističnim valnim duljinama ekscitacije i emisije s vremenima zadržavanja, spektrima i fluorescencijom standardnih spojeva. Masene koncentracije identificiranih spojeva određene su pomoću baždarnih krivulja načinjenih analizom vanjskog standarda (Tomaz i Maslov, 2016.). pH vrijednost vina određena je mjerenjem na digitalnom pH-metru (Lab 845, SI Analytics),

Korištene statističke analize podataka uključivale su analizu varijance (ANOVA i t-test), pri čemu se srednje vrijednosti označene različitim slovima statistički razlikuju pri $p \leq 0,05$. Za analizu ukupne varijabilnosti sadržaja pojedinačnih antocijana i pH vrijednosti kod analiziranih uzoraka vina korištena je i analiza glavnih komponenti (PCA) primjenom statističkog programa XLSTAT v.2022.1.1. (Addinsoft).

Rezultati i rasprava

Prva istraživanja s *L. thermotolerans* i crnim vinima rezultirala su značajnim povećanjem (~ 10%) intenziteta boje u sekvencijalnim fermentacijama sa *S. cerevisiae* u usporedbi sa samostalnim *S. cerevisiae* tretmanom (Benito i sur., 2015., 2017.). Ta pojava pripisuje se jačem intenzitetu boje antocijana pri nižoj pH vrijednosti i povećanom stvaranju mliječne kiseline tijekom fermentacije s *Lt*. Kasnija istraživanja pokazuju smanjenje sadržaja antocijana u vinu u fermentaciji s *Lt.* uz povećanje udjela pojedinih antocijana (Benito i sur., 2019.). Analiza rezultata pojedinačnih antocijana (Tablica 1.) obuhvaćala je četiri glukozidna, te četiri alkilirana antocijana pri čemu je zabilježena značajna razlika u njihovom ukupnom udjelu na razini tretmana te značajno najveći udio u tretmanu *Spont.* Slične rezultate objavili su Del Fresno i sur. (2017.) i Escott i sur. (2018.) dok su Hranilović i sur. (2017.) zabilježili značajno povećanje antocijana u tretmanu s *Lt.*.

Malvidin-3-glukozid bio je najzastupljeniji antocijan u svim tretmanima, s najnižom koncentracijom u tretmanu sekvencijalne fermentacije *Lt. x Sc*. Isti tretman bilježi i najniže koncentracije glukozidnog i alkiliranog petunidina što je u skladu s istraživanjem Escribano Viana i sur. (2019.), ali značajno najviše peonidin-3-*O*-glukozida i malvidin-3-*O*-acetilglukozida, što je u odnosu na tretman *Sc.,* u skladu s prethodnim istraživanjima (Escott i sur., 2018., Chen i sur., 2018.). Niži sadržaj ukupnih antocijana kod tretmana *Lt. x Sc.* može se povezati sa značajno nižom vrijednosti pH, a koji ima važnu ulogu u brzini reakcija polimerizacije i kopigmentacije polifenolnih spojeva (He i sur. 2012.).

Rezultati pojedinačnih antocijana obrađeni su i analizom glavnih komponenata (Grafikon 1.). Temeljem položaja tretmana u koordinatnom sustavu definiranog s prve dvije glavne komponente koje objašnjavaju preko 90 % ukupne varijabilnosti te korelacije sadržaja pojedinačnih i ukupnih antocijana te pH vrijednosti vina, vidljivo je kako vina spontane fermentacije odlikuje viši sadržaj većine antocijana i niži sadržaj malvidin-3-*O*-acetilglukozida u odnosu na vina oba tretmana. Specifičnost tretmana, *Lt. x Sc*. dodatno je visok sadržaj malvidin-3-*O*-acetil glukozida dok tretman *Sc.* ponajviše definira najviša pH vrijednost, te niži sadržaj svih antocijana osim malvidin-3-*O*-glukozida.

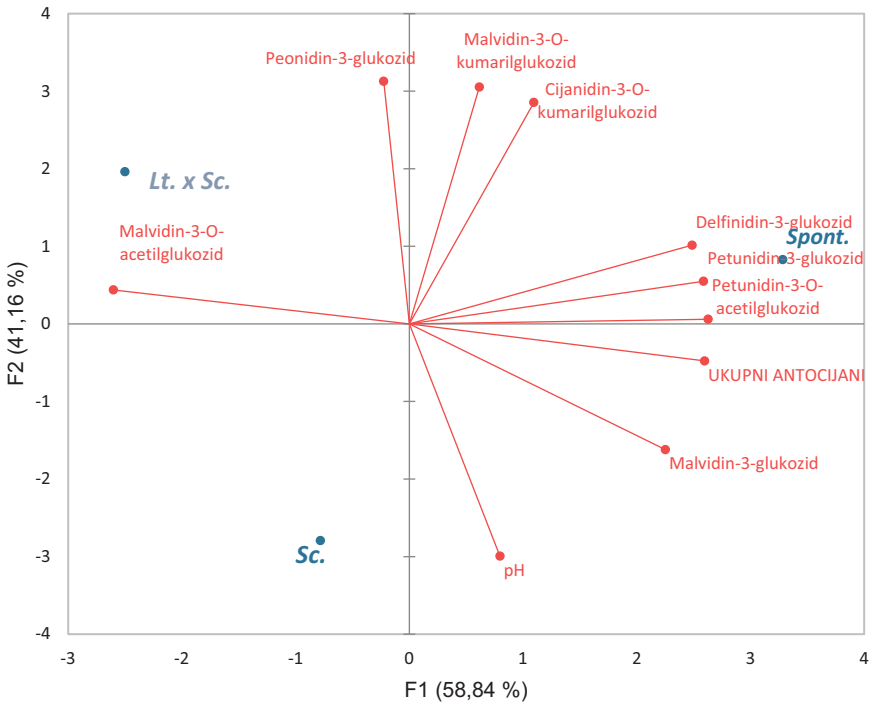
Zaključak

Analizom rezultata fermentacijskog pokusa s tri različita tretmana, utvrđene su značajne razlike s obzirom na istraživane parametre. Vino ´Trnjak´ iz tretmana sekvencijalne fermentacije s kvascima *L. thermotolerans* x *S. cerevisiae* imao je značajno najniži sadržaj pojedinačnih antocijana u usporedbi s ostalim tretmanima. Isti tretman značajno je utjecao na smanjenje pH vrijednosti što je jedan od glavnih učinaka primijenjene kombinacije kvasaca u sekvencijalnoj fermentaciji.

Tablica 1. Koncentracija pojedinačnih antocijana i pH vrijednost u vinu ´Trnjak´ berbe 2021.

Spoj (mgL^{-1})	***Spont.***	***Lt. x Sc.***	***Sc.***
Delfinidin-3-*O*-glukozid	0,25 a	0,01 b	0,00 b
Petunidin-3-*O*-glukozid	2,43 a	1,31 c	1,45 b
Peonidin-3-*O*-glukozid	1,85 b	2,55 a	0,36 c
Malvidin-3-O-glukozid	118,32 a	106,06 b	115,61 a
Petunidin-3-*O*-acetilglukozid	6,14 a	0,53 c	2,10 b
Malvidin-3-*O*-acetilglukozid	22,08 b	23,02 a	22,62 ab
Cijanidin-3-*O*-kumarilglukozid	0,19 a	0,16 a	0,05 b
Malvidin-3-*O*-kumarilglukozid	6,78 a	6,78 a	5,86 b
$\sum$	158,05 a	140,42 c	148,04 b
pH vrijednost	3,7 b	3,63 c	3,76 a

Vrijednosti su izražene kao srednja vrijednost (n=3). Značajne razlike pri $p \leq 0,05$ prikazane su različitim slovima (a,b,c) u istom redu. *Spont.*=spontana fermentacija; *Lt. x Sc.*= sekvencijalna fermentacija *L. thermotolerans* x *S. cerevisiae*; *Sc.= S. cerevisiae*



Spont.=spontana fermentacija; *Lt. x Sc.*= sekvencijalna fermentacija *L. thermotolerans* x *S. cerevisiae*; *Sc.= S. cerevisiae*

Grafikon 1. Analiza osnovnih komponenti (PCA)- pojedinačni antocijani i pH vrijednost u vinu ´Trnjak´

Literatura

Benito Á., Calderón F., Benito, S. (2019). The influence of non-*Saccharomyces* species on wine fermentation quality 540 parameters. Fermentation 5, 1–18.

Benito Á., Calderón F., Benito S. (2017). The Combined Use of *Schizosaccharomyces pombe* and Lachancea thermotolerans—Effect on the Anthocyanin Wine Composition. Molecules, 22, 739.

Benito Á., Calderón F., Palomero F., Benito S. (2015). Combine use of selected *Schizosaccharomyces pombe* and *Lachancea thermotolerans* yeast strains as an

alternative to the traditional malolactic fermentation in red wine production. Molecules. 20:9510–9523.

Chen K., Escott C., Loira I., del Fresno J.M., Morata A., Tesfaye W., Calderon F., Suárez-Lepe J.A., Han S., Benito S. (2018). Use of non-*Saccharomyces* yeasts and oenological tannin in red winemaking: influence on colour, aroma and sensorial properties of young wines. Food Microbiol 69:51–63.

Del Fresno J.M., Morata A., Loira I., Banuelos M.A. Escott C., Benito S., Chamorro C. G., Suarez-Lepe J.A. (2017). Use of non-*Saccharomyces* in single-culture, mixed and sequential fermentation to improve red wine quality. Eur. Food Res. Technol., 243, 2175–2185.

Echeverrigaray S., Scariot F. J., Menegotto M., Longaray Delamare A. P. (2020). Anthocyanin adsorption by *Saccharomyces cerevisiae* during wine fermentation is associated to the loss of yeast cell wall/membrane integrity. Int. J. Food Microbiol. 314:108383.

Escott C., Del Fresno J. M., Loira I., Morata A., Tesfaye W., González M. C. (2018). Formation of polymeric pigments in red wines through sequential fermentation of flavanol-enriched musts with non-*Saccharomyces* yeasts. Food Chemistry. 239: 975-983

Escribano-Viana R., Portu J., Garijo P., López R., Santamaría P., López-Alfaro I. (2019). Effect of the sequential inoculation of non-*Saccharomyces/Saccharomyces* on the anthocyans and stilbenes compositionof Tempranillo wines. Front. Microbiol. 10:773.

He F., Liang, N., Mu L., Hong-Pan Q., Wang J., Reeves M. J., Duan C. (2012). Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution. Molecules, 17(2): 1483-1519.

Hranilovic A., Bely M., Masneuf-Pomarede I., Jiranek V., Albertin W. (2017). The evolution of Lachancea thermotolerans is driven by geographical determination, anthropisation and flux between different ecosystems. PLoS ONE, 12(9), Article 0184652.

Hranilovic A., Gambetta J. M., Schmidtke L., Boss P. K., Grbin P. R., Masneuf-Pomarede I. (2018). Oenological traits of *Lachancea thermotolerans* show signs of domestication and allopatric differentiation. Scientific Reports, 8(1).

Kapsopoulou K., Mourtzini A., Anthoulas M., Nerantzis E. (2007). Biological acidification during grape must fermentation using mixedcultures of *Kluyveromyces thermotolerans* and *Saccharomyces cerevisiae*. World Journal of Microbiology and Biotechnology. 23: 735–739.

Maletić E., Karoglan Kontić J., Pejić I., Preiner D., Zdunić G., Bubola M., Stupić D., Andabaka Ž., Marković Z., Šimon S., Žulj Mihaljević M., Ilijaš I., Marković D. (2015). Zelena knjiga Hrvatske izvorne sorte vinove loze. Državni zavod za zaštitu prirode. Zagreb.

Maletić E., Karoglan Kontić J., Preiner D., Jeromel A., Patz C.D., Dietrich H. (2009). Anthocyanin profile and antioxudative capacitiy of some autochtonous Croatian red wines. Journal of Food, Agriculture and Environment. 7(1): 48-51.

Mills D.A., Johannsen E.A., Cocolin, L. (2002). Yeast diversity and persistence in Botrytis-affected wine fermentations. Appl. Environ. Microbiol. 68, 4884–4893

Mirošević N., Turković Z. (2003). Ampelografski atlas. Golden marketing – Tehnička knjiga, Zagreb, Hrvatska

Morata A., Escott C., Loira I., Del Fresno J.M., González C., Suárez-Lepe J.A. (2019). Influence of Saccharomyces and non-*Saccharomyces* yeasts in the Formation of Pyranoanthocyanins and Polymeric Pigments during Red Wine Making. Molecules. 24(24):4490.

Morata A., Gómez-Cordovés M. C. Calderón, F., Suárez, J. A. (2006). Effects of pH, temperature and SO2 on the formation of pyranoanthocyanins during red wine fermentation with two species of Saccharomyces. Int. J. Food Microbiol. 106, 123–129.

Morata A., Gómez-Cordovés M. C., Colomo B., Suárez J. A. (2003). Pyruvic acid and acetaldehyde production by different strains of *Saccharomyces cerevisiae*: relationship with Vitisin A and B formation in red wines. J. Agric. Food Chem. 51, 7402–7409.

Morata A., González C., Suárez-Lepe J. A. (2007). Formation of vinylphenolic pyranoanthocyanins by selected yeasts fermenting red grape musts supplemented with hydroxycinnamic acids. Int. J. Food Microbiol. 116, 144–152.

Morata A., Loira I., Heras J. M., Callejo M. J., Tesfaye W., González C. (2016). Yeast influence on the formation of stable pigments in red winemaking. Food Chem. 197, 686–691.

Ribereau-Gayon P., Glories Y., Maujean A. (2006). Handbook of Enology. West Sussex: John Wiley & Sons, Ltd.

Sokolić I. (2006). Veliki vinogradarsko-vinarski leksikon. Vlastita naklada, Novi Vinodolski, Hrvatska.

Suárez-Lepe J. A., Morata A. (2012). New trends in yeast selection for winemaking. Trends Food Sci. Technol. 23, 39–50.

Tomaz I., Maslov L. (2016). Simultaneous Determination of Phenolic Compounds in Different Matrices using Phenyl-Hexyl Stationary Phase, Food Analytical Methods, 19(2): 401-410.

Influence of yeasts on anthocyanin composition of Trnjak wine

Abstract

Saccharomyces cerevisiae is the most commonly used selected yeast in wine production while among non-*Saccharomyces* yeast *Lachancea thermotolerans* is getting more attention. It stands out for its ability to acidify musts with higher pH values, especially from warm wine-growing regions. During vinification, the formation of wine color and anthocyanin composition can be influenced by yeast, ie. the adsorption characteristics of the yeast cell wall. The stability, aroma and color of wine depends on the yeast strain used during the fermentation process. Metabolites produced by *S. cerevisiae* or non-*Saccharomyces* yeasts can react with anthocyanins and by formation of pyranantocyanins can have influence on the color intensity and stability. The aim of this study was to compare the influence of sequential inoculation of commercial strains of *L. thermotolerans* and *S. cerevisiae* with treatments with *S. cerevisiae* alone and epiphytic yeasts on the anthocyanin composition of Trnjak wine. The results of the analyzes indicated significantly higher concentrations of anthocyanins in treatments with *S. cerevisiae* and epiphytic yeasts.

Key words: *Saccharomyces cerevisiae*, *Lachancea thermotolerans*, non-*Saccharomyces* yeasts, anthocyanins, sequential fermentation

IZVORNI ZNANSTVENI RAD

Utjecaj antioksidansa u predkulturi mikroreznica *in vitro* na regeneraciju vinove loze nakon krioprezervacije

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Sažetak
Protokole krioprezervacije potrebno je testirati za autohtone sorte na zaraženom i zdravom sadnom materijalu, jer zaraženost može predstavljati problem u kulturi tkiva. Antioksidansi mogu potaknuti rast biljaka. Cilj rada bio je uspostaviti regeneraciju biljaka inficiranih genotipova u *in vitro* uvjetima, te na njima testirati standardni protokol krioprezervacije za vinovu lozu. Pupovi inficiranih genotipova veličine 1,5 mm postavljeni su na različite MS (Murashige i Skoog) medije uz dodatak 0,5 mg L^{-1} 6-benzilaminopurina (BAP) i 0,05 mg L^{-1} indol-3-octene kiseline (IAA) sa antioksidansima (M1: 0,1 mM salicilne kiseline, M2: 1 mM askorbinske kiseline, M3: 1 mM glutationa i M4:kombinacija tri antioksidansa navedenih koncentracija), te je praćen njihov rast *in vitro*. Iz potjeralih biljaka izolirani su vršni meristemi, te je proveden pokus krioprezervacije (LS-20´, ½ PVS2-30´i PVS2-50´ kontrola i smrzavanje). Najveći rast *in vitro* postignut je na mediju sa dodatkom glutationa (M3). Zaraženost je imala utjecaj u regeneraciji biljaka *in vitro*, ali nije utjecala na regeneraciju nakon krioprezervacije.

Ključne riječi: rast *in vitro*, inficirani genotipovi, antioksidansi, regeneracija, krioprezervacija

Uvod

Vinova loza je najrasprostranjenija voćna vrsta u svijetu, koja svojom ukupnom proizvodnjom dominira nad ostalim kulturama (Maletić i sur., 2008). 'Plavac mali crni' je autohtona sorta srednje i južne Dalmacije (Mirošević i Karoglan Kontić, 2008). Po zastupljenosti u sortimentu Republike Hrvatske je na trećem mjestu, iza Graševine i Malvazije istarske. Kakvoća najviše ovisi o položaju, a za uzgoj ove sorte ističu se položaji Dingač, Postup, Sv. Nedjelja, Ivan Dolac (Maletić i sur., 2015). 'Pošip bijeli' je autohtona sorta otoka Korčule (Mirošević i Karoglan Kontić, 2008). Na otoku Korčuli, Čarskom i Smokvičkom polju ima najviše 'Pošipa bijelog', a nalazi se i na susjednim otocima te priobalju. Danas je jedna od najpoznatijih bijelih sorata Dalmacije (Maletić i sur., 2015).Brzo klonsko razmnožavanje u *in vitro* uvjetima, odličan je način dobivanja velike količine biljnog materijala bez patogena. Virusom inficirani biljni materijal, slabije reagira na uvjete kulture tkiva. Uz brzo klonsko razmnožavanje, moguće je u *in vitro* uvjetima smrznuti biljni materijal, te ga na taj način pohraniti na duži vremenski period. Nova metoda bazirana je na pohrani biljnog materijala u tekućem dušiku (liquid nitrogen, LN) pri niskim temperaturama (-196 ºC) koje uvjetuju potpuno zaustavljanje metaboličkih funkcija biološkog materijala, te na taj način omogućuju čuvanje uzoraka na duži period (Reed, 2008). Postoji nekoliko protokola za krioprezervaciju vinove loze, ali ne postoji standardni protokol koji je učinkovit u svakom istraživačkom laboratoriju za veći broj sorata vinove loze. Sorte vinove loze različito reagiraju na krioprezervaciju te je potrebno prvo ispitati

protokol kako bi se postigla visoka stopa regeneracije. Antioksidans je svaka tvar koja odgađa ili sprječava oksidaciju spojeva koji su podložni oksidaciji, poput lipida, proteina, deoksiribonukleinske kiseline (DNK) i ugljikohidrata. Funkcija antioksidansa u biološkim sustavima je onemogućavanje djelovanja slobodnih radikala (Halliwell i sur., 1995). Cilj ovog rada je pokazati kako virusima inficirani genotipovi rastu u *in vitro* uvjetima, te kako reagiraju na osnovni standardni protokol krioprezervacije.

Materijal i metode

Odabrane su dvije autohtone sorte 'Plavac mali crni' i 'Pošip bijeli'. Biljni materijal sorata 'Plavac mali crni' i 'Pošip bijeli' za unos u *in vitro* kulturu je dobiven prikupljanjem virusom zaraženih i zdravih reznica iz vinograda sa pokušališta Jazbina. Potjerali izdanci, sterilizirani su po standardnom protokolu (ispiranje tekućom vodom, potapljanje u 70 % etanolu 20 minuta, izlaganje 5 % otopini natrijevog hipoklorita 15 minuta, te tri puta ispiranje sterilnom vodom u laminaru) te su korišteni za postavljanje pokusa u predkulturi mikroreznica. Za postavljanje na hranjivi medij korišteni su nodijski odsječci veličine 1,5 cm sa jednim pupom tzv. mikroreznice. Nakon postavljanja na medije, mikroreznice su rasle u uvjetima klima komore (Aralab Fitoklima PL 1200) na konstantnih 26±0,5 °C pod bijelim LED svjetlom u intenzitetu od 100 µmol sm^{-2} uz fotoperiod od 16 sati svjetla i 8 sati mraka. U ovom istraživanju je korišten hranjivi medij laboratorijske oznake B inicijalni medij sastava MS (Murashige i Skoog, 1962.) medij uz dodatak biljnih hormona citokinina 6-benzilaminopurin (BAP) u koncentraciji od 0,5 mg L^{-1} i auksina indol-3-octena kiselina (IAA) u koncentraciji od 0,05 mg L^{-1}, 30 g L^{-1} saharoze, 8 g L^{-1} agara i pH podešen na 5,8 prije autoklaviranja. U B inicijalni medij za postavljanje pokusa su dodani antioksidansi u koncentracijama kako je navedeno u tablici 1. Nakon provedene krioprezervacije eksplantati su postavljeni u Petrijeve zdjelice (90 mm) po 15 eksplantata na regeneracijski medij (RM) i na osnovu toga je napravljena statistička obrada podataka.

Tablica 1. Prikaz oznaka medija i naziva medija sa koncentracijama antioksidansa korištenih u istraživanju

Oznaka medija	Naziv medija i koncentracija antioksidansa
M_1	B inicijalni + 0.1 mM salicilna kiselina (SA)
M_2	B inicijalni + 1 mM askorbinska kiselina (AA)
M_3	B inicijalni + 1 mM glutation (GSH)
M_4	B inicijalni + 0.1mM SA+1mM AA+1mM GSH
RM	MS medij + 0,25 mg L^{-1}BAP-a

Korištene su 2 sorte, odnosno njihovi klonovi ('Plavac mali' (PMC) i 'Pošip bijeli' (POŠ)), te su uzorci uzimani sa nekoliko trseva (PMC sa 6 trseva, a POŠ sa 2 trsa). U testiranju predkulture mikroreznica, svaki pojedini trs je promatran pojedinačno, dok se u testiranju protokola krioprezervacije zanemarilo podrijetlo materijala i uzorci su testirani na razini sorte. Nakon uzimanja materijala provedeno je testiranje na viruse. Sanitarni status korištenih klonova tj.trseva je prikazan u tablici 2. Prisustvo virusa GFLV, GFLaV-1 i GFLaV-3 je utvrđeno ELISA testom. Mikroreznice su postavljene na medije sa antioksidansima (salicilna kiselina, askorbinska kiselina i glutation, te njihova kombinacija). Na jedan medij je postavljeno 10-12 mikroreznica sa svakog pojedinog trsa, kada je bilo dovoljno potjeralog materijala. U slučaju ograničene količine potjeralog materijala, prvo su postavljane mikroreznice na medije M_1 i M_4 (sa salicilnom kiselinom i kombinacijom antioksidansa) pa na medij M_2 i na kraju na medij M_3.

Tablica 2. Prikaz klona i broja trsa sorata 'Plavac mali' i 'Pošip bijeli', korištenih u istraživanju i njihov sanitarni status

Sorta	Klon i broj trsa	Sanitarni status
'Plavac mali crni'	PMC16-12	bez virusa
	PMC16-3	(LR3)
	PMC16-8	(GFLV+LR1)
	PMC16-18	(LR1)
	PMC19-13	bez virusa
	PMC19-15	(GFLV)
'Pošip bijeli'	POŠ089-3	bez virusa
	POŠ089-11	(LR1+LR3)

Nakon sadnje na medij sa antioksidansima, a u svrhu dobivanja materijala za pokuse krioprezervacije, pratili su se rast i razvoj biljaka. Četiri tjedna nakon sadnje, zabilježen je postotak regeneriranih biljaka. Regenerirane biljke su one koje su razvile dva do tri listića i s njih su uzimani vegetacijski vršci, tehnikom disekcije, za pokus krioprezervacije.Vegetacijski vršci veličine 1 - 1,5 mm su disekcijom izdvojeni s mikroreznica postavljenih na medije sa antioksidansima. Vegetacijski vršci stavljeni su na medij za pretkulturu (M4) i zatim je nakon 72 sata proveden postupak krioprezervacije. Postupak krioprezervacije zbog ograničene količine materijala proveden je za svaku na razini dviju sorata ('Plavac mali' i 'Pošip bijeli') koristeći se pri tome svim mikroreznicama dobivenim u prethodnom pokusu. Krioprezervacija je provedena po metodi droplet-vitrifikacije prema Bettoni i sur., (2019). U postupku krioprezervacije prvi tretman je uranjanje eksplantata u LS otopinu (eng. *loading solution*) u trajanju od 20 minuta pri sobnoj temperaturi. LS otopina sadrži 2 M glicerola i 0.4 M saharoze u MS mediju. Postupak dehidracije je izveden 50 % -tnom otopinom PVS2 pri sobnoj temperaturi u trajanju od 30 minuta i 100 %-tnom otopinom PVS2 pri temperaturi od 0 °C u trajanju od 50 minuta. Sterilna petrijeva zdjelica s otopinom i eksplantatima je bila smještena u posudici sa ledom kako bi se održala temperatura od 0 °C. Nakon toga su kapljice PVS2 otopine u volumenu od 0.01 mL dodane pipetom na komadić aluminijske folije. Eksplantati su stavljeni u kapljice PVS2 otopine. Zatim su se komadići aluminijske folije sa eksplantatima stavili u tekući dušik na 50 minuta. Eksplantati su stavljeni u US otopinu nakon vađenja iz tekućeg dušika. US otopina (eng. *unloading solution*) služi za vraćanje stanične tekućine i temperature eksplantata u stanje kakvo je bilo prije postupka krioprezervacije, a sadrži 0,2 M saharoze. Eksplantati su u US otopini bili 20 minuta. Nakon provedenog postupka eksplantati su stavljeni na regeneracijski medij (RM). Opažanje regeneracije je provedeno 8 tjedana nakon postupka krioprezervacije. Utjecaj svih nezavisnih varijabli vezanih uz prvi dio pokusa za dobivanje mikroreznica (medij, genotip, sanitarni status) te utjecaj sorte (u pokusu krioprezervacije) testirani su odvojeno jednosmjernom analizom varijance. Usporedba srednjih vrijednosti provedena je pomoću Duncan's multiple range testa. Sve analize provedene su korištenjem statističkog softvera XLSTAT 2022.1.1. (Addinsoft).

Rezultati i rasprava

Utjecaj medija sa dodatkom različitih antioksidansa, genotipa i sanitarnog statusa prikazan je u tablici 3. na regeneraciju mikroreznica u predkulturi. Najveći postotak regeneriranih razvio se na mediju sa dodatkom glutationa (94,44 %), što je statistički značajno u odnosu na medij sa dodatkom salicilne kiseline (61,76 %) koji je ostvario najmanji rezultat. Uspjeh regeneriranih biljaka na medijima sa askorbinskom kiselinom i kombinacijom antioksidansa je približno jednak. Regeneracija biljaka na razini genotipa ostvarila je približno jednake vrijednosti (70,19 – 85,92 %). Sanitarni status dao je široki raspon ostvarene regeneracije,

gdje je sanitarni status GLRaV-1 ostvario najvišu regeneraciju (89,58 %), statistički značajno različitu od sanitarnog statusa GLRaV-3 (45,83 %). Jedino je sanitarni status GLRaV-3 rezultirao samostalno nižim uspjehom regeneracije na razini svih genotipova. Ista činjenica je ranije potvrđena kod testiranja utjecaja sanitarnog statusa na svim trsevima genotipa PMC-16 (rezultati nisu prikazani), a uzrok mogu biti različite mutacije ili utjecaj različitih genotipova korištenih u istraživanju. Slabiji uspjeh rasta u *in vitro* uvjetima inficiranih genotipova sorata u istraživanju, ali i drugih sorata ranije je također potvrđen (Marković et al., 2015). Stoga je potrebno definirati protokol za *in vitro* uvjete za svaku pojedinu sortu, da bi se biotehnološki postupci ozdravljivanja mogli primijeniti.

Tablica 3. Utjecaj medija sa dodatkom različitih antioksidansa, genotipa i sanitarnog statusa na regeneraciju mikroreznica u kulturi tkiva

izvor varijabilnosti	razina	uspjeh regeneracije
Medij - antioksidansi	M_3	94.44 a
	M_2	79.29 ab
	M_4	78.60 ab
	M_1	61.76 b
Genotip	POŠ089	85.92 a
	PMC19	78.06 a
	PMC16	70.19 a
Sanitarni status	GLRaV-1	89.58 a
	bez virusa	79.09 a
	GFLaV1+GFLaV3	76.39 ab
	GFLV+GLRaV-1	75.00 ab
	GFLV	71.03 ab
	GLRaV-3	45.83 b

Proveden je pokus krioprezervacije na sortama 'Plavac mali' i 'Pošip bijeli' (Tablica 4.). Kontrola je korak koji prethodni smrzavanju i podrazumijeva izlaganje krioprotektantu PVS2 50 minuta. Smrzavanje je korak u kojem su eksplantati prošli sve korake protokola i završno su potopljeni u tekući dušik. Kao što je vidljivo iz prikazanih rezultata, sorta 'Pošip bijeli' je dala bolje rezultate u oba koraka, međutim bez signifikantno značajne razlike među njima. U kontrolnom koraku sorta 'Plavac mali' je dala slabije rezultate (29.03%) u odnosu na smrzavanje (34.03%), što je suprotno od očekivanog. S obzirom da je prvi put ovaj protokol testiran na ovim sortama, može se zaključiti da su relativno visoke vrijednosti postignute za kontrolni, kao i za korak smrzavanja kod standardnog protokola krioprezervacije.

Tablica 4. Rezultat krioprezervacije metodom droplet-vitrifikacije na sortama 'Plavac mali' i 'Pošip bijeli'

Tretman	Sorta	Udio potjeralih eksplantata (%)
Kontrola	POŠ	52.70 a
	PMC	29.03 a
Smrzavanje	POŠ	47.20 a
	PMC	34.03 a

Slika 3. Eksplantat sorte 'Plavac mali crni' četiri tjedna nakon provedene krioprezervacije (kontrola)

Slika 4. Eksplantat sorte 'Pošip bijeli' četiri tjedna nakon provedene krioprezervacije (smrzavanje)

Zaključak

S ciljem postavljanja banke gena vinove loze, na temelju rezultata ovog istraživanja možemo zaključiti da je moguće testirati protokole krioprezervacije na zaraženom biljnom materijalu, te da se uz pomoć antioksidansa može pospješiti rast istih u *in vitro* uvjetima. Međutim, za primjenu ovih tehnika potrebno je napraviti daljnja testiranja *in vitro* uvjeta i protokola krioprezervacije.

Napomena

Istraživanja neophodna za ovaj rad dio su hrvatsko-srpskog bilateralnog projekta „*In vitro* razmnožavanje, krioprezervacija i kvantifikacija biološke aktivnosti plodova jagodastih vrsta voćaka i vinove loze “.

Literatura

Bettoni J.C., Bonnart R., Shepherd A., Kretzschma A., Volk G.M. (2019). Successful cryopreservation of Vitis vinifera ‘Chardonnay’ from both in vitro and growth chamber source plants. Acta Horticulturae. 1234:211–218.

Halliwell B., Aeschbach R., Löliger J., Aruoma O.I. (1995). The characterization of antioxidants. Food and Chemical Toxicology. 33:601–617.

Maletić. E., Karoglan Kontić, J., Pejić, I. (2008). Vinova loza – ampelografija, ekologija, oplemenjivanje, Zagreb, Hrvatska: Školska knjiga.

Maletić E., Karoglan Kontić J., Pejić I., Preiner D., Zdunić G., Bubola M., Stupić D., Andabaka Ž., Marković Z., Šimon S. (2015). Zelena knjiga: hrvatske izvorne sorte vinove loze. Zagreb, Hrvatska: Državni zavod za zaštitu prirode.

Marković Z., Preiner D., Bošnjak A.M., Safner T., Stupić D., Andabaka Ž., Maletić, E., Chatelet P., Engelmann F., Kontić J.K. (2014). *In vitro* introduction of healthy and virus-infected genotypes of native Croatian grapevine cultivars. Cental European Journal of Biology. 9:1087–1098.

Mirošević N., Karoglan Kontić J. (2008). Vinogradarstvo. Zagreb, Hrvatska: Nakladni zavod globus.
Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiologia Plantarum. 15:473-497.
Reed B.M. (2008). Plant cryopreservation: a practical guide. 4-87. New York , SAD: Springer.

Influence of antioxidants in preculture of *in vitro* microcuttings on grapevine regeneration after cryopreservation

Abstract
Cryopreservation protocols need to be tested for autochthonous cultivars on an infected and healthy plant material cause virus infection can be difficult in tissue culture. Antioxidants can improve the plant growth. The aim of this work was to establish the plant regeneration of infected plants in tissue culture conditions and testing a standard cryopreservation protocol for grapevine on them. Buds 1.5.mm-size of infected genotypes were inoculated on different MS (Murashige i Skoog) media with addition of 0.5 mg L^{-1} 6-benzilaminopurina (BAP) and 0.05 mg L^{-1} indol-3-acetic acid (IAA) with antioxidants (M1: 0.1 mM salycilic acid, M2: 1 mM ascorbic acid, M3: 1 mM glutathione and M4:combination of three antioxidants with mentioned concentrations) and *in vitro* growth was observed. From shooted plants apical meristems were excised and cryopreservation protocol was performed (LS-20´, ½ PVS2-30´and PVS2-50´ control and freezing). The highest *in vitro* growth was achieved on medium with addition of glutathione (M3). Infection has an effect on regeneration of plants, but has not effect on regeneration after cryopreservation.

Key words: growth *in vitro*, infected genotypes, antioxidants, regeneration, cryopreservation

IZVORNI ZNANSTVENI RAD

Impact of pre-fermentative mash cooling and heating on anthocyanin concentration and color of Teran wines

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Abstract
Six vinification treatments, including control wine (TK7) were carried out to investigate: the impact of 48-h pre-fermentative mash cooling (8 °C) followed by maceration of 13 days (TC15), 28 days (TC30) and *saignée* technique (TCS15); and the impact of 48-h heating (50 °C) followed by maceration of 13 days (TH15) and 28 days (TH30) on anthocyanin concentration and color in Teran wines. Anthocyanins, intensity, hue and composition of color were analyzed by UV/Vis spectrometry. The results showed that total anthocyanins and color hue values in all treatments were higher compared to control wine. The highest concentrations of free anthocyanins were found in wines with pre-fermentative heating treatment. Color intensity exposed the best results in 15-day macerations regardless of pre-fermentative treatment.

Key words: Teran wines, pre-fermentation treatments, anthocyanins, color intensity, color hue

Introduction

The color of red wines is one of the most important sensory attributes and is related to the extraction of anthocyanins from grape skins during winemaking. There are several hundred known anthocyanins and anthocyanin-derived pigments with diverse structures and properties (Sáenz-Navajas et al., 2011), responsible for color in red grapes and wines. They are accumulated in berry skins during the grape ripening (Perez-Lamela et al., 2007). Sacchi et al. (2005) report that the following winemaking variables and techniques increase phenolic composition: fermentation temperature, thermovinification, must freezing, *saignée*, pectolytic enzyme treatments, and extended macerations. In wines, monomeric anthocyanins can also undergo a wide variety of reactions and associations and various anthocyanin-derived new pigments are formed, which are extremely crucial for color stability (He et al., 2012). Many winemaking techniques have been developed to improve color extraction to obtain products with the market demanding characteristic (Puertas et al., 2008). Basic thermovinification entails short heating of the skins from 50 to 80 °C extracting them with the juice, pressing, and then cooling before fermentation. If heating at the same temperature before fermentation is extended for a longer period (for instance, up to 24 h), the process is called pre-fermentative heat treatment (Escudier et al., 2008; Rossi et al., 2022). The heat damages the hypodermal cell membranes, releasing anthocyanins, and it also denatures polyphenol oxidase, preventing browning. Since there is no alcohol present at the time of heating, it would not be expected to increase tannin extraction (Sacchi et al., 2005). Pre-fermentative cold maceration, also known as cold soaking or cryomaceration, is being increasingly used by enologists worldwide in order to improve some important quality characteristics of wines such as color and aroma. This technique consists in maintaining the crushed grapes at low temperatures (5–10 °C) (Heredia et al., 2010). In pre-fermentation

juice runoff which is also known as *saignée*, the juice is removed before fermentation, thus increasing the skin to juice ratio (Sacchi et al., 2005). Teran (*Vitis vinifera* L.) is the most widespread red autochthonous cultivar on the Istrian peninsula, Croatia (Plavša et al., 2012; Rossi et al., 2022). The aim of this study was to investigate how pre-fermentation cooling or heating treatments, *saignée* technique, and various maceration durations affect the anthocyanin concentrations and parameters of color in Teran red wine.

Material and methods

The grapes of *cv*. Teran (*Vitis vinifera* L.) were grown in Western Istria, the town of Poreč, in a typical Istrian terroir. The harvest was held in 2020 when the sugar content was measured at 18.9 °Brix, 8.0 g L^{-1} of total acidity expressed as tartaric acid, and pH 3.2. On the same day, manually harvested grapes were destemmed and crushed with standard equipment and homogenized. Red grape mash was equally divided according to the plan of the experiment (Table 1). Five vinification treatments were submitted to pre-fermentative mash cooling at 8 °C (cryomaceration) or mash heating at 50 °C for 48 hours, proceeded with fermentation at 24 °C and followed by prolonged maceration in two periods of duration: 13 days, respectively 15 days in total including pre-fermentative process (TC15; TH15) and 28 days, in total 30 days (TC30; TH30). In one of the treatments with pre-fermentative mash cooling, the *saignée* technique was performed before fermentation. A proportion (33 %) of the total juice quantity was racked and concentrated mash proceeded with fermentation (24 °C) and prolonged maceration of 13 days, 15 days in total (TCS15). This experiment has also included a control treatment (TK7), with a standard technique of a 7-day maceration and a maceration/fermentation temperature of 24 °C. Every treatment was performed in three replications in 220 L-stainless steel tanks. All grape mashes were inoculated with 30 g hl^{-1} of selected *Saccharomyces cerevisiae* dry yeast (Fermol Mediterranee, AEB), and chaptalized with 3 kg hl^{-1} of saccharose. After the end of the maceration process, fermented mashes were pressed and wine was racked in clean tanks. After six months the wine was bottled and stored prior to analysis.

Table 1. Overview of the experiment: pre-fermentative treatments, winemaking techniques and maceration duration in Teran wine treatments

Treatment*	Pre-fermentative treatment	Fermentation and maceration			Maceration duration including pre-fermentative treatment
	Duration – 48 hours	Winemaking technique	Maceration/ fermentation temperature	Maceration duration	
TCS15	Cooling - at 8 °C	*Saignée* technique	24 °C	13 days	15 days
TC15		Prolonged maceration		13 days	15 days
TC30				28 days	30 days
TH15	Heating - at 50 °C			15 days	15 days
TH30				28 days	30 days
TK7	/	Standard technique		7 days	/

*T-Teran; C-cooling; H-heating; S- *saignée;* K-contol; 7,15,30 – days of maceration duration in total

The color intensity and composition i.e. partial contribution to the full color were determined according to Glories (1984) using a Cary 50 UV/Vis spectrophotometer (Varian Inc., Harbour City, CA, USA). The method is based on the determination of absorbances values on three different wavelengths; 420 nm, 520 nm, and 620 nm. Glories (1984) modified the definition of color intensity (CI') as a sum of absorbances (A) on those three wavelengths: CI'= A420 + A520 + A620. Partial contribution to the full color was calculated using this

formulas: for % of red color = (A520*100)/100; % of yellow = (A420*100)/100; % of blue = (A620*100)/100. Color hue was determined using the method by Sudraud (1958) defined as a ratio of absorbances A420 and A520. Total and free anthocyanin content was analyzed according to the methods described by Di Stefano (1989). Briefly, methods are based on the reading of absorbances at wavelengths between 536 and 540 nm using the solutions of wine and other chemicals previously prepared according to the described instructions. The highest absorbance is calculated and given results are expressed as mg l^{-1}. One-way analysis of variance (ANOVA) and Fisher's least significance difference (LSD) test was used to compare mean values ($p \leq 0.05$). Statistical analyses were performed using Statistica 10.0. software (Sta-Soft Inc. Tulsa, OK).

Results and discussion

Total anthocyanin concentration in all treatments differed significantly in comparison to the control wine ($p \leq 0.05$) (Figure 1). The highest value was found in treatment subjected to the pre-fermentative heating process, TH15. Treatments submitted to the diverse duration of prolonged maceration TCS15, TC15 and, TH30 showed no significant difference. Boulton (2001) reports that many studies showed additional contact time between skins and wine cannot provide additional anthocyanin content or color. Regarding free anthocyanins, it was found that treatments that underwent pre-fermentative heating process (TH15 and TH30) were statistically the highest and different compared to others (Figure 2). There was no difference between pre-fermentative cooling processes (TCS15, TC15 and, TC30) regardless of maceration duration. Heredia et al. (2010) exhibit that low temperatures can influence phenolic co-pigmentation power which means they can reduce free anthocyanin content as in our case in comparison to heating treatment.

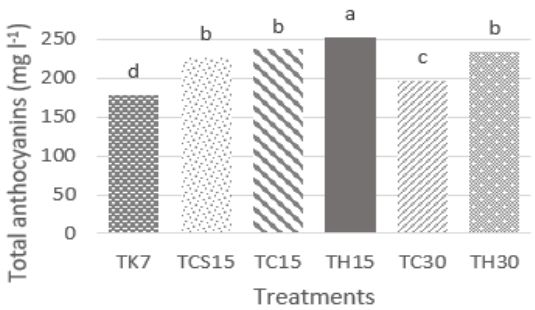

Figure 1. Total anthocyanin concentrations (mg L^{-1}) in Teran wines

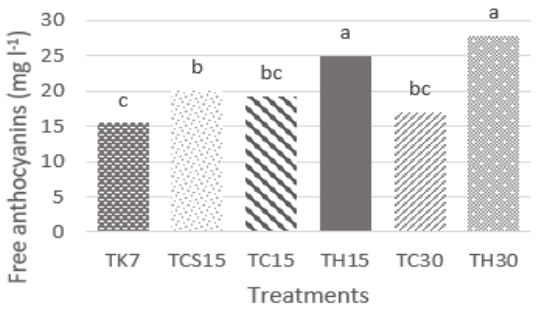

Figure 2. Free anthocyanin concentrations (mg L^{-1}) in Teran wines

Lower-case letters above column represent significant differences at $p \leq 0.05$ level according to the LSD test

The color intensity results showed that treatments TCS15, TC15, and TH15 were significantly different in comparison to other samples ($p \leq 0.05$) (Table 2). Also was found that intensity in 30-day maceration treatment (TC30) was statistically lower than in control wine (TK7). A decrease in color intensity in TC30 treatment could be related to a lower concentration of total anthocyanins (Figure 2). Gonzalez-Neves et al. (2010) suggest that the decrease of anthocyanins in prolonged macerations is a result of post-fermentation fixation by yeasts and solids. Color hue results presented that control wine (TK7) was significantly the lowest in comparison to other treatments. The highest value was obtained in TC30 treatment, and compared to the other 30-day maceration treatment (TH30) was different. There was no statistical difference in color hue values between cooling and heating pre-fermentative processes in treatments with 15-day maceration (TCS15, TC15, TH15) (Table

2). There was a significant difference in the distribution of yellow color between cooling and heating processes but only in 30-days maceration treatments (TC30 and TH30) (Table 2). The highest value was obtained in TC30 treatment, which might be related to color hue results, because several authors noted that tone (hue) and proportion of yellow increase during winemaking and aging due to oxidation processes (Puertas et al., 2008). Treatments TCS15, TC15 and, TH15 did not differ significantly in distribution of yellow color. Regarding blue color distribution, it increased in all treatments, except TC15, in comparison to control wine (TK7). The highest distribution value of blue color was found in treatment TH30. Other treatments (TCS15, TH15 and, TC30) were identical regardless of pre-fermentative process and maceration duration. Boulton (2001) report that blue color increases with winemaking and also with aging due to anthocyanins condensation and polymerization with other polyphenols, which might be the case in wines from our experiment. The distribution of red color was significantly the highest in control wine (TK7). On the contrary Puertas et al. (2008) obtain that percentage of red color is the lowest in control wine in comparison to other investigated treatments. Red color values obtained in treatments with 15-day maceration were equal, and higher than in 30-day maceration treatments despite to used pre-fermentative process (Table 2).

Table 2. Color intensity, color hue values and percentage of red, yellow and blue color in Teran red wines

	Color intensity	Color hue	Red color (%)	Yellow color (%)	Blue color (%)
TCS15	0.94 ± 0.0058a	0.73 ± 0.0038c	49.40 ± 0.0364b	35.87 ± 0.1631c	14.73 ± 0.1268b
TC15	0.94 ± 0.0222a	0.72 ± 0.0068c	49.66 ± 0.1491b	35.89 ± 0.2293c	14.45 ± 0.0813c
TH15	0.95 ± 0.0288a	0.72 ± 0.0075c	49.56 ± 0.2094b	35.82 ± 0.223cd	14.63 ± 0.0267b
TC30	0.73 ± 0.0014d	0.76 ± 0.0047a	48.55 ± 0.1875c	36.81 ± 0.0854a	14.64 ± 0.1026b
TH30	0.85 ± 0.0022b	0.74 ± 0.0044b	48.68 ± 0.1098c	36.26 ± 0.1308b	15.06 ± 0.0235a
TK7	0.8 ± 0.0038c	0.1 ± 0.0069d	50.20 ± 0.2616a	35.51 ± 0.1751d	14.28 ± 0.1379c

Each value is the mean ± standard deviation, n=3. Lower-case letters represent significant differences at $p \leq 0.05$ level (LSD test).

Conclusions

Obtained results demonstrated the notable impact of pre-fermentative cooling and heating, together with prolonged macerations of Teran mash on free and total anthocyanins, color intensity, color hue, and percentage of red, yellow and blue color. Both pre-fermentative cooling and heating treatments positively affected the color intensity and hue, while pre-fermentative heating treatment significantly enhanced free anthocyanin concentration in Teran wines. On the basis of obtained results, wine producers can choose the appropriate winemaking technology for improving the quality of their products.

Acknowledgement

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References

Boulton R. (2001). The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: A Critical Review. Am J Enol Vitic 52: 67-87.

Di Stefano R., Cravero M.C., Gentilizi N. (1989). Metodi per lo studio dei polifenoli dei vini. L'Enotecnico. 5:83–89.

Glories Y. (1984). La Couleur des Vins Rouges. Con. Vigne et du Vin 18(4): 253–271.

Gomez-Cordoves C. and Gonzalez-SanJose M.L. (1995) Interpretation of color variables during the aging of red wines: relationship with families of phenolic compounds. J. Agric. Food Chem. 43(3): 557-561.

González-Neves G., Gil G., Barreiro L., Favre G. (2010) Pigment profile of red wines *cv*. Tannat made with alternative winemaking techniques. Journal of Food Composition and Analysis. 23(5):447–54.

He F., Liang N-N., Mu L., Pan Q-H., Wang J., Reeves M.J., Duan C.Q. (2012) Anthocyanins and their variation in red wines I. monomeric anthocyanins and their color expression. Molecules. 17(2):1571–601.

Heredia F.J., Escudero-Gilete M.L., Hernanz D., Gordillo B., Meléndez-Martínez A.J., Vicario I.M., Gonzalez-Miret M.L. (2010). Influence of the refrigeration technique red wines obtained by pre-fermentative cold maceration. Food Chemistry. 18(2):377–83.

Maza M., Álvarez I., Raso J. (2019) Thermal and Non-Thermal Physical Methods for Improving Polyphenol Extraction in Red Winemaking. Beverages. 5(3):47.

Pérez-Lamela C., García-Falcón M.S., Simal-Gándara J., Orriols-Fernández I. (2007) Influence of grape variety, vine system and enological treatments on the colour stability of young red wines. Food Chemistry. 101(2):601–6.

Plavša T., Jurinjak N., Antunović D., Peršurić Đ., Kovačević Ganić K. (2012). The influence of skin maceration time on the phenolic composition and antioxidant activity of red wine Teran (Vitis vinifera L.). Food Technol Biotechnol 50:152-158.

Puertas B., Guerrero R.F., Jurado M.S., Jimenez M.J., Cantos-Villar E. (2008) Evaluation of alternative winemaking processes. Food sci technol int. 14(5):21–7.

Rossi S., Bestulić E., Horvat I., Plavša T., Lukić I., Bubola M., Kovačević Ganić K., Ćurko N., Jagatić Korenika A.-M., Radeka S. (2022) Comparison of different winemaking processes for improvement of phenolic composition, macro- and microelemental content, and taste sensory attributes of Teran (*Vitis vinifera* L.) red wines. LWT. 154:112619.

Sacchi K.L., Bisson L.F., Adams D.O. (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. Am. J. Enol. Vitic. 56 (3):197–206.

Sáenz-Navajas M-P., Echavarri F., Ferreira V., Fernández P. (2011) Pigment composition and color parameters of Spanish red wines. Eur Food Res. 232(5):877–87.

Sudraud P. (1958). Interpretation des courbes d'absortion des vins rouges. Annales de Technologie Agricole 7:203–208.

Utjecaj predfermentacijskog hlađenja i zagrijavanja masulja na koncentraciju antocijana i boju vina sorte Teran (*Vitis vinifera* L.)

Sažetak

Provedeno je šest vinifikacijskih tretmana, uključujući kontrolu (TK7) kako bi se istražio: utjecaj 48 satnog predfermentacijskog tretmana hlađenja masulja (8 °C) koji se nastavio maceracijom od 13 dana (TC15), 28 dana (TC30), te tretmanom *saignée* tehnologije (TCS15); i utjecaj 48 satnog tretmana grijanja (50 °C), koji se nastavio maceracijom od 13 (TH15) i 28 dana (TH30) na koncentraciju antocijana i boje u vinima sorte Teran. Antocijani i parametri boje analizirani su spektrofotometrijski. Utvrđeno je da su ukupni antocijani i nijansa boje u svim tretmanima viši u odnosu na kontrolu. Najveće koncentracije slobodnih antocijana pronađene su u vinima s predfermentacijskim grijanjem. Najbolji rezultati intenziteta boje dobiveni su u tretmanima s 15 dana maceracije bez obzira na predfermentacijski tretman.

Ključne riječi: Teran, predfermentacijski tretmani, antocijani, intenzitet boje, nijansa boje

IZVORNI ZNANSTVENI RAD

Floristic analysis of weed community in orchards on a family farm Željko Novoselić

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Abstract
The aim of the study was to analyse weeds in cherry orchards. The paper explored the weed flora of orchards in Šag with an area of ten ha. During 2020-2021., 43 plant species that taxonomically belong to 18 families, were recorded. Most species belong to the family Asteraceae (23.25%), followed by the family Fabaceae with 11.60%. All other families are represented in a smaller percentage. Three invasive species have also been identified. Taxonomic analysis shows the dominance of broadleaf weeds (90.70%) and life expectancy analysis shows the dominance of perennial weeds (58.10%).

Key words: weeds flora, orchard, Šag

Introduction

The tradition of growing fruit trees is several centuries old. Fruit trees have been grown on almost all rural farms and partly in urban areas. Having in mind natural factors, the Republic of Croatia has extremely suitable conditions for the production of various fruits.
Weeds are as old as agriculture. They compete with the crops and reduce yield and productivity. They are companions of individual crops, adapted to common life and agrotechnical measures in anthropogenic ecosystems. That habitats are more or less under the influence of a man (Taseva, 2005).
In intensive fruit growing, knowledge of weeds is important in order to choose the most suitable controlling method. Very often two agrotechnical interventions are applied at the same time, for example the inter-row space is grassed and the weeds within the rows are controlled with herbicides (Ciglar, 1998). Depending on the climatic conditions, grassy areas need to be mowed several times in a season. In the Republic of Croatia, several different herbicides for weed control in orchards have been registered. A single application does not achieve a long-term solution. Therefore it is important to know the weed flora and the spectrum and mode of action of the herbicide (Taseva, 2005).

Material and methods

Osijek-Baranja County is located in the east of Croatia and includes the areas around the lower course of the Drava river before its confluence with the Danube. The area of this county is 4,152 km^2, and the altitude is 90 m. The climate is moderately warm and rainy with precipitation evenly distributed throughout the year. The average annual temperature is 10 ^{0}C. In the coldest period in January, temperatures can be below -25 ^{0}C, and in the warmest period in July and August, the maximum can exceed 40 ^{0}C. The soil in this county is of extremely high quality and suitable for agricultural production.
Family farm Željko Novoselić was founded in 2006 in Šag (45°38'21"N, 18°28'07"E), a settlement within the town of Valpovo in Osijek-Baranja County. He is exclusively engaged

in fruit growing, especially in growing cherries on several plots with a total area of ten ha. The research was conducted during the vegetation season in 2020-2021.
The soil in the orchard was maintained by grazing and mowing, and by leaving mulch in the orchard. In early May and early June, the herbicide was applied to control weeds within rows.
Fieldwork included the determination of plants found in the orchard. Determination of plant taxa was performed by using standard determination keys: Javorka and Csapody (1975), Domac (2002), Rogošić (2011), Franjić and Škvorc (2010, 2014). The nomenclature of plant taxa is harmonized according to the Flora Croatica Database (Nikolić, 2020). Invasive taxa were determined by Nikolić et al. (2014).
Life forms are divided into five basic groups according to Raunkiaer's system (Pignatti, 1982) and Rauš and Šegulja (1983): Ch – chamaephyta, G – geophyte, H – hemicryptophyte, P – phanerophyte, T – therophyte. Floral elements and duration of life classified by Knežević (2006): (1-annual, 2-biannual, per-perennial; euroas-euroasian, centeur-central European, submideu-submideuropean, subcirpol-subcircumpolar, pon-pontic, adv-adventitious plant, wide-widespread).

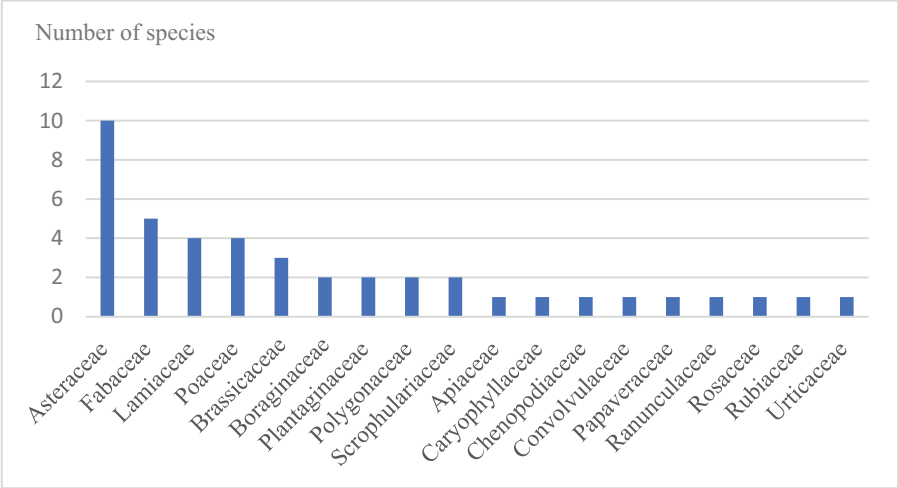
Results and discussion
43 plant species that taxonomically belong to 18 families (Table 1.), are identified during vegetation season 2020-2021.

Table 1. The list of weed species in orchards

Family	Species	Life form	Duration of life	Floral element
Apiaceae	*Daucus carota* L.	H	2	euroas
Asteraceae	*Achillea millefolium* L.	H	per	euroas
	Ambrosia artemisiifolia L.	T	1	adv
	Bellis perennis L.	H	per	centeur
	Cichorium intybus L.	T	per	euroas
	Cirsium arvense L.	G	per	euroas
	Erigeron annus (L.) Pers.	T	1-2	adv
	Matricaria chamomilla L.	T	1	euroas
	Hypochaeris radicata L.	H	per	euroas
	Senecio vulgaris L.	T	1-2	euroas
	Taraxacum officinale Web.	H	per	euroas
Boraginaceae	*Anchusa officinalis* L.	H	per	centeur
	Myosotis arvensis (L.) Hill	T	1-2	euroas
Brassicaceae	*Capsella bursa-pastoris* (L.) Med.	T	1	wide
	Cardaria draba (L.) Desv.	H	2	submideu
	Rorippa sylvestris (L.) Bess.	H	per	euroas
Caryophyllaceae	*Stellaria media* (L.) Vill.	T	1-2	wide
Chenopodiaceae	*Chenopodium album* L.	T	1	wide
Convolvulaceae	*Convolvulus arvensis* L.	G	per	wide
Fabaceae	*Coronilla varia* L.	H	per	pon
	Lotus corniculatus L.	H	per	centeur
	Trifolium pratense L.	H	per	euroas
	Trifolium repens L.	H	per	euroas
	Vicia cracca L.	H	per	euroas
Lamiaceae	*Ajuga reptans* L.	H	per	submideu
	Glechoma hederifolia L.	H	per	euroas

	Lamium purpureum L.	T	1-2	centeur
	Prunella vulgaris L.	H	per	euroas
Papaveraceae	*Papaver rhoeas* L.	T	1	euroas
Plantaginaceae	*Plantago lanceolata* L.	H	per	euroas
	Plantago major L.	H	per	euroas
Poaceae	*Bromus sterilis* L.	T	1	euroas
	Poa annua L.	T	1-2	wide
	Poa pratensis L.	H	per	subcirpol
	Setaria viridis (L.) PB	T	1	euroas
Polygonaceae	*Polygonum aviculare* L.	T	1	wide
	Rumex acetosa L.	H	per	euroas
Ranunculaceae	*Ranunculus repens* L.	H	per	euroas
Rosaceae	*Potentilla reptans* L.	H	per	euroas
Rubiaceae	*Galium mollugo* L.	H	per	submideu
Scrophulariaceae	*Veronica arvensis* L.	T	1-2	centeur
	Veronica persica Poir.	T	1-2	adv
Urticaceae	*Urtica dioica* L.	H	per	euroas

The dominance of broadleaf weed species was determined. There are 39 species, which makes 90.70% of the total determined species. There are four grassy weed species, which makes 9.30% of the total determined species. The most numerous species is the Asteraceae family with ten species, which makes 23.25%. Similar results are reported by Kojić et al. (2004) on research conducted in Bosnia and Herzegovina. Fabaceae family has five species, which makes 11.60%. Lamiaceae and Poaceae family are represented with four species (9.30%). Nine families are represented with only one species (Graph 1.).



Graph 1. Number of species by family

The analysis of duration of life shows that perennial species occur the most (25 species, 58.10%). Similar results are given by Dujmović Purgar (2010). The dominance of hemicryptophytes (24 species, 56%), therophytes (17 species, 39%), and the least detected species (2 species, 5%) are geophytes. The dominance of hemicryptophytes in the studied orchard is similar with the research of other authors in our neighbour countries (Kojić et al., 2004, Kovačević, 2014). Species *Trifoloium repens, Trifolium pratense, Plantago lanceolata*, which are hemicryptophytes with a large cover, occur as well in the orchards of the "Jazbina" experimental site (Pajač, 2007).

Phytogeographic analysis of weeds determined the dominance of plant species of the Eurasian floral element (24 species, which is 56%). The results of this analysis coincide with the research of the flora of the wider area. Of the invasive alien species of the vascular flora, three of them were found: *Erigeron annus, Ambrosia artemisiifolia*, and *Veronica persica*. Two species belong to the Asteraceae family and one to the Scrophulariaceae family. The importance of recognizing invasive species should be emphasized due to their aggressiveness, which affects biodiversity, changes the environment, affects human health and causes damage in agriculture (Ostojić et al., 2007). It is concerned about the spread of the aeroallergenic species *Ambrosia artemisiifolia* from the surrounding ruderal habitats to agricultural areas. This invasive species is a public health and economic problem (Rašić, 2011, Dujmović Purgar, 2010). Among the weed species of the investigated orchard, honey plant species were also recorded: *Cichorium intybus, Cirsium arvense, Lamium purpureum* and *Taraxacum officinale* (Dujmović Purgar and Hulina, 2007).

Species composition in orchards show that weed species, such as *Chenopodium album* heavily infested orchards (Lisek and Sas-Paszt, 2015). *Chenopodium album, Taraxacum officinale* and *Convolvulus arvensis* are cosmopolitan weeds that occur in the orchards of many countries, such as Korea, Argentina, Portugal, Bulgaria and Turkey (Ciglar, 1998., Lipecki, 2004., Lisek, 2012., Tasseva, 2005., Sa et al., 1989.). According to the same author, *Stellaria media, Capsella bursa-pastoris* and *Polygonum aviculare* are species which commonly occur in orchards. *Chenopodium album* is present among orchards in great amount regardless of whether it is conventional or organic cultivation (Lisek and Sas-Paszt, 2015).

Anthropogenic influence has great importance on species composition. A lack of tillage causes increased perennial weeds. *Convolvulus arvensis* and *Cirsium arvense* are perennial weeds, and weed control is difficult against them (Tasseva, 2005.).

Conclusion

Great attention needs to be paid to weed control because they cause great damage in fruit production. The composition of the weed flora depends on agrotechnical measures, cultivation form of fruit trees, row spacing and nutrition. Weed control in the orchard can be carried out by agrotechnical and chemical measures. Agrotechnical measures include tillage between rows and within rows of fruit trees. In order to prevent financial losses, it is necessary to know the interaction of weed and cultivated species. The profit is ensured by quality and timely care of the orchard. The key to successful weed control lies in combining and balancing all available methods of control measures in accordance with the biology and ecology of weed flora and pedoclimatic characteristics of the soil. The representation of such integrated protection system is constantly growing.

References

Ciglar, I. (1998). Integrirana zaštita voćnjaka i vinograda. Zrinski d. d., Čakovec. 301.

Domac, R. (2002). Flora Hrvatske, Školska knjiga, Zagreb. 504.

Dujmović Purgar, D. (2010). Korovna flora vrtova Plešivičkog prigorja (SZ Hrvatska), Agronomski glasnik, 2-3: 111-124.

Dujmović Purgar, D., Hulina, N. (2007.): Medonosne biljne vrste Plešivičkog prigorja (SZ Hrvatska). Agronomski glasnik 1: 3-22.

Franjić, J., Škvorc, Ž. (2014.): Šumsko zeljasto bilje Hrvatske. Sveučilište u Zagrebu. Šumarski fakultet. Zagreb. 626.

Javorka i Csapody (1975.):Iconographia florae partis austro – orientalis Europae Centralis. Akadémiai Kiadó, Budapest. 576.

Knežević, M. (2006.): Atlas korovne, ruderalne i travnjačke flore. Poljoprivredni fakultet u Osijeku. 402.

Kojić, M., Mitrić, S., Janjić, V., Đurić, G. (2004.): Korovska flora voćnjaka Bosne i Hercegovine. Acta Botanica, Vol.13(2):569-578.
Kovačević, Z. (2014.): Asocijacija Convolvulo-Polygonetum aviculare (ass. Nova) u korovskoj vegetaciji vinograda rejona Hercegovina. Agroknowledge Journal, vol. 15(3): 281-298.
Lipecki, J. (2004.): Orchard weed in Lublin region twenty years on – preliminary report. Journal of fruit and ornamental plant research. Vol. 12. 105-110.
Lisek, J. (2012.): Synanthropic orchard flora in West Mazovia- Central Poland. Journal of fruit and ornamental plant research. Vol. 20(2): 71-83.
Lisek, J., Sas-Paszt, L. (2015.): Biodiversity of weed communities in organic and conventionali orchards. Journal of horticultural research. Vol. 23(1): 39-48.
Nikolić, T. Nikolić, T., Mitić, B., Boršić, I. (2014.): Flora Hrvatske – invazivne vrste. Alfa, Zagreb. 296.
Nikolić, T. (2020.): Flora Croatica Database. Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu. hirc.botanic.hr/fcd/
Ostojić, Z., Galzina, N., Goršić, M., Šćepanović, M. (2007.): Invazivne korovne vrste u Republici Hrvatskoj i svijetu. Glasilo biljne zaštite 1-dodatak.
Pajač, I. (2007.): Inventarizacija samonikle vaskularne flore u voćnjacima pokušališta „Jazbina“. Diplomski rad, Agronomski fakultet, Zagreb. 33.
Pignatti, S., (1982.): Flora d Italia. I-III. Edagriole, Bologna. 107.
Rašić, S. (2011.): Ambrozija (*Ambrosia artemisiifolia* L.) – agronomski i javno-zdravstveni problem na području Baranje. Doktorska disertacija. Sveučilište J. J. Strossmayera u Osijeku i Institut Ruđer Bošković, Zagreb. 110.
Rauš, Đ., Šegulja, N. (1983.): Flora Slavonije i Baranje. Glasnik za šumske pokuse, 21: 179-211.
Rogošić, J. (2011.):Bilinar cvjetnjača hrvatske flore s ključem za određivanje bilja. Sv. 2. Sveučilište u Zadru. 571.
Tasseva, V. (2005.): Species composition of weed vegetation in different apple growing technologies. Notulae Botanicae Hortti Agrobotanici Cluj-Napoca, 33: 59-64.
Sa, G., Vasconcelos, T., Nazare, F. (1989.): Weed flora of some orchards in Portugal. Influence of ecological factors. Proceedings of the 4th EWRS symposium on weed problems in Mediterranean climates. Vol. 1. Problems of weed control in fruit, horticultural crops and rice. 51-58.

Floristička analiza korovne zajednice u voćnjacima na OPG-u Željko Novoselić

Sažetak

Cilj istraživanja bio je analizirati korove u nasadima višanja. U radu je istraživana korovna flora voćnjaka u Šagu površine deset hektara. Tijekom 2020.-2021. zabilježene su 43 biljne vrste koje taksonomski pripadaju u 18 porodica. Većina vrsta pripada porodici Asteraceae (10 vrsta, 28,25 %), a slijedi porodica Fabaceae s 5 vrsta (11,60 %). Sve ostale porodice zastupljene su u manjem postotku. Također su identificirane tri invazivne vrste. Taksonomska analiza pokazuje dominaciju širokolisnih korova (90,70 %), a analiza životnog vijeka pokazuje dominaciju višegodišnjih korova (58,10 %).

Ključne riječi: korovna flora, voćnjak, Šag

STRUČNI RAD

Značaj autohtonih sorata vinove loze (*Vitis vinifera* L.) u ukupnoj proizvodnji grožđa i vina u Republici Hrvatskoj

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Sažetak
Povijesni dokumenti o uzgoju vinove loze na području Republike Hrvatske svjedoče da je krajem 19. stoljeća bilo oko 400 autohtonih sorata. Današnjim genetičkim istraživanja utvrđena su 103 različita genotipa. Cilj rada je iz dokumenata službene statistike prikazati udio autohtonih sorata u površinama pod vinogradima te udio u ukupnoj proizvodnji grožđa i vina u Hrvatskoj. Autohtone sorte u Hrvatskoj u 2020. godini zauzimaju 39,1 % ukupnih vinogradarskih površina. U 2019. godini udio autohtonih sorata u proizvodnji grožđa je bio 31,1 %, a vina 32 %. Malvazija istarska, Plavac mali i Plavina zauzimaju 21% površina, a Malvazija Istarska, Plavac mali crni i Pošip bijeli 19% proizvodnje grožđa i 20 % proizvodnje vina. Iako autohtone sorte zauzimaju skoro trećinu proizvodnje svakako bi kroz državne poticaje i sredstva iz Europskih fondova i dalje trebalo poticati povećanje vinogradarskih površina pod autohtonim sortama.

Ključne riječi: genetski resursi, autohtone sorte, grožđe, vino.

Uvod

Njastariji zapisi spomena imena pojedinih autohtonih sorti Hrvatske datiraju iz 14. stoljeća Malvasija dubrovačka i 16. stoljeća Tribidrag (Maletić i sur., 2008.). Krajem 19. stoljeća u uzgoju bilo više od 400 autohtonih sorata. Hrvatski ampelograf Stjepan Bulić u svom djelu „Dalmatinska ampelografija“ opisao je 200 sorata vinove loze na području Dalmacije (Bulić, 1949.). U Hrvatskoj krajem 19 stoljeća je bilo 170 000 ha pod vinogradima od toga 90 000 ha u Dalmaciji (Maletić i sur., 2008.). Kraj 19. st. obilježen je nizom događaja koji su do danas uvjetovali pad površina pod vinogradima i „eroziju“ sortimenta. Prvi događaj bila je „Vinska klauzula“, koju je 1893.godine potpisala Austro-Ugarska Monarhija s Italijom čime joj je omogućila povlašteni uvoz talijanskih vina dok su hrvatska ostala u nepovoljnom položaju. Pojava filoksere 1894. godine dodatno je pogodovala propasti vinogradarsta u Dalmaciji prvenstveno kroz iseljavanje stanovništva u zemlje novog svijeta, napuštanje vinograda i gubitak sorata (Maletić i sur., 2015a.). Iako sustavne brige o očuvanju sortimenta nije bilo sve do kraja 20. stoljeća većina sorata preživjela je do danas. Sve sorte koje su se povijesno pokazale kvalitetnije ili tehnološki i tržišno prihvatljivije poput Malvazije Istarske, Plavca malog crnog, Pošipa bijelog, Plavine crne i danas su zastupljene na većim površinama. Sorte poput Debita, Kraljevine i Škrleta opstale su u najvećoj mjeri zbog tradicijske važnosti u pojedinim regijama. Neke od sorata koje su osjetljive na bolesti ili zahtjevnije za uzgoj zbog čega su dovedene pred prag izumiranja (Dobričić, Šipelj i druge). Inicijativom znanstvene zajednice prije 20 godina pokrenuti su projekti identifikace, inventarizacije i klonske selekcije određenih autohtonih sorata: Škrlet, Kraljevina, Plavac mali i Pošip. Time je uspostavljen sustav brige i očuvanja populacije autohtonih sorata vinove loze. Zadnjim znanstvenim rezultatima potvrđene su 103 autohtone sorte vinove loze

u Hrvatskoj (Maletić i sur., 2015b.). Ulazak Hrvatske na zajedničko tržište Europske unije 2013. godine donosi ekspanziju turizma, ali i bolje mogućnosti financiranja poljoprivredne proizvodnje. U tim uvjetima tržište prepoznaje važnost autohtonih sorti. Cilj rada je na osnovu podataka iz službene statistike proizvodnje grožđa i vina koju vodi Agencija za plaćanje u poljoprivredi, ribarstvu i ruralnom razvoju utvrditi udio autohtonih sorata u ukupnim površinama te udio u proizvodnje grožđa i vina u 2019. i 2020. godini.

Materijali i metode

U svrhu istraživanja korišteni su podaci iz službenog Vinogradarskog registra koji se vodi pri Hrvatskoj agenciji za plaćanje u poljoprivredi, ribarstvu i ruralnom razvoju (https://www.apprrr.hr/registri/). Podaci iz Registra odnose na površine pod vinovom lozom, proizvodnju grožđa i proizvodnju vina za svaku od sorata prijavljenu u Registar. Podaci se odnose na period 2020. godine za površine pod vinogradima, a 2019. godine za proizvodnju grožđa i vina. Podaci su obrađeni na način da su sorte definirane kao introducirane i autohtone. Kao parametar za definiranje autohtonosti korištena je knjiga „Zelena knjiga autohtonih sorata vinove loze“ autora Maletić i sur. (2015a.). U navedenoj knjizi su osnovu referentnih ampelografskih metoda propisanih od strane Međunarodne organizacije za vinogradarstvo i vinarstvo (The International Organisation of Vine and Wine, „OIV“) uključujući i DNA identifikaciju definirane sve relevantne informacije i dokazi o autohtonosti pojedinih sorata na području RH. Važno je spomenuti da se sorta Graševina, iako najzastupljenija sorta u Hrvatskoj vodi kao introducirana sorta pošto genetički nije utvrđeno njezino izravno hrvatsko podrijetlo. U stručnoj literaturi kao podrijetlo se navodi područje panonske nizine (Robinson i sur., 2012.). Također, u uzgoju je dosta zastupljena i u susjednim zemljama Sloveniji, Srbiji, Italiji, Mađarskoj. Analiza podataka iz registra rađena je na osnovu izračunavanja postotnog udjela autohtonih sorata u ukupnim površinama pod vinogradima (izraženo u ha) i ukupnoj proizvodnji grožđa (izraženo u t) i vina (izraženo u hL).

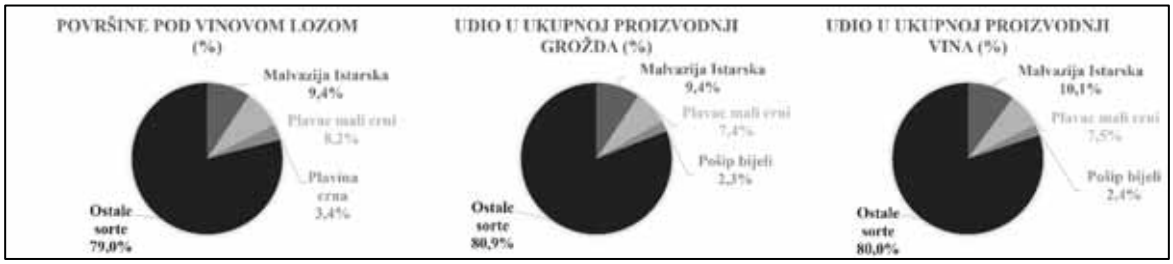
Rezultati i rasprava

Istraživanjem je utvrđeno da od ukupno 17370,24 ha pod vinovom lozom u RH u 2020. godini na autohtone sorte otpada 6783,4 ha ili 39,1% (Graf 1). Od ukupne proizvodnje grožđa od 83408 tona u 2019. godini na autohtone otpada 25916,42 ili 31,1%. Ukupna proizvodnje vina u 2019. godini u RH je iznosila 524596,8 hl na autohtone otpada 168128 hl ili 32%. Ukupno je na listi proizvodnih površina evidentirano 103 autohtone sorte a u proizvodnji grožđa i vina 76 autohtonih sorata.

Graf 1. Udio autohtonih sorata u ukupnim površinama, proizvodnji grožđa i vina u Hrvatskoj

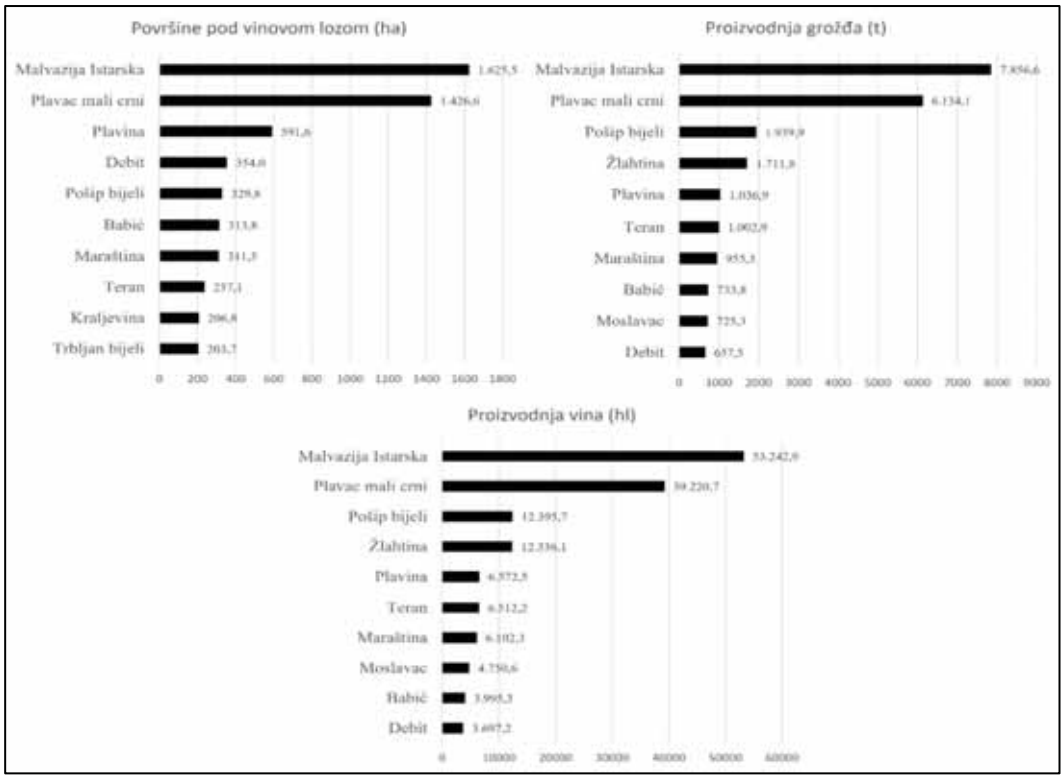
U grafu 2. prikazan je udio triju najzastupljenijih autohtonih sorata u površinama pod vinovom lozom, proizvodnji grožđa i vina. Tri najzastupljenije sorte po površinama su Malvazija Istarska, Plavac mali crni i Plavina crna koje zauzimaju 21% ukupnih površina pod vinogradima ili 3643,8 ha. Po roizvodnji grožđa najzastupljenije su Malvazija istarska,

Plavac mali crni i Pošip bijeli na koje otpada 19,1% proizvodnje grožđa u RH ili 13990,7 tona grožđa. U proizvodnji vina najzastupljenije su također Malvazija Istarska, Plavac mali crni i Pošip bijeli sa udjelom od 20% odnosno 92463,7 hl u ukupnoj proizvodnji vina.



Graf 2. Udio triju najzastupljenijih autohtonih sorata u ukupnim površinama pod vinom lozom i ukupnoj proizvodnji grožđa i vina u Hrvatskoj

Analizirajući samo autohtone sorte 10 najzastupljenijih sorata po površinama su Malvazija Istarska Plavac mali crni, Plavina crna, Debit, Pošip bijeli, Babić, Maraština, Teran, Kraljevina, Trbljan bijeli (Grafikon 3). Ovih deset sorata čini ukupno 82,6% ukupnih površina od autohtonih sorata pri čemu samo prve tri zauzimaju 53.7%. Po proizvodnji grožđa Malvazija Istarska, Plavac mali crni, Pošip bijeli, Žlahtina, Plavina crna, Teran, Maraština, Babić, Moslavac, Debit čine ukupno 87,8 % u proizvodnji grožđa od autohtonih sorata od čega samo prve tri zauzimaju 61,5%. U proizvodnji vina najzastupljeniji su Malvazija Istarska, Plavac mali crni, Pošip bijeli, Žlahtina, Plavina, Teran, Maraština, Moslavac, Babić, Debit. One čine 88,5% ukupne količine proizvedenog vina od svih autohtonih sorata. Malvazija Istarska, Plavac mali crni i Pošip bijeli zauzimaju 62,4% proizvodnje vina od autohtonih sorata.



Graf 3. Deset najzastupljenijih autohtonih sorata vinove loze po površinama (ha), proizvodnji grožđa (t) i vina (hl) u Hrvatskoj

U vinogradarskom registru pored spomenute 3 najznačajnije sorate nalazi se još 100 autohtonih sorata za koje se vode površine pod vinogradima odnosno 73 kod kojih je prijavljena proizvodnja grožđa i vina. Analizom podataka ostalih 100 sorata zauzima 46,3% površina autohtonih i 10% ukupnih površina pod vinogradima u RH. Kod proizvodnje grožđa i vina ostale 73 autohtone sorte čine otprilike 38.5% proizvodnje grožđa od autohtonih odnosno 10% od ukupne proizvodnje grožđa u RH. Kod proizvodnje vina isti broj sorata čini 37.6% proizvodnje vina od autohtonih i ukupno 12% ukupne proizvodnje vina u RH

Zaključak

Iako su negativni povijesni trendovi zadnjih stotinjak godina rezultirali degradacijom najvećeg broja sorata njihov udio u današnjim vinogradarskom površinama i proizvodnji grožđa i vina i dalje je značajan, gotovo trećinu. Najzastupljenije sorte su Malvazija Istarska, Plavac mali crni, Pošip bijeli i Plavina crna koje čine više od 60 % proizvodnje grožđa i vina autohtonih sorata i 20 % ukupne proizvodnje u Hrvatskoj. Opstojnost ovih četiriju sorata u vinogradarsko vinarskoj proizvodnji nije upitna. Međutim treba i dalje poticati sadnju novih površina ili konverziju starih vinograda novim selekcioniranim bezvirusnim sadnim materijalom. Ostalih 70-ak autohtonih sorata čini svega 10 % ukupne proizvodnje grožđa i vina. Iako ovaj udio nije beznačajan kroz poticaje u proizvodnji i popularizaciju na tržištu udio ovih sorata trebalo bi također povećavati.

Napomena

Zahvaljujemo Hrvatskoj agenciji za plaćanje u poljoprivredi, ribarstvu i ruralnom razvoju na ustupljenim podacima iz Vinogradarskog registra Republike Hrvatske.

Literatura

Bulić, S. (1949). Dalmatinska ampelografija. Poljoprivredni nakladni zavod. Zagreb

Maletić, E., Karoglan Kontić, J., Pejić, I. (2008). Vinova loza-ampelografija, ekologija, oplemenjivanje. Školska knjiga. Zagreb.

Maletić, E., Karoglan Kontić, J., Pejić, I., Preiner, D., Zdunić, G., Bubola, M., Stupić, D., Andabaka, Ž., Marković, Z., Šimon, S., Žulj Mihaljević M., Ilijaš, I., Marković, D. (2015a). Zelena knjiga hrvatske izvorne sorte vinove loze. Zagreb. Državni zavod za zaštitu prirode

Maletić, E., Pejić, I., Karoglan Kontić, J., Zdunić, G., Preiner, D., Šimon, S., Andabaka, Ž., Žulj Mihaljević, M., Bubola, M., Marković, Z., Stupić, D., Mucalo, A. (2015b) Ampelographic and genetic characterization of Croatian grapevine varieties. Vitis. (54): Posebno izdanje; 93-98

Robinson, J., Harding, J., Vouillamoz, J. (2012). Wine Grapes- A complete guide to 1368 vine varieties, including their origins and flavours. Penguin Book Ltd. 80 Strand, London WC2R ORI, England

Vinogradarski registar Republike Hrvatske (2021). Agencija za plaćanje u poljoprivredi, ribarstvu i ruralnom razvoju.

Importance of autochthonous varieties in the total production of grapes and wine in the Republic of Croatia

Abstract
Historical documents on the cultivation of autochtonous grapevine varieties in the Republic of Croatia testify that at the end of the 19th century there were about 400 autochthonous varieties. Genetic research in a last twenty years identified 103 different genotypes in Croatian grapevine germplasm. The aim of this paper is to show the share of autochthonous varieties under vineyards and in the production of grapes and wine in the Republic of Croatia. Results are based on data from official statistics. According to the analysis of data, autochtonous varieties in Croatia in 2021 take share of 39.1% of the total vineyard area. In 2019, the share of autochtonous varieties in grape production was 31.1% and wine 32 %. Malvazija Istarska, Plavac mali crni and Plavina crna take share of 21% of the area and Malvazija istarska, Plavac mali crni and Pošip bijeli make 19% of grape production and 20% of wine production. Although autochthonous grape varieties take share of almost a third of the grape and wine production, it should be necessarily to encourage the planting new vineyard areas under autochthonous varieties.

Key words: genetic resources, autochtonous varieties, grape, wine

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Poljoprivredna tehnika

09

Agricultural technics

IZVORNI ZNANSTVENI RAD

Postrna sjetva soje u udvojene redove

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Sažetak
Prikazani su rezultati komparativnog istraživanja utjecaja načina sjetve kod postrno sijane soje *Flavia „*00“ grupe zrenja na pokušalištu Privlaka (Vukovarsko-srijemska županija). Sjetva u udvojene redove na razmak 48 + 22 obavljena je sijačicom *MaterMac* Twin Row-2. Kao usporedba s obzirom na ostvarenje prinosa (kg ha^{-1}), za standardnu sjetvu, korištena je pneumatska sijačica *Gaspardo* SP 7 na razmak redova 50 cm. Utvrđeno je da s obzirom na dobiveni prosječni prinos Twin Row: 2826,71 kg zrna ha^{-1} te kod standardne sjetve 2821,62 kg zrna ha^{-1}, između načina sjetve soje nije utvrđena statistička značajna razlika. LSD $_{005}$ testom utvrđeno je da je kod udvojene sjetve ostvarena niža vlažnost zrna (Twin Row: LSD$_{005}$=7,81; Standardna sjetva: LSD $_{0,05}$ =8,83).

Ključne riječi: postrna sjetva, soja, sjetva u udvojene redove, twin row sijačica, prinos

Uvod

Proizvodnja soje u periodu 2014. do 2019. godine u svijetu (*FAOSTAT*, 2021.) iznosila je između 117 i 121 milijuna ha s prosječnim prinosom od 2,60 do 2,86 t ha^{-1}. Najveći udio ukupne proizvodnje soje nalazi se u Sjevernoj i Južnoj Americi. Najveći proizvođači (*FAOSTAT*, 2021.) soje u svijetu su: SAD, Brazil, Argentina, Kina, Indija, Paragvaj, Kanada, Ukrajina, Rusija i Bolivija s ukupnom proizvodnjom od 94,48 % ukupne svjetske proizvodnje. U RH soja se sije u rasponu 70 do 85 000 ha^{-1} s prosječnim prinosima zrna 2,2 do 3,2 t ha^{-1} ovisno o meteorološkim pogodnostima u godini proizvodnje. U agroekološkim uvjetima Republike Hrvatska, soja se može uzgajati u redovnim rokovima sjetve ili kao postrni usjev. Sjetva soje na prostorima Republike Hrvatske obavlja se na više načina. Najzastupljenija sjetva je žitnim sijačicama sa sjetvom svakog drugog reda, kao i sjetva pneumatskim sijačicama na međuredni razmak od 50 ili 70 cm. Sjetva soje u udvojene redove zastupljena je vrlo malo i prvenstveno je sastavni dio znanstvenih istraživanja. Cilj istraživanja bio je utvrditi razliku u visini prinosa sjetvom postrne soje u udvojene redove (*Twin Row*) i sjetve soje na međuredni razmak od 50 cm.

Materijal i metode

Lokacija postavljenog istraživanja i osnovni podaci o sjetvi

Istraživanje utjecaja sustava sjetve na prinosa zrna kg ha^{-1} pri sjetvi postrne soje obavljeno je na pokušalištu „Privlaka“, na k.č. 901, Arkod ID 3807231, površine 0,49 ha (45,18822222 N, 18,82302778 E). Kao usporedba s obzirom na ostvarenje prinosa (kg ha^{-1}), za standardnu sjetvu, korištena je pneumatska sijačica *Gaspardo* SP 7. Sijačica je bila podešena na teorijsku sjetvu od 689 000 biljaka ha^{-1} odnosno pri korištenju sjetvene ploče n=72 otvora ostvaren je razmak između biljaka od 2,9 cm u redu (sjetvena kombinacija A23/B17/72). Standardna sjetva obavljena je na razmak redova od 50 cm odnosno 200 redova ha^{-1}, a u *twin row* tehnologiji ostvareno je 284 reda ha^{-1} s razmakom udvojenih redova od 22 cm. Za sjetvu u udvojene redove korištena je sijačica *MaterMacc* Twin Row-2, a sjetva je obavljena upotrebom sjetvene ploče n=72 otvora ø 3,5 mm s prijenosnim odnosom ($n_{kotač}$-$n_{sjetvena\ ploča}$)

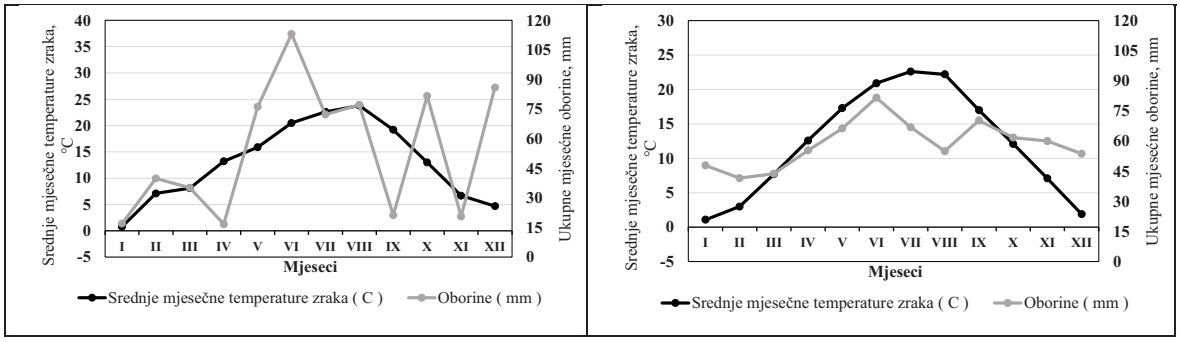
i = 0,504. Pri ovoj prijenosnoj radnoj kombinaciji ostvaren je teorijski razmak zrna od 4,11 cm i sklop od 690 901 biljaka ha^{-1}, odnosno 1 901 biljka po ha^{-1} više nego kod standardne sjetve. Prosječna dubina sjetve kod sustava standardne sjetve iznosila je 6,00 cm uz standardnu devijaciju od 0,797, a zabilježena prosječna dubina sjetva u udvojene redove iznosila je 6,20 cm uz standardnu devijaciju od 1,010. Prosječna brzina gibanja sijačice u sjetvi sijačicom *MaterMac* Twin Row-2 iznosila je 7,15 km h^{-1}, a kod sijačice *Gaspardo* SP 7 od 7,05 km h^{-1} uz standardnu devijaciju 0,708. Standardna sjetva je obavljena 1. srpnja a sjetva u udvojene redove 2. srpnja 2020. godine. Korištena je sorta *Flavia (*00 grupa zrenja-vrlo rana sorta) koju karakterizira izrazito brz početni porast, srednje visine biljke (90 - 100 cm u povoljnim uvjetima), ljubičaste boje cvijeta s dobrom otpornosti na plamenjaču (*Peronospora manshurica)* i visoke apsolutne mase 1000 zrna. U najnižoj varijanti sjetve korištena je preporučena sjetvena norma sorte *Flavia od* 55 do 60 zrna m^{-2} dok su druge dvije varijante posijane sa 64 i 69 biljaka m^{-2}. Korišteno je sjeme treće reprodukcije, mehanički očišćeno preko sita radi uklanjanja polovica te drugih primjesa bez prethodne inokulacije.

Opis tipa tla i kemijske analize tla

Sjetva je obavljena u tlo srednje teškog mehaničkog sastava te je prema rezultatima teksturne analize svrstano u ilovasto tlo. Analizom je utvrđeno da pH reakcija u vodenoj fazi iznosila 6,51, dok je pH djelovanjem u klorovodičnoj kiselini iznosio 5,11. Sadržaj humusa bio je 2,31 % čime se ovo tlo svrstava u umjereno humozna tla. Sadržaj fosfora iznosio je 17,89 mg, a kalija 19,15 mg na 100 g tla. Temeljem toga može se zaključiti da je tlo dobro opskrbljeno navedenim hranivima.

Klimatske prilike u vrijeme istraživanja

Klimatske prilike u vrijeme provođenja istraživanja u 2020. godini kao i vrijednosti višegodišnjih prosjeka temperatura i oborina prikazani su Walterovim dijagramom (Grafikon 1.) za automatsku meteorološku postaju RC Gradište (45.15900 N 18.70400 E)



Grafikon 1. Klima dijagram prema Walteru za 2020. (lijevo) i 1981 - 2022. godine (desno)

Iz Grafikona 1. zapaža se da su prva četiri mjeseca vegetacije 2020. godine period s povećanim srednjim mjesečnim temperaturama u odnosu na višegodišnji prosjek, posebice u veljači kada je razlika prosječne temperature zraka u 2020. godini u odnosu na višegodišnji prosjek iznosila + 4,1°C. Ukupna zabilježena količina oborina u siječnju i travnju bila je 230 % niža od višegodišnjeg prosjeka. Lipanj je po temperaturnim pokazateljima bio u granicama višegodišnjeg prosjeka sa 113,1 l oborine što je 31,5 % više od višegodišnjeg prosjeka. U lipnju je došlo do popunjavanja ranije spomenutog deficita vode u tlu što je bilo vrlo poticajno za planirani sjetveni pokus. U pogledu količine oborina srpanj je bio s većom količinom oborina za 8,7 % te su spomenute količine uistinu pogodovale ubrzanom klijanju i nicanju posijanog pokusa. U kolovozu je količina oborina bila viša za 40,36 % što je itekako odgovaralo u provedbi pokusa posebice u zatvaranju redova krajem mjeseca što je bilo vrlo

važno radi uspješnosti oplodnje cvijeta te nalijevanja i formiranja mahuna. U kolovozu je bilo za 1,6 a u rujnu 2,2 °C toplije od višegodišnjeg prosjeka što je odgovaralo polaganom kretanju zriobe postrne soje kako se žetva ne bi odvijala u studenom kao što se znalo ranije događati. Količina oborina u rujnu 2020. godine bila je 49 mm niža od višegodišnjeg prosjeka što nije ostavilo posljedice na rezultate pokusa iz razloga jer su u rujnu izražene jutarnje rose. U listopadu je količina oborina bila viša od višegodišnjeg prosjeka za 19,9 litara m^2, sa nešto većom prosječnom srednjom mjesečnom temperaturom zraka za + 0,9 °C.

Postavljanje pokusa

Tlo je pripremljeno odmah nakon žetve ječma (26. lipnja. 2020.) radi čuvanja vlage te dobivanja povoljne sjetve strukture. Sjetva pokusa je obavljena šesti dana nakon završetka pripreme tla. U periodu između završene obrade tla i sjetve pokusa, došlo je do vizualno lako primjetnog isušenja sjetvenog sloja. Nakon nicanja usjeva unutar svakog sustava sjetve obilježeno po 12 parcela površine 5 m^2. Svaka parcela kao podvarijanta pokusa odijeljena je od drugih podvarijanti drvenim kolčićima radi lakšeg praćenja i mjerenja rezultata. Podvarijante su postavljene u središtu pokusne parcele udaljene od ruba parcele >20 m kako bi se izbjegao njihov negativan učinak na rezultate pokusa. Nakon što je obavljeno označavanje podvarijanti unutar pokusne parcele, u fenofazi prve troliske obavljeno je prebrojavanje biljaka i prorjeđivanje sklopa skalpelom s ciljem formiranja teorijskog sklopa pokusnih podvarijanti unutar obje glavne varijante na 59, 64 te 69 biljaka m^{-2}. Nakon rezanja i zbrinjavanja stabljika soje u polju 22. listopada, naredni dan na Fakultetu agrobiotehničkih znanosti Osijek (FAZOS) je odrađen izvršaj mahuna *Wintersteiger* Ld 180 uređajem, te vaganje i mjerenje vrijednosti vlage dobivenog zrna soje.

Rezultati i rasprava

Veći broj autora (Mascagni i sur., 2008.; Bruns, 2011.) u nekoliko istraživanja na različitim tlima i klimatskim uvjetima navode rezultate da su zabilježeni prinosi zrna soje veći u udvojenoj sjetvi u odnosu na standardnu jednorednu sjetvu. Gulluoglu i sur., (2016.) navode da su kod sjetve soje iza 15. srpnja u regiji Cukurova u tehnologiji udvojenih redove (80 x 25 x 80) ostvarili prinos od 4 906 kg ha^{-1} odnosno veći za 24,5 % u odnosu na standardnu sjetve na razmak redova od 70 cm koristeći 31,6 i 69,9 biljaka m^{-2}. Isto tako Mascagni i sur. (2008.) navode povećanje prinosa sjemena soje od 12,6 i 13,1 % u sjetvi u udvojenim redovima u Louisiani. Gričar (2007.) navodi povećanje prinosa za 17 % pri sjetvi u istoj tehnologiji udvojenih redova. Sjetva soje u udvojenim redovima u odnosu na sjetvu u jedan red po biljci ostvaruje veći broj mahunica, te veći prinos (Bell, 2005.). Slična razmišljanja o mogućnošću povećanja prinosu postoje i za sjetvu kukuruza i suncokreta (Banaj i sur. 2019.; Banaj i sur. 2021.) u istoj tehnologiji u odnosu na standardnu sjetvu u jedan red (Ebelhar, 2010.; Smith, 2010.) što doprinosi razvoju navedene tehnologije. Iz Tablice 1. vidljivo je da nisu ostvareni planirani teorijski sklopovi, a jedan od razloga je vrlo loša i krupna struktura pripreme tla kao i nešto lošiji ponik biljaka, a posebno u tehnologiji udvojenih redova gdje je sijačica usijavala sjeme u gaženi dio prolaska pneumatika traktora. Analizom varijance dobivenih prinosa zrna kod različitih sustava sjetve nije utvrđena statistička razlika. Prosječan prinos zrna po podvarijantama kod sustava sjetve u udvojene redove iznosio je zadovoljavajućih 2 805 do 2 860 kg zrna ha^{-1}, a kod standardne sjetve 2 807 do 2 832 kg zrna ha^{-1}. LSD_{005} testom (Tablica 2.) utvrđeno je da je kod udvojene sjetva ostvarena niža vlažnost zrna (Twin Row: LSD_{005}=7,81; Standardna sjetva: $LSD_{0,05}$ =8,83).

Tablica 1. Ostvarenja sklopa te prosječni prinosi i vlage zrna soje ovisno o načinu sjetve

Mjerena vrijednost	Standardna sjetva			Twin Row sjetva		
	Planirani teorijski sklop biljaka ha^{-1}					
	590 000	640 000	690 000	590 000	640 000	690 000
	Prosječni ostvreni sklop biljaka ha^{-1}					
$\overline{x}$	502 500	602 500	645 000	525 000	600 000	655 000
σ	26299,556	35000,000	36968,455	34156,500	29439,200	34156,500
KV (%)	5,23	5,81	5,73	6,50	4,90	5,21
	Prosječni prinos zrna soje kg ha^{-1}					
$\overline{x}$	2 807	2 832	2 825	2 863	2 810	2 805
σ	284,137	57,880	134,508	196,039	53,810	68,040
KV (%)	10,12	2,04	4,76	6,84	1,91	2,42
	Prosječna vlažnost zrna soje (%)					
$\overline{x}$	8,32	8,75	9,42	7,55	7,75	8,15
σ	0,556	0,718	1,268	0,310	0,500	0,953
KV (%)	6,67	8,21	13,45	4,11	6,45	11,70

Rezultati razlika između svojstava istraživanja s obzirom na sustav sjetve (LSD_{005} test) prikazani su u Tablici 2.

Tablica 2. $LSD_{0,05}$ test za glavna svojstva istraživanja (α=0,05)

Svojstvo	Prinos (kg ha^{-1})	LSD $_{005}$	Vlaga zrna (%)	LSD $_{005}$
Twin Row	2826,71 ^{A}	121,63	7,81 ^{B}	0,682
Standardna	2821,62 ^{A}		8,83 ^{A}	

Sjetvom u udvojene redove soja brže zatvara međuprostor redova i time omogućava veću površinu listova, a radi povećane lisne površine listova i poboljšano usvajanje hraniva i korištenja vode u tlu (Bruns, 2011.). Uspješnost primjene tehnologije u udvojene redove ovisi o dostupnosti vode (Jamieson i sur., 1995.), hranjivih tvari i temperatura. Ova tehnologija sjetve soje prihvaćena je među proizvođačima u Mississippiju i u velikom dijelu srednjeg juga Sjedinjenih Država (Mississippi, Arkansas, Louisiana, Missouri) kao strategija za maksimiziranje prinosa sjemena soje (Bowers i sur., 2000.; Bruns, 2011.). Procijenjeno je da gotovo 80 % ha soje u delti Mississippija 2010. posijano u tehnologiju udvojenih redova (Bruns, 2011.).

Zaključak

Temeljem dobivenih saznanja mogu se donijeti slijedeći zaključci

- Sjetva je obavljena u ilovasto tlo srednje teškog mehaničkog sastava, umjereno humozno s 2,31 % humusa. Temeljem sadržaja fosfora od 17,89 mg i kalija 19,15 mg na100 g tla tlo svrstavamo u grupu tala kao dobro opskrbljeno tlo.
-Sjetve kod oba sustava sjetve obavljene su 1. i 2 srpnja u vrlo loše pripremljeno tlo s orašastom strukturom isušenih grudva tla, a pogotovo na mjestima koja su nastala gaženjem prilikom predsjetvene pripreme.
- Razlike u prinosima po podvarijantama oba sustava sjetve nisu bile statistički značajne.
- Kod sustava sjetve u udvojene redove zabilježene su nešto niže prosječne vlažnosti zrna u vrijeme berbe u svim podvarijantama.

Literatura

Banaj A., Banaj Đ., Petrović D., Stipešević B., Tadić V. (2021). Sustavi sjetve kao čimbenik prinosa zrna u proizvodnji suncokreta. Poljoprivreda. 27(2): 84-90.

Banaj A., Banaj Đ., Tadić V., Petrović D., Stipešević B. (2019). Utjecaj sustava sjetve na prinos zrna kukuruza različitih FAO grupa. Poljoprivreda. 25(2): 62-70.

Bell A. (2005). Higher yields with twin-row soybeans. Available at http://delta-farmpress.com/higher-yields-twin-row-soybeans (verified 25 Feb. 2011). Delta Farm Press, Penton Media, New York.

Bowers G.R., Rabb J.L., Ashlock L.O., Santini J.B. (2000). Row spacing in the early soybean production system. Agronomy Journal. 92: 524–531.

Bruns H. A. (2011). Planting Date, Rate, and Twin-Row vs. Single-Row Soybean in the Mid-South. Agronomy Journal. 103 (5): 1308-1313.

Ebelhar M.W. (2010). Twin-row corn boosts yields. Corn & Soybean Digest. Available at http://cornandsoybeandigest.com/corn/twin-row-corn-boosts-yields (verified 25 Feb. 2011). Penton Media, New York.

Grichar W. J. (2007). Row spacing, plant populations, and cultivar effects on soybean production along the Texas gulf coast. Available at www. plantmanagementnetwork. org/cm/. Crop Manage. doi: 10.1094/CM-2007–0615–01-RS.

Gulluoglu L., Bakal H., Arioglu H. (2016). The effects of twin-row planting pattern and plant population on seed yield and yield components of soybean at late double-cropped planting in Cukurova region. Turkish Journal of Field Crops. 21(1): 60 – 66. DOI: 10.17557/tjfc.66663

Jamieson P., Martin,R., Francis G., Wilson D. (1995). Drought effects on biomass production and radiation-use efficiency in barley. Field Crops Research. 43: 77–86.

Mascagni H.J., Clawson E., Lancios D., Boquet D., Ferguson R. (2008). Comparing single-row, twin-row configurations for Louisiana crop production. Louisiana agriculture. 51 (3): 16.

Smith C. (2010). Twin-row corn. Corn South. Available at http://www.cotton-farming.com/home/Corn_South/issues/2010-01/2010_JanTwinRowCorn.html (verified 25 Feb. 2011). One Grower Publ., Memphis, TN.

Post sowing of soybeans in double rows

Abstract

The results of a comparative study of the influence of sowing methods in the soybean Flavia "00" maturity group at the Privlaka experimental field (Vukovar-Srijem County) are presented. Sowing in double rows at a distance of 48 + 22 was done with a MaterMac Twin Row-2 drill. As a comparison with respect to yield realization, for standard sowing, a pneumatic seed drill Gaspardo SP 7 with a row spacing of 50 cm was used. It was found that with respect to the obtained average yield of Twin Row: 2826.71 kg of grain ha^{-1} and with standard sowing 2821.62 kg of grain ha^{-1}, no statistically significant difference was found between soybean sowing methods. LSD_{005} test showed that with double sowing, lower grain moisture was achieved (Twin Row: LSD_{005} = 7.81; Standard sowing: LSD_{005} = 8.83).

Key words: post – harvest seeding, soybean, double rows sowing, twin row planter, grain yield

IZVORNI ZNANSTVENI RAD

Primjena MaterMacc Twin Row–2 sijačice u sjetvi kukuruza

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Sažetak

Istraživanja su provedena na pokušalištu Fakulteta agrobiotehničkih znanosti Osijek uporabom dva hibrida sjemenske kuće *Pioneer* – hibrid *P9903* i hibrid *P0023,* zasijanih 11. travnja 2019. godine u optimalnom agrotehničkom roku. Standardna sjetva obavljena je pneumatskom sijačicom *OLT PSK*-4 na razmak redova 70 cm, a sjetva u *Twin Row* tehnologiji sijačicom *MaterMacc* Twin Row-2 s razmakom udvojenih redova od 22 + 48 cm. Standardnom sjetvom hibrida P9903, ostvaren je prinos zrna od 13 578 kg ha^{-1} (52 753 biljaka ha^{-1}) što je za 0,53 % niži od prinosa u *Twin Row* sjetvi. Sjetvom na razmak redova od 70 cm, hibrid *P0023* ostvario je sklop od 70 645 biljaka ha^{-1} s prinosom zrna od 14 930 kg ha^{-1}. Dobiveni prinos je za 2,87 % viši u odnosu na *Twin Row* sjetvu (14 514 kg ha^{-1}) koji je ostvaren pri sklopu od 78 810 biljaka ha^{-1}.

Ključne riječi: kukuruz, sjetva, prinos, twin row, sijačica

Uvod

Sjetva kukuruza na prostoru Republike Hrvatske obavlja se na razmaku redova od 70 i 75 cm. U novije vrijeme provode se znanstvena istraživanja sjetve kukuruza u udvojene redove, u svijetu poznate kao *Twin Row* tehnologija. Proizvođači *Twin Row* sijačica, proizvode sijačice s razmacima udvojenih redova od 20, 22 ili 25 cm, a središnji razmak susjednih udvojenih redova iznosi 70 ili 75 cm tako da je obavljanje berbe moguće i sa standardnim beračima za kukuruz. Takva tehnologija sjetve omogućava bolji raspored unutar reda te veće iskorištenje sunčeve svjetlosti, hraniva i vlage iz tla i u većini eksperimenata doprinosi ostvarenju jednakog ili većeg prinosa po hektaru. Primarna komponenta prinosa je broj biljaka ili klipova po jedinici površine i ta komponenta ima veliki utjecaj na visinu prinosa. Standardnim sustavom sjetve kukuruza za zasijavanje prosječnog sklopa od 65 000 biljaka na 1 ha, s razmakom od 70 cm može se zasijati 142 reda dužine 100 m, pri čemu je udaljenost do najbliže susjedne biljke 21,8 cm, dok se sjetvom kukuruza u udvojene redove s razmakom udvojenih redova od 22 cm može posijati 284 reda dužine 100 m, no, udaljenost do najbliže biljke je 43,6 cm, što rezultira dvostruko većim vegetacijskim prostorom za mladu biljku. Ovakav način sjetve u SAD-u je u potpunosti razvijen, dok se u Republici Hrvatskoj *Twin Row* tehnologija obavlja u znanstvene svrhe. Upravo i cilj istraživanja bio je utvrditi razliku u visini prinosa sjetvom kukuruza u udvojene redove (*Twin Row*) i klasične sjetve kukuruza na međurednom razmaku od 70 cm.

Materijal i metode

Lokacija postavljenog istraživanja i osnovni podaci o sjetvi

Istraživanje je obavljeno na pokušalištu Fakulteta agrobiotehničkih znanosti Osijek (FAZOS) – *Tenja* (45°31'1,83" s. z. š./18°46'37,5" i. z. d. - k. o. Tenja; kč. 1308 i 1309). Sjetva dva hibrida kukuruza obavljena je 11. travnja 2019. u optimalnom agrotehničkom roku. Standardna sjetva je obavljena sijačicom *OLT PSK4*, a sjetva u udvojene redove (*Twin Row*), sijačicom *MaterMacc Twin Row-2*.

Opis tipa tla i kemijske analize tla

Na pokušalištu *Tenja* prevladava eutrično smeđe tlo, koje pripada odjelu automorfnih tala, klasi kambičnih tala. Prema teksturi tlo pripada u praškaste ilovače te je malo porozno, s osrednjim kapacitetom tla za vodu u oraničnome i podoraničnom horizontu (Banaj A., 2020). Tlo je alkalno u svim horizontima (pH u vodi 8,44 i KCl 7,55) s dosta humoznim oraničnim slojem (3,71 %.). Sadržaj fosfora iznoso je 15,58 mg (umjerena opskrbljeno tlo), a kalija 24,29 mg na 100 g tla (dobro opskrbljeno tlo).

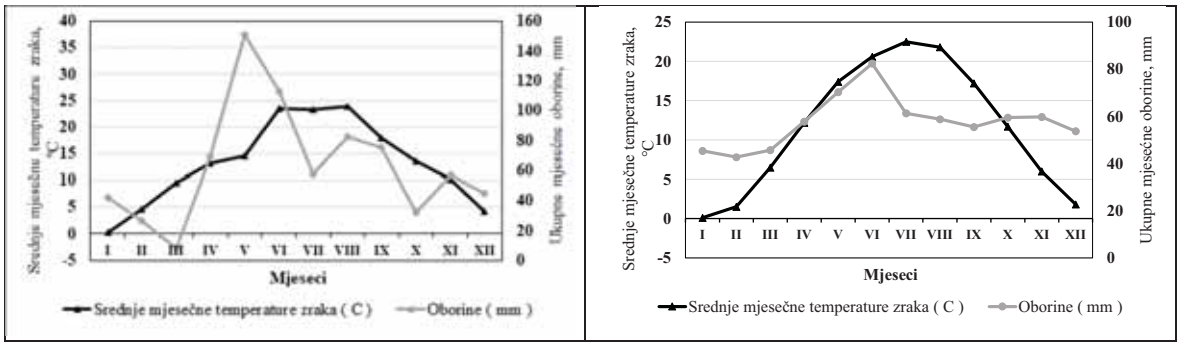
Klimatske prilike u vrijeme istraživanja

Klimatske prilike u vrijeme provođenja istraživanja u 2020. godini nisu značajnije odstupale od vrijednosti višegodišnjeg prosjeka. U Tablici 1. prikazane su srednje mjesečne temperature zraka i ukupne mjesečne količine oborina (mm) za vegetacijsku 2020. godinu.

Tablica 1. Klimatski uvjeti u 2019. godini izmjereni na glavnoj meteorološkoj postaji Osijek-Klisa (45.46000° 18.82000°)

Mjesec	Srednje mjesečne temperature zraka (°C)		Oborine (mm)	
	2019.	1899. - 2018.	2019.	1899. - 2018.
I.	0,3	0,1	42,4	45,4
II.	4,6	1,5	26,8	42,7
III.	9,6	6,5	8,4	45,7
IV.	13,2	12,2	68,6	57,8
V.	14,6	17,4	150,8	70,3
VI.	23,6	20,6	112,8	82,4
VII.	23,3	22,5	57,4	61,3
VIII.	24,0	21,8	82,2	58,8
IX.	18,0	17,2	75,0	55,5
X.	13,5	11,7	32,3	59,5
XI.	10,3	6,0	57,1	59,8
XII.	4,3	1,8	44,9	53,7
Ukupno IV.-X.	19,4	18,6	546,8	386,1
Ukupno I.-XII.	13,2	11,6	758,7	692,9

Izvor: *DHMZ* (2021.); *IV.-X.* – vegetacija kukuruza



Grafikon 1. Klima dijagram prema Walteru za 2019. (lijevo) i 1899. - 2018. godine (desno)

Iz Tablice 1. i Grafikona 1. vidljivo je da je u prva četiri mjeseca vegetacije 2019. godine imamo razdoblje s povećanim srednjim mjesečnim temperaturama u odnosu na višegodišnji prosjek, posebice u veljači i ožujku kada je razlika prosječne temperature zraka u 2019.

godini u odnosu na višegodišnji prosjek iznosila + 3,1 °C. Ukupna zabilježena količina oborina u prva tri mjeseca bila je samo 42,30 % u odnosu na višegodišnjeg prosjek. Lipanj je po temperaturnim pokazateljima bio topliji za + 3 °C sa 112,8 l oborine što je 36,8 % više od višegodišnjeg prosjeka. U lipnju je došlo do popunjavanja ranije spomenutog deficita vode u tlu, što je bilo vrlo pogodno za daljni razvoj usjeva. U pogledu količine oborina srpanj je bio s nešto manjom količinom oborina od 3,9 mm. U kolovozu je količina oborina bila viša za 23,4 mm s prosječnom višom temperaturom zraka za 2,2 °C. Količina oborina u rujnu 2019. godine bila je za 24,5 mm viša od višegodišnjeg prosjeka s višom prosječnom temperaturom zraka od + 0,8 °C.

Rezultati i rasprava

Iz Tablice 1. može se zaključiti da je u vrijeme berbe utvrđeno kod oba hibrida smanjenje sklopa kod standardne sjetve između 1 745 do 8 845 biljaka, a kod tehnologije twin row od 4 355 do 14 117 u odnosu na teorijski sklop biljaka ha^{-1}. Autori Banaj i sur. (2017.) navode da od planiranog teorijskog sklopa pri sjetvi do berbe nedostaje od 8 do 13 000 biljaka ha^{-1}.

Tablica 1. Utvrđeni sklop biljaka po ha^{-1} kod hibrida *P9903* i hibrida *P0023*

Sustav sjetve i teorijski sklop	Hibrid *P9903*			Hibrid *P0023*		
	Sklop biljaka po ha^{-1} u vrijeme nicanja					
	$\bar{x}$	σ	KV (%)	$\bar{x}$	σ	KV (%)
Standardna I - 60000	52753	16829,589	31,90	55735	4078,639	7,32
Twin Row I - 66355	57510	2719,093	4,73	64610	4260,000	6,59
Standardna II -75000	60883	20358,738	33,44	70645	2688,017	3,80
Twin Row II - 84523	72420	3666,424	5,06	78810	1420,000	1,80

Standardnom sjetvom I (Tablica 2.) hibrida *P9903*, ostvaren je sklop od 52 753 biljaka ha^{-1} s prinosom od 13 578 kg ha^{-1}. Dobiveni prinos je za 0,53 % niži od prinosa dobivenog u Twin Row sjetvi I (13 651 kg ha^{-1}) s prosječnom vlažnošću zrna 25,2 %. Twin Row sjetvom II istoga hibrida ostvaren je sklop od 72 420 biljaka ha^{-1}, s prinosom od 14 560 kg ha^{-1} s prosječnom vlagom zrna od 26,38 %. Ostvareni prinos je za 1,28 % viši u odnosu na prinos dobiven standardnom sjetvom II. Slične rezultate navode (Tadić i sur., 2017.) da su u Twin Row sjetvi ostvarili više prinose zrna kg ha^{-1}za 2,40 % (hibrid *ZP 560*) i 6,48 % kod hibrida *ZP 488*. Blandino i sur. (2013.) navode da je na 66 % ispitivanih lokacija došlo do povećanja uroda zrna u sjetvi u udvojenim redovima u prosjeku 5,5 %. U ovom istraživanju povećanje prinosa iznosilo je od 0,6 do 0,9 t ha^{-1}. Slične rezultate bilježe autori Jurković i sur. (2017.) gdje je povećanje uroda zrna kod sustava sjetve u udvojene redove kod hibrida P0412 iznosilo 5,53 % a kod hibrida BC 525 14,95 % više u odnosu na standardnu sjetvu.

Tablica 2. Ostvareni prinosi zrna (svedeno na vlagu od 14 %) kg ha^{-1} i vlaga zrna kukuruza kod standardne i Twin Row sjetve kod hibrida *P9903*

Sustav sjetve i ostvareni sklop	Hibrid *P9903*					
	Prinos zrna kg ha^{-1}			Vlaga zrna (%)		
	$\bar{x}$	σ	KV (%)	$\bar{x}$	σ	KV (%)
Standardna I - 52753	13578	1222,729	9,01	25,02	0,881	3,52
Twin Row I - 57510	13651	1049,062	7,68	25,76	1,440	5,59
Standardna II - 60883	14389	1173,001	8,15	25,26	1,972	7,81
Twin Row II - 72420	14560	620,489	4,26	26,38	1,636	6,20

Kod hibrida *P0023* (Tablica 3.), standardnom sjetvom I ostvaren je sklop u vrijeme berbe od 55 735 biljaka ha^{-1}, s prinosom zrna od 13 659 kg ha^{-1}. To je za 4,86 % više u odnosu na

Twin Row sjetvu II. Prosječna vlaga zrna kod standardne sjetve iznosila je 25,92 %, a kod Twin Row sjetve 26,88 %. Kod Twin Row sjetve II pri sklopu od 78 810 biljaka ha^{-1} ostvaren je prinosom od 14 514 kg ha^{-1} s prosječnom vlagom zrna od 28,04 %. Dobiveni prinos je za 2,87 % niži od prinosa ostvarenoga standardnom sjetvom II.

Tablica 3. Ostvareni prinosi zrna (svedeno na vlagu od 14 %) kg ha^{-1} i vlaga zrna kukuruza kod standardne i Twin Row sjetve kod hibrida *P0023*

Sustav sjetve i ostvareni sklop	Hibrid *P0023*					
	Prinos zrna kg ha^{-1}			Vlaga zrna (%)		
	$\overline{x}$	σ	KV (%)	$\overline{x}$	σ	KV (%)
Standardna I - 55735	13659	777,709	5,69	25,92	2,265	8,74
Twin Row I - 64610	13025	840,360	6,45	26,88	2,485	9,25
Standardna II - 70645	14931	843,664	5,65	25,72	2,183	8,49
Twin Row II - 78810	14514	630,531	4,34	28,04	1,494	5,33

Autor Ogrizović (2015.) navodi da je sjetvom u udvojene redove utvrđeno povećanje uroda za 3,26 % u odnosu na standardnu sjetvu. Autori (Banaj i sur. (2017.) navode da je prinos hibrida *Kamparis*, ostvaren Twin Row sjetvom iznosio čak za 10,07 % više u usporedbi sa standardnom sjetvom. Kod hibrida *Balasco* prinos zrna ostvaren Twin Row sjetvom bio je niži za 5,94 %. Isti autori Banaj i sur. (2017.) utvrđuju veći prinos uroda zrna kod uzgoja kukuruza P0023 u tehnologiji udvojenih redova od 10, 35 %, a kod hibrida P0412 u istom sustavu sjetve ostvaren je veći urod zrna od 10, 59 % više u odnosu na standardnu sjetvu. Jurković i suradnici (2018.) bilježe povećanje uroda kod hibrida OS 403 u udvojenim redovima za 3,56 %, a kod hibrida OS 378 od 7,66 % više u odnosu na standardnu sjetvu. Autori Banaj i sur. (2019.) utvrdili su da je hibrid FAO grupe 290 u udvojenoj sjetvi ostvario veći urod zrna od 3,77 do 9,66 %, hibrid FAO grupe 380 6,46 do 10,97 %, hibrid FAO grupe 410 3,45 do 9,95 % te hibrid FAO grupe 450 11,72 %. Hibrid FAO grupe 590 ostvario je u 2018. godini sjetvom u trake manji prinos za 0,59 % s obzirom na standardnu sjetvu.

Zaključak

Na temelju dobivenih saznanja mogu se donijeti sljedeći zaključci;

- Temeljem meteoroloških podataka prvenstveno promatrajući srednje mjesečne temperature zraka i mjesečne količine oborina nameće se zaključak da je vegetacijska godina 2019. bila pogodna za proizvodnju kukuruza na pokušalištu Tenja.
- U standardnoj sjetvi s pneumatskom sijačicom *OLT PSK-4,* hibrid sjemenske kuće *Pioneer P9903* u sklopu 52 753 biljaka ha^{-1} nakon nicanja ostvario je prinos naturalnog zrna od 13 578 kg ha^{-1} s prosječnom vlažnošću od 25,02 %.
-Sjetvom istog hibrida u udvojene redove ostvaren je sklop od 57 510 biljaka ha^{-1} s prinosom suhog zrna od 13 651 kg ha^{-1} s prosječnom vlažnošću od 25,76 % što čini povećanje u odnosu na standardnu sjetvu od 0,53 %.
- Povećanjem sklopa na 60 883 biljaka ha^{-1} kod sjetve istog hibrida na standardni način dobiven je prinos zrna 14 389 kg ha^{-1} dok je prinos u sustavu sjetve u udvojene redove iznosio 14 560 kg ha^{-1} s prosječnom vlagom zrna od 26,38 %.
- Standardnom sjetvom hibrid *P0023* ostvario je prinos suhog zrna od 13 659 kg h^{-1}, a kod sjetve u udvojene redove 13 025 kg h^{-1} s prosječnom vlagom 26 88 %.
- Isti hibrid pri standardnoj sjetvi u sklopu biljaka 70 645 ha^{-1} ostvario je prinos od 14 931 kg h^{-1}, a u Twin row sjetvi nešto niži od 14 514 kg h^{-1}.
- Jednogodišnjim istraživanjem problematike razmaka redova u sjetvi kukuruza na veći broj međusobno povezanih čimbenika među kojima je svakako prinos u potpunosti sa

znanstvenog stajališta nemoguće je dobivene rezultate potvrditi kao statistički opravdane, te se predlaže nastavak postupka istraživanja.

Literatura

Banaj A., Šumanovac L., Hefer G., Tadić V., Banaj Đ. (2017). Yield of corn grain by sowing in twin rows with MaterMacc-2 planter. In *Proceedings of the 45th International Symposium on Agricultural Engineering, Actual Tasks on Agricultural Engineering*, 21-24 February 2017, Opatija, Croatia 141-152.

Banaj A., Banaj Đ., Tadić V., Petrović D., Stipešević B. (2019). Utjecaj sustava sjetve na prinos zrna kukuruza različitih FAO grupa. Poljoprivreda. 25(2): 62-70.

Banaj A. (2020). Kvaliteta rada pneumatskih sijaćica s podtlakom pri različitim sustavima sjetve kukuruza. Doktorska disertacija, Fakultet agrobiotehničkih znanosti Osijek, Osijek.

Banaj A., Banaj Đ., Petrović D., Stipešević B., Tadić V. (2021). Sustavi sjetve kao čimbenik prinosa zrna u proizvodnji suncokreta. Poljoprivreda. 27(2): 84-90.

Blandino M., Amedeo R., Giulio T. (2013). Aumentare la produttivitŕ del mais con alti investimenti e file binate. Un test in dodici localitŕ vocate conferma la validitŕ delle nuove agrotecniche. Terra e Vita, Tecnica e Tecnologia. 7.

Jurković D., Kajić N., Banaj A., Tadić V., Banaj Đ., Jović J. (2017). Twin row technology maize sowing. *Proceedings of the 8th International Agricultural Symposium*: Agrosym, 62-66.

Jurković D., Kajić N, Banaj A., Banaj Đ. (2018). Utjecaj načina sjetve na prinos zrna kukuruza. Proceedings of the *53rd Croatian and 13th International Symposium on Agriculture*, 299-303.

Ogrizović B. (2015). Rezultati setve kukuruza Twin – Row sejalicom u region Sombor, 43. *International Scientific Symposium: Actual Tasks on Agricultural Engineering*, Agronomy faculty in Zagreb; Opatija, Croatia, 319-329.

Tadić V., Banaj A., Banaj Đ., Petrović D., Knežević D. (2017). Twin row technology for maize seeding. *The Third International Symposium on Agricultural Engineering*, 69-74. Belgrade–Zemun, Serbia.

Application of MaterMacc Twin Row-2 drill for seeding maize

Abstract

Research has been conducted at FAZOS experimental site with two hybrids from seed company Pioneer – hybrid P9903 and hybrid P0023, seeded on 11. April 2019 in optimal agrotechnical term. Standard seeding has been done with pneumatic seed drill OLT PSK-4 with 70 cm distance between rows, whereas Twin Row seeding has been done with MaterMacc Twin Row-2 seed drill, with distance between twin rows 22 + 48 cm. With standard seeding of hybrid P9903, grain yield of 13578 kg ha^{-1} (52753 plants ha^{-1}) has been achieved, which was for 0.53% lower grain yield in comparison with Twin Row grain yield. Seeding at 70 cm between-row distance of hybrid P0023 achieved plant population of 70645 plants ha^{-1}, with grain yield of 14 930 kg ha^{-1}. Harvested grain yield was for 2.87% higher in comparison with Twin row seeding (14 514 kg ha^{-1}) which was achieved by plant population of 78 810 plants ha^{-1}.

Key words: maize, seeding grain yield, twin row, seed drill

IZVORNI ZNANSTVENI RAD

Analiza održavanja poljoprivredne tehnike

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Sažetak
U radu su prikazani rezultati istraživanja analize održavanja poljoprivredne tehnike u eksploataciji. Obavljen je intervju sa rukovateljima (81) koji upravljaju s istraživanom poljoprivrednom tehnikom, a u vidu utvrđivanja provođenja mjera dnevnog i tjednog održavanja. Mjere dnevnog održavanja se u većem dijelu ne provode, a što je suprotno od mjera tjednog održavanja koji se većim dijelom provode. Rukovatelje istraživanih strojeva treba potaknuti i po potrebi poslati ponovno na edukacije kako bi kvalitetnije obavljali mjere održavanja i s time produžili radni vijek poljoprivredne tehnike.

Ključne riječi: dnevno i tjedno održavanje, poljoprivredna tehnika, rukovatelj.

Uvod

Tehnološki procesi u poljoprivrednoj proizvodnji su se kroz povijest do danas intenzivno mehanizirali. Razvoj poljoprivredne tehnike rezultirao je suvremenim konstrukcijama, koje uz sofisticiranost i složenost zahtjevaju educiranog rukovatelja i kvalitetno obavljanje redovitih mjera održavanja. Poljoprivredni traktor, koji se koristi u svim segmentima poljoprivredne proizvodnje, namijenjen je za vuču, nošenje i transport poljoprivrednih strojeva. Današnje poljoprivredne traktore karakterizira veći udio informacijske tehnologije s unaprijeđenim konceptom prijenosnika snage, visoko sofisticiranom hidraulikom i visokom razinom u smjeru ergonomije (Gospodarski list, 2012.).

Bekčić (1981.) navodi da je za kvalitetno održavanje potrebna dobra strategija održavanja, budući da isto obavlja veći broj djelatnika. Metoda upravljanja koja se primjenjuje u svrhu realizacije ciljeva održavanja naziva se strategija održavanja. U suvremenom održavanju najčešće se primjenjuju dvije vrste održavanja: korektivno i plansko održavanje (preventivno i prediktivno). Prema standardu EN 13306 (2018.) korektivno održavanje je oblik održavanja koji se provodi nakon što se kvar dogodio. Preventivno održavanje je održavanje koje se obavlja u unaprijed određenim intervalima koje određuje proizvođač sa ciljem smanjenja vjerojatnosti pojave kvara. Prediktivno održavanje je održavanje prema stanju koje podrazumijeva praćenje trajnosti dijelova kako bi se zamjenski dijelovi pripremili prije otkaza te kako bi ih se moglo pravodobno i brzo zamijeniti. Metode koje se mogu koristiti za praćenje degradacije dijelova su infracrveno snimanje, mjerenje razine buke, temperature, vibracija itd.

Mishra i Satapathy (2021.) navode da bi se poboljšale eksploatacijske karakteristike strojeva u poljoprivredi, pravilan odabir plana održavanja bio je glavni zahtjev kako u zemljama u razvoju tako i razvijenim zemljama. Razvoj plana održavanja i odabir optimalnog održavanja strojeva omogućen je primjenom všekriterijskog odlučivanja (MCDM – multi-criteria decision-making).

Uloga servisno preventivnog održavanja je kontinuirano provođenje održavanja svih tehničkih parametara na propisanoj razini te na taj način se postiže dobar rad svih sklopova

stroja i maksimalan učinak. Provođenjem stalnih servisa sprječavaju se veći kvarovi, a eksploatacijski (uporabni) vijek značajno se produžuje. Servisno – preventivno održavanje sastoji se od tehničkog (dnevno i tjedno) i servisnog održavanja. Tehničko održavanje obavlja rukovatelj prije početka rada sa strojem, a po naputku za rukovanje i održavanje proizvođača stroja. Servisno održavanje u jamstvenom roku provodi proizvođač tj. ovlašteni servis (jamstveni rok 1000 radnih sati ili 1 godina dana). Svi servisi unutar i izvan jamstvenog roka upisuju se u servisnu knjižicu u svrhu praćenja samog stroja (Emert i sur., 1995.). Uporabom dijagnostike u servisno-preventivnom održavanju utvrđuje se tehničko stanje stroja te je preporuka obavljanja iste jednom godišnje (Cvetičanin, 1982.).

Neadekvatnim garažiranjem poljoprivrednih strojeva, isti su izloženi utjecaju negativnih atmosferskih čimbenika koje za posljedicu imaju smanjenje uporabnog vijeka stroja te s tim direktno utječu na povećanje troškova poljoprivredne mehanizacije (Banaj i sur., 2003.). Autori (Barač i sur., 2016.; Jurić, 2001.; Landeka, 1995.) navode kako je važna konzervacija i zaštita poljoprivrednih strojeva te kako korozija može dovesti do pucanja i raspadanja određenih elemenata i sklopova te prouzročiti kvar.

Cilj istraživanja ovoga rada je analizirati i utvrditi mjere održavanja poljoprivredne tehnike na poljoprivrednom gospodarstvu te dati smjernice za poboljšanje.

Materijal i metode

Poljoprivredno gospodarstvo na kojem je obavljeno istraživanje nalazi se u okolici Donjeg Miholjca i registrirano je 1998. godine. Uz proizvodnju žitarica, uzgoj stoke i peradi te ukrasnog bilja, gospodarstvo je registrirano i za trgovačko posredovanje na domaćem i stranom tržištu, proizvodnju hrane i pića, cestovni prijevoz robe i opskrbu električnom energijom. Temeljni kapital poslovnog subjekta financiran je 100 % domaćim kapitalom, a u prethodnom razdoblju nije se mijenjao iznos temeljnog kapitala. Poljoprivredno gospodarstvo je u 2019. godini imalo 108 zaposlenih. Isto se primarno bavi ratarstvom (obrađuje 5000 ha) i stočarstvom (broj stoke 3000 grla).

Poljoprivredno gospodarstvo posjeduje ukupno 27 poljoprivrednih traktora različitih proizvođača, snage i godina proizvodnje. Istraživanje glede provođenja redovitih mjera dnevnog i tjednog održavanja obavljeno je za 19 traktora marke John Deere, 4 traktora marke Claas te po jedan od marke TD, Ursus, Yanmar i Zetor. Istraživanje je obavljeno intervjuiranjem rukovatelja poljoprivrednih traktora (broj rukovatelja 81) koji su prethodno educirani za rad na pojedinom traktoru, a dobiveni rezultati su analizirani i za iste je učinjena jednosmjerna analiza varijance.

Rezultati i rasprava

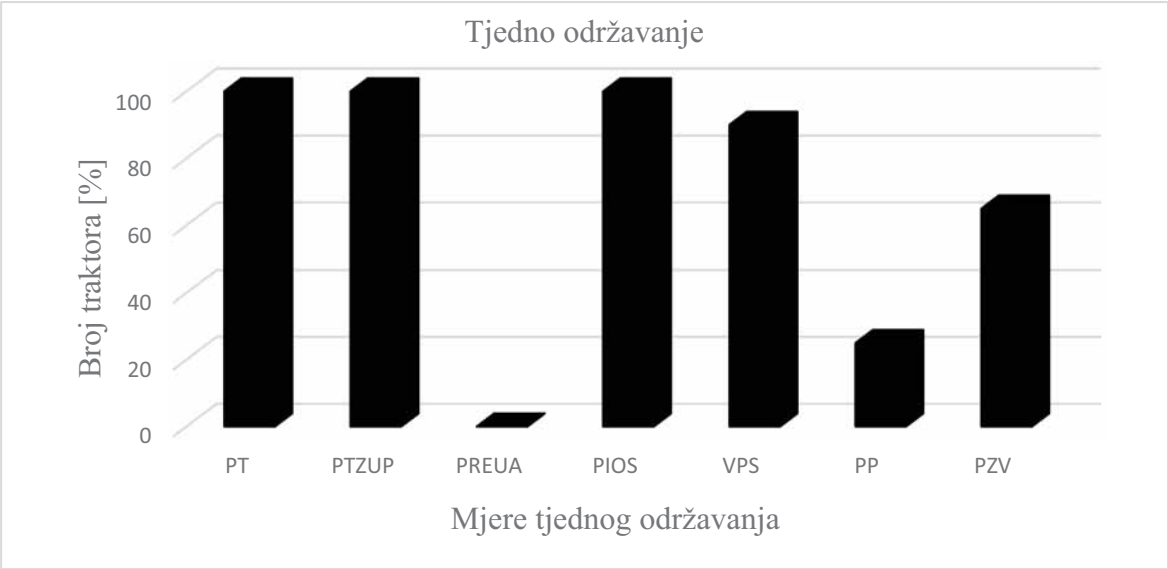
Rezultati dobiveni glede redovitih mjera dnevnog održavanja za pojedine traktore predočeni su grafikonom 1, gdje su na osi x predočene mjere dnevnog održavanja: provjera razine ulja u motoru (PRUM), provjera razine ulja mjenjača – hidraulike (PRUMH), provjera rashladne tekućine (PRT), provjera sustava komprimiranog zraka (PSKZ), provjera filtera goriva (PFG), čišćenje filtera zraka (ČFZ), čišćenje filtera zraka kabine (ČFZK), čišćenje hladnjaka (ČH), provjera ispravnosti kočnica (PIK), provjera razine kočione tekućine (PRKT), provjera zategnutosti remena (PZR), provjera slobodnog hoda kola upravljača (PSHKU), provjera slobodnog hoda pedale spojke (PSHPS), provjera ispravnosti signalizacije i osvjetljenja (PISIO) te vizualni pregled stroja (VPS).

Redovite mjere održavanja koje rukovatelji obavljaju u okviru tjednog održavanja predočene su grafikonom 2, gdje su na osi x navedene mjere: podmazivanje traktora (PT), provjera tlaka zraka u pneumaticima (PTZUP), provjera razine elektrolita u akumulatoru (PREUA),

pranje i odmašćivanje stroja (PIOS), vizualni pregled stroja (VPS), podmazivanje poteznice (PP) te provjera zategnutosti vijaka (PZV).

Grafikon 1. Rezultati provođenja mjera dnevnog održavanja (%)

Analizom varijance utvrđena je signifikantnost kod mjera dnevnog i tjednog održavanja. Rezultati istraživanja ukazuju da se redovite mjere dnevnog održavanja ne obavljaju sukladno naputku za rukovanje i održavanje proizvođača pojedinog tipa traktora (Grafikon 1). Rukovatelji najmanje obavljaju mjere PSKZ (5 %), PRKT (10 %), PZR (10 %) i PSHKU (10 %). Sve mjere koje je propisao proizvođač moraju se provoditi s ciljem sprječavanja nastanka kvarova, očuvanja traktora, produženja radnog vijeka traktora i bolje iskorištenosti pri radu (Emert i sur., 1995.; Barač i sur., 2016., 2018.; Plaščak i sur., 2015.; Vidaković i sur., 2018.) te sigurnosti i zdravlja rukovatelja.



Grafikon 2. Rezultati provođenja mjera tjednog održavanja (%)

Rukovatelji tijekom tjednog održavanja u najmanjoj mjeri obavljaju mjere održavanje PREUA (0 %) i PP (25 %) (Grafikon 2). Kao razlog za neprovođenje mjera PREUA

rukovatelji navode da se radi o novim akumulatorima te ovu mjeru obavljaju nakon šest mjeseci eksploatacije akumulatora, dok izostanak mjera PP objašnjavaju da je istu potrebno obaviti po potrebi, a ne sukladno naputku proizvođača. Mishra i Satapathy (2021.) ukazuju da obavljanje tjednog održavanja i razvoj optimalnog plana preventivnog održavanja može biti korisno u minimiziranju ukupnih troškova održavanja i maksimiziranju parametara sustava. Planovi održavanja mogu pozitivno utjecati na rukovatelje u obavljanju agrotehničkih operacija, korištenjem ispravnog traktora, navode isti autori.

Zaključak

Provedenim istraživanjem na poljoprivrednom gospodarstvu doneseni su sljedeći zaključci:

- Posjeduju svu potrebnu dokumentaciju za poljoprivredne traktore (naputke za rukovanje i održavanje, radioničke priručnike, kataloge rezervnih dijelova i servisne knjižice)
- Rukovatelji poljoprivrednog traktora su stručno osposobljeni i educirani za rad, te bi daljnje poboljšanje trebalo usmjeriti u smjeru organizacije provođenja mjera održavanja;
- Mjere dnevnog i tjednog odražavanja se obavljaju, ali ne u potpunosti što nije dobro;
- Kako je poljoprivredna tehnika izrazito skupa, s neobavljanjem kvalitetnog održavanja direktno se utječe na povećanje troškova proizvodnje.

Literatura

Banaj Đ., Šmrčković P. (2003). Upravljanje poljoprivrednom tehnikom. Poljoprivredni fakultet u Osijeku, Osijek, Hrvatska.

Barač Ž., Jurić T., Plaščak I., Heffer G., Kramer M. (2016). Ustroj i značaj servisno-preventivnog održavanja u "PP Orahovica" obzirom na zaštitu okoliša. *25. međunarodni znanstveni stručni skup Organizacija i tehnologija održavanja*, Lacković, Z. (ed.), 51-56. Osijek, Hrvatska.

Barač Ž., Plaščak I., Jurić T., Jurišić M., Heffer G., Vidaković I., Zimmer D., Majstorović S. (2018). Održavanje linije strojeva za uzgoj lijeske – studija slučaja. *27. međunarodni znanstveni stručni skup Organizacija i tehnologija održavanja*, Glavaš, H., Barić, T., Nyarko, E. K. (ed.), 199-204. Osijek, Hrvatska.

Bekčić M. (1981). Održavanje i remont mehanizacije. Viša tehnička mašinska škola Beograd, Beograd, Srbija.

Cvetičanin R. (1982). Mogućnosti dijagnosticiranja stanja ispravnosti tehničkih sredstava u poljoprivredi. *Zbornik radova XI Internacionalnog simpozija Servis, održavanje, remont i snabdjevanje rezervnim dijelovima tehničkih sistema poljoprivrede*, 199-204. Osijek, Hrvatska.

EN 13306. (2018). Maintenance - Maintenance terminology. [EN 13306: 2018]. Brussels, Belgija. European committee for standardization.

Emert R., Jurić T., Filipović D., Štefanek E. (1995). Održavanje traktora i poljoprivrednih strojeva. Sveučilište Josipa Jurja Strossmayera u Osijeku, Osijek, Hrvatska.

Gospodarski list. (2012). Spremanje strojeva preko zime. Available from: https://gospodarski.hr/rubrike/spremanje-strojeva-preko-zime/

Jurić T., Emert R., Šumanovac L., Horvat D. (2001). Provođenje mjera održavanja na obiteljskim gospodarstvima. *29. International symosium on agricultural engineering*, Filipović, D. (ed.), 43-51. Opatija, Hrvatska.

Landeka S. (1995). Motori i traktori. Vinkovci, Hrvatska.

Mishra D., Satapathy S. (2021). Reliability and maintenance of agricultural machinery by MCDM approach. International Journal of System Assurance Engineering and Management.

Plaščak I., Barač Ž., Jurić T., Marinović D., Juratović I. (2015). Audit središnje remontne radionice za popravak poljoprivrednih strojeva – analiza slučaja. *24. međunarodni znanstveni stručni skup Organizacija i tehnologija održavanja*, Lacković, Z. (ed.), 157-162. Donji Miholjac, Hrvatska.

Vidaković I., Plaščak I., Heffer G., Barač Ž., Zimmer D., Marinović D. (2018). Utjecaj sustava za obradu ispušnih plinova dizel motora na svojstva motornog ulja. *11th International Scientific/Professional Conference Agriculture in Nature and Environment Protection*, Jug, D., Brozović, B. (ed.), 307-311. Vukovar, Hrvatska.

Agricultural machinery maintenance analysis

Abstract

The paper presents research results of agricultural machinery analysis maintenance in exploitation. An interview was conducted with the operators (81) who manage the researched agricultural machinery, in the form of determining the implementation of daily and weekly maintenance measures. Daily maintenance measures are mostly not implemented, which is the opposite of weekly maintenance measures. Operators of the researched machines should be encouraged and, if necessary, sent back to training in order to perform better maintenance measures and thus extend the working life of agricultural machinery.

Key words: daily and weekly maintenance, agricultural machinery, operator

IZVORNI ZNANSTVENI RAD

Energetska analiza miskantusa uzgojenog na tlu kontaminiranom teškim metalima

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Sažetak
Korištenje višegodišnjih energetskih kultura kao „alata“ u procesu fitoremedijacije može se okarakterizirati kao višefunkcionalna tehnika koja se manifestira kroz čišćenje kontaminiranih tala uz istodobnu proizvodnju biomase. Kako bi se provela valorizacija biomase, kao ulazne sirovine u procesima izgaranja, neophodno je utvrditi energetsku analizu koja objedinjuje (I) energetski relevantne parametre i (II) elementarnu analizu. Cilj rada je utvrditi utjecaj razine koncentracije kadmija i žive apliciranih u tlo na energetsku analizu biomase miskantusa nakon procesa fitoremedijacije. Osim udjela kadmija, svi su istraživani parametri u skladu s tipičnim vrijednostima miskntusa navedenih u ISO 17225-1:2014 normi za kruta biogoriva.

Ključne riječi: biomasa, fitoremedijacija, energetsko korištenje, kadmij, živa

Uvod

Fitoremedijacija se smatra ekološki održivom, energetski učinkovitom i jeftinom biološkom tehnikom, koja se temelji na mogućnosti biljke da apsorbira, imobilizira ili razgradi različite onečišćujuće tvari iz onečišćenog tla (Ashraf i sur., 2019.; Dastyar i sur., 2019.). Kako bi se povećao potencijal fitoremedijacije sve se više pažnje pridaje višegodišnjim energetskim kulturama. Energetske kulture svojim morfološkim i fizološkim predispozicijama izravno utječu na efikasnost apsorpcije i/ili translokacije teških metala. Uz virdžinijski sljez, divlje proso, divovsku trsku jedna od najznačajnih energetskih kultura u procesu čišćenja kontaminiranih tala je i miskantus (*Miskantus x giganteus*). Općenito, biomasa miskantusa pokazuje mogućnost konverzije u različite oblike energije i biogoriva. Tehnologije termokemijske pretvorbe poput izgaranja, pirolize, torefakcija te rasplinjavanjem su konvencionalni, ali ujedno i obećavajući načini konverzije biomase dobivene procesom fitoremedijacije (Yadav i sur., 2018.). Izgaranje biomase kontaminirane teškim metalima i dalje dovodi do redovitih emisija (CO, NOx, leteći pepeo), a također proizvodi čvrste i plinovite spojeve metala (Kovacs i Szemmelveisz, 2017.). Laval-Gilly i sur. (2017.) su utvrdili da niske koncentracije metala u nadzemnoj biomasi trebaju olakšati korištenje kontaminirane biomase, dok Pogrzeba i sur. (2013.) navode da bi se biomasa nakon procesa fitoremedijacije trebala tretirati kao opasan materijal. S ciljem spriječavanja ponovne remisije metala u atmosferu, posebno onih koji su vrlo hlapivi na temperaturi izgaranja, potrebno je provesti izgaranje na suvremenim ložištima koji svojim konstrukcijskim rješenjima omogućuju ekološki prihvatljivo korištenje kontaminirane biomase (Pogrzeba i sur. 2013.; Kovacs i Szemmelveisz, 2017.). Cilj rada je utvrditi utjecaj razine koncentracije kadmija i žive apliciranih u tlo na energetsku analizu biomase miskantusa nakon procesa fitoremedijacije.

Materijali i metode

In situ uzgoj na kontaminiranom tlu je proveden u stakleniku Zavoda za Opću proizvodnju bilja Sveučilišta u Zagrebu Agronomskog fakulteta. Pokus je postavljen prema slučajnom bloknom rasporedu sadnjom rizoma miskantusa u plastične posude. Na tlo su primijenjena četiri tretmana (C, T1, T1 + SS, T2) s različitim koncentracijama, odnosno T I – kontrola; T II – 10 mg/kg tla Cd + 2 mg/kg tla Hg; T III - 10 mg/kg soil Cd + 2 mg/kg soil Hg + mulj, T IV - 100 mg/kg soil Cd + 20 mg/kg soil Hg. Treća skupina (T1 + SS) tretirana je identičnim koncentracijama Cd i Hg u tlu kao u L1, ali uz dodatak kanalizacijskog mulja u ekvivalentu od najviše 1,66 t ST ha^{-1} sukladno NN 38/2008.

Hidrometeorološki uvjeti, svojstva tla i kanalizacijskog mulja detaljno su objašnjeni u Zgorelec i sur., (2019.). Za potrebe laboratorijskih istraživanja analizirana biomasa je uzorkovana tijekom tri vegetacijske godine u odnosu na apliciranu razinu onečišćenja. U ovome radu su prikazane njihove prosječne vrijednosti tijekom tri godine istraživanja. Energetska analiza je provedena na Zavodu za poljoprivrednu tehnologiju, skladištenje i transport Sveučilišta u Zagrebu Agronomskog fakulteta i Institutu za medicinska istraživanja i medicinu rada. Od energetski relevantnih parametara utvrđen je udio vlage (HRN EN 18134-2:2015), pepela (HRN EN ISO 18122:2015), fiksiranog ugljika (EN 15148:2009), hlapivih tvari (EN 15148:2009) te donja ogrijevna vrijednost (EN 14918:2010 i izračun), dok je elementarna analiza obuhvatila: ugljik, vodik, dušik, sumpor (EN 15104:2011 / EN 15289:2011), kisik (izračun), kadmij i živu (ICP-MS 7500 cx, Agilent Technologies, Waldbronn, Germany).

Rezultati i rasprava

Kako bi se provela energetska analiza biomase, kao ulazne sirovine u procese izgaranja, određeni su energetski relevantne parametre i elementarni sastav biomase. Energetski parameti objedinjuju udio vlage, pepela, fiksiranog ugljika, hlapivih tvari i donju ogrijevnu vrijednosti. Uz bazne elemente odnosno ugljik, vodik, dušik, kisik i sumpor važno je utvriti i prisutnosti ostalih mikro i makro elemnata koji svojim vrijednosti mogu uzrokovati probleme tijekom korištenja u ložištima. Nakon procesa fitoremedijacije poseban naglasak treba usmijeriti na koncentraciju teških metala u uzgojenoj biomasi kako bi odabrana energetska konverzija bila ekološki održiva. Rezultati svih provedenih laboratorijskih analiza istraživane biomase prikazani su u tablici 1.

Udio vlage, uz pepeo i donju ogrjevnu vrijednost, predstavlja temeljni parametar u energetskoj valorizaciji biomase. Obzirom na period (rano proljeće) uzorkovanja biomase utvrđeni udio vlage je u skladu s očekivanim vrijednostima (~ 20 %). Analiza vrijednosti pepela, fiksiranog ugljika i hlapivih tvari ukazuje na statistički značajnu promjenu sadržaja navedenih parmetara u odnosu na postavljene tretmane. Utvrđeni udjeli navedenih parametara su u skladu (pepeo), minimalno (hlapive tvari) ili značajnije (fiksirani ugljik) odstupaju u odnosu na literaturne navode, koji se kod miskantusa kreću od 1.9 % do 9.6 % za pepeo (Visser i sur., 2007.; Garcia i sur., 2012.), fiksirani ugljik od 9.5 % do 16 % (Jeguirim i sur., 2010.; Howell i sur., 2019.) te hlapive tvari od 70.7 % do 87.2 %. (Khodier i sur., 2012.; Nhuchhen i Salam, 2012.). Donja ogrijevna vrijednosti (Hd) definirana je kroz količinu potencijalno dostupne energije u odnosu na zadanu masu sirovine te se obično izražava u MJ/kg. Postavljeni tretmani ne pokazuju statistički značajan utjecaj na Hd. Autori Osman i sur. (2018.) te Mos i sur. (2013.) utvrđuju količinu energije u biomasi miskantusa u intervalu od 15.35 MJ/kg do 17.5 MJ/kg.

Tablica 1. Energetska analiza biomase miskantusa (s.t.) u odnosu na različite tretmane kontaminacije

Tretman	T I	T II	T III	T IV
		Energetski relevantni parametri		
Vlaga (%)	19,81 ± 0,65	20,87 ± 1,79	19,83 ± 2,69	19,75 ± 4,16
Pepeo (%)	**2,72 a**± 0,19	**2,43 b** ± 0,01	**2,33 b** ± 0,06	**2,05 c** ± 0,22
Cfix (%)	**6,44 a** ± 0,08	**6,71 a** ± 0,83	**5,65 b** ± 0,25	**5,33 bc** ± 0,85
HT (%)	**87.49 bc** ± 0,59	**87.18 c** ± 0,70	**88.14 b** ± 0,69	**89.00 a** ± 1,25
Hd (MJ/kg)	16,28 ± 0,48	16,71 ± 0,65	16,73 ± 0,33	16,38 ± 0,26
		Elementarni parametri		
Dušik (%)	**0,41 b** ± 0,01	**0,41 b** ± 0,01	**0,43 a** ± 0,01	**0,41 b** ± 0,01
Ugljik (%)	48,98 ± 0,49	47,95 ± 0,52	48,61 ± 1,09	48,84 ± 0,54
Sumpor (%)	0,33 ± 0,06	0,30 ± 0,01	0,29 ± 0,02	0,30 ± 0,01
Vodik (%)	5,33 ± 0,05	5,32 ± 0,09	5,25 ± 0,05	5,28 ± 0,09
Kisik (%)	44,95 ± 0,41	46,02 ± 0,60	45,43 ± 1,08	46,17 ± 0,50
Cd (mg/kg)	**0,121 c** ± 0,043	**1,685 b** ± 0,366	**1,951 b** ± 0,217	**3,277 a** ± 0,044
Hg (mg/kg)	0,013 ± 0,003	0,013 ± 0,001	0,009 ± 0,001	0,024 ± 0,017

T I – kontrola; T II – 10 mg/kg tla Cd + 2 mg/kg tla Hg; T III - 10 mg/kg soil Cd + 2 mg/kg soil Hg + mulj, T IV - 100 mg/kg soil Cd + 20 mg/kg soil Hg

Temeljem vrijednosti prikazanih elementa u tablici 1. može se vidjeti statistički značajan utjecaj istraživanih razina kontaminacije na udjele dušika i kadmija. Ugljik, vodik i kisik su usko povezni s ogrijevnom vrijednošću, pri tome ugljik i vodik imaju pozitivan, a kisik negativan utjecaj na kvalitetu biomase. Literaturni podaci ukazuju na vrijednost sadržaja ugljika od 46,75 % (Meehan i sur., 2013.) do 49,80 % (Howell i sur., 2019.), vodika od 4,80% do 7,32 % (Werle i sur., 2017.) te kisika od 44,20 % (Werle i sur., 2017.) do 50,01 % (Osman i sur., 2018.). Dušik i sumpor su nepoželji elementi u sastavu biomase i utječe na emisije NO_x i SO_x tijekom izgaranja. Wilik and Magdzriaz (2010.) i Osman i sur. (2018.) navode udio ugljika u rasponu od 0,36 % do 1,21 %.

Od prikazanih elemenata Cd i Hg se smatraju visoko toksičnim teškim metalima (Ashraf i sur., 2019.), a ujedno ih karakterizira i visok stupanj hlapivosti tijekom procesa izgaranja. Visoka hlapivost je posebno naglašena kod Hg (Nzihou i Stanmore, 2013.), dok se Cd obično nalazi u aerosolnoj frakciji (Obernberger i sur., 2006.). Nzihou i Stanmore (2013.) navode da se Cd i Hg nalaze u finim česticama lebdećeg pepela (fly ash) te zaključuju kako je Hg jedini teški metal koji se nakon proizvodnje energije ne zadržava u pepelu. Sadržaj Cd i Hg u nadzemnoj biomasi miskantusa prvenstveno je vezan uz koncentraciju u kojoj su ti elementi prisutni u tlu (Zhang i sur., 2015.; Fernando i Oliveira, 2004.), a tipične varijacije na nekontaminiranom tlima kreću se između 0,05 – 0,2 mg/kg (Cd) i < 0,02 – 0,1 mg/kg (Hg) (CEN/TS 14961:2005).

Zaključak

Temeljem provedenih istraživanja može se zaključiti kako različiti tretmani onečišćenja tla statistički značajno utječu na analizirane vrijednosti pepela, fiksiranog ugljik, hlapivih tvari, dušika i kadmija. Najviše vrijednosti pepela i fiksiranog ugljika utvrđene su na kontrolnoj varijati pokusa, a hlapivih tvari na tretmanu s najvišom koncetracijom teških metala. Primjena kanalizacijskog mulja je utjecala na povećanje udjela dušika u biomasi. Usporedno s ISO 17225-1:2014 normom za kruta biogoriva udio žive je u skladu s tipičnim vrijednostima (< 0,02 – 0,1 mg/kg) miskantusa, dok udio kadmija ne odstupa od tipičnog intervala (0,05 – 0,2 mg/kg) samo kod kontrolnog tretmana.

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Literatura

Ashraf S., Ali Q., Zahir Z. A., Ashraf S., Asghar H. N. (2019). Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. Ecotoxicology Environmental Safety. 174: 714-727.

CEN/TS 14961:2005 (E), Solid biofuels—Fuel Specification andClasses, CEN/TC 335—Solid biofuels.

Dastyar W., Raheem A., He J., Zhao M. (2019). Biofuel production using thermochemical conversion of heavy metal-contaminated biomass (HMCB) harvested from phytoextraction process. Chemical Engineering Journal. 358: 759-785.

Fernando A., i Oliveira J. S. (2004). Effects on Growth, Productivity and Biomass Quality of *Miscanthus x giganteus* of soils contaminated with heavy metals. In Biomass for Energy, Industry and Climate Protection: *Proceedings of the 2nd World Biomass Conference*. 10-14 Rome, Italy.

Garcia R., Pizarro C., Lavín A.G., Bueno J.L. (2012). Characterization of Spanish biomass wastes for energy use. Bioresource Technology. 103: 249 - 258.

Howell A., Stahlfeld K., Mohammed A.B., Vijlee S., Belmont E. (2019). Gas independence of *Miscanthus×giganteus* torrefied in nitrogen (N_2) and carbon dioxide (CO_2) using calibrated thermogravimetric analysis. Bioresource Technology Reports. 7: 100238.

Jeguirim M., Dorge S., Trouvé G. (2010). Thermogravimetric analysis and emission characteristics of two energy crops in air atmosphere: *Arundo donax* and *Miscanthus giganthus*. Bioresource Technology. 101(2): 788-793.

Khodier A.H.M., Hussain T., Simms N.J., Oakey J.E., Kigallon P.J. (2012). Deposit formation and emissions from co-firing miscanthus with Daw Mill coal: Pilot plant experiments. Fuel. 101: 53-61.

Kovacs H. i Szemmelveisz K. (2017). Disposal options for polluted plants grown on heavy metal contaminated brownfield lands – A review. Chemosphere. 166: 8-20.

Laval-Gilly P., Henry S., Mazziotti M., Bonnefoy A., Comel A., Falla J. (2017). *Miscanthus x giganteus* composition in metals and potassium after culture on polluted soil and its use as biofuel. BioEnergy Research. 10(3): 846-852.

Meehan P.G., Finnan J.M., Mc Donnell K.P. (2013): The effect of harvest date and harvest method on the combustion characteristics of *Miscanthus× giganteus*., GCB Bioenergy: Bioproducts for a Sustainable Bioeconomy. 5(5): 487-496.

Mos M., Banks S.W., Nowakowski D.J., Robson P.R.H., Bridgwater A.V., Donnison I.S. (2013). Impact of *Miscanthus x giganteus* senescence times on fast pyrolysis bio-oil quality. Bioresource Technology. 129: 335-342.

Nhuchhen D.R., i Salam P.A. (2012) Estimation of higher heating value of biomass from proximate analysis: A new approach. Fuel. 99: 55 – 63.

Nzihou A., i Stanmore B. (2013). The fate of heavy metals during combustion and gasification of contaminated biomass—a brief review. Journal of Hazardous Materials. 256: 56-66.

Osman A.I., Ahmed A.T., Johnston C.R., Rooney D.W. (2018). Physicochemical characterization of miscanthus and its application in heavy metals removal from wastewaters. Environmental Progress and Sustainable Energy. 37(3): 1058-1067.

Pogrzeba M., Krzyżak J., Sas-Nowosielska A., (2013). Environmental hazards related to *Miscanthus x giganteus* cultivation on heavy metal contaminated soil. In *E3S Web of Conferences*. Édition Diffusion Presse Sciences. 1: 29006.

Visser P., i Pignatelli V., (2007). Utilisation of *Miscanthus* productivity. Published at Miscanthus for energy and fibre, Jones, M. B. i Walsh, M. (eds.), 109-155. London, UK: Eartscan.

Werle S., Ziółkowsk, Ł., Pogrzeba M., Krzyżak J., Ratman-Kłosińska I., Burnete D. (2017). Properties of the waste products from the heavy metal contaminated energy crops gasification process. *Proceedings of ECOpole*.11.

Wilk M., i Magdziarz. A. (2017). Hydrothermal carbonization, torrefaction and slow pyrolysis of *Miscanthus giganteus*. Energy. 140: 1292-1304.

Yadav K.K., Gupta N., Kumar A., Reece L.M., Singh N., Rezania S., Khan S.A. (2018). Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecological engineering. 120: 274-298.

Zgorelec Z., Bilandzija N., Knez K., Galic M., Zuzul S. (2020). Cadmium and Mercury phytostabilization from soil using *Miscanthus× giganteus*. Scientific Reports. 10(1): 1-10.

Zhang J., Yang S., Huang Y., Zhou S. (2015). The tolerance and accumulation of *Miscanthus sacchariflorus* (maxim.) Benth., an energy plant species, to cadmium. International Journal of Phytoremediation. 17(6): 538-545.

Energy analysis of miscanthus grown on soil contaminated with heavy metals

Abstract

The use of perennial energy crops as a "tool" in the process of phytoremediation can be characterized as a more functional technique that manifests itself through the cleaning of contaminated soils with the simultaneous production of biomass. In order to valorize biomass as a raw material in combustion processes, it is necessary to establish an energy analysis that combines (I) energy-relevant parameters and (II) elemental analysis. The aim of this study is to determine the influence level of cadmium and mercury concentration applied to the soil on the energy analysis of miscanthus biomass after the phytoremediation process. In addition to the cadmium content, all investigated parameters are in accordance with the typical values of miscanthus specified in the ISO 17225-1: 2014 standard for solid biofuels.

Key words: biomass, phytoremediation, energy utilisation, cadmium and mercury concentration

PREGLEDNI RAD

Umjetne neuronske mreže kao statistički alat u predviđanju donje ogrjevne vrijednosti biomase

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Sažetak
Pri korištenju biomase kao sirovine za proizvodnju goriva i toplinske energije, potrebno je odrediti njezina energetska svojstva. Primjenom modela umjetnih neuronskih mreža moguće je s velikom točnošću predvidjeti donju ogrjevnu vrijednost pojedinih energetskih kultura, čime se olakšava pronalaženje optimalnog rješenja. Sposobnost samoorganizacije, optimiziranog učenja i visoke tolerancije na pogreške neuronskim mrežama omogućuje pouzdano i precizno izračunavanje donje ogrjevne vrijednosti biomase. Ispravnom konfiguracijom mreže i prikupljanjem podataka, modeli umjetnih neuronskih mreža mogu se koristiti za predviđanje donje ogrjevne vrijednosti u realnom vremenu.

Ključne riječi: biomasa, ogrjevna vrijednost, umjetne neuronske mreže.

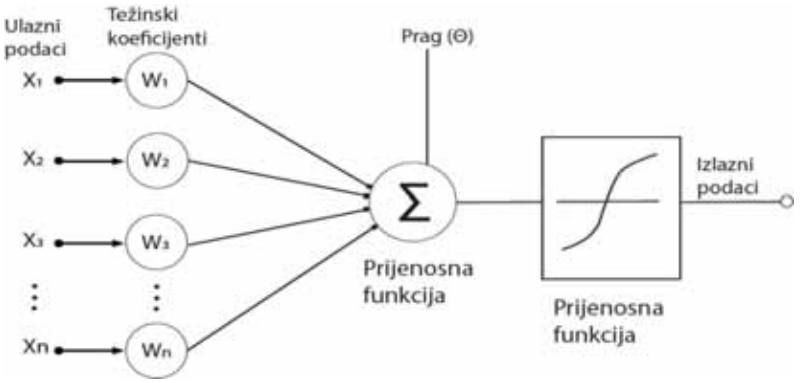
Uvod

Ogrjevna vrijednost goriva važno je svojstvo koje definira energetski sadržaj sirovine. Donja ogrjevna vrijednost definirana je kao energija koja se oslobađa po jedinici mase ili volumena pri potpunom izgaranju, a obično se izražava u megadžulima po kilogramu (MJ/kg) (Ghugare i sur., 2014.). Pomoću već razvijenih matematičkih modela za predviđanje moguće je proučiti učinak pojedinih varijabli u sustavu te utvrditi njihovu povezanost sa krajnjim rezultatom. Za razliku od navedenih modela, umjetne neuronske mreže (*eng. Artificial Neural Networks)* pokazuju veliku učinkovitost u modeliranju kompleksnih procesa uvjetovanih velikim brojem varijabli (Izadifar i Demneh, 2006.). Nakon odabira odgovarajućih ulaznih podataka, umjetne neuronske mreže imaju sposobnost ispravno generalizirati podatke te ih prilagoditi ukoliko su nepotpuni. Građa modela umjetnih neuronskih mreža uključuje nekoliko organiziranih slojeva između kojih se odvijaju matematičke operacije. Poput ljudskog mozga neuronske mreže se treniraju iskustvom i stvaranjem obrasca kojim je najlakše izračunati željeni izlaz. Za testiranje točnosti modela neuronskih mreža potrebno je usporediti ekperimentalne rezultate sa rezultatima dobivenim putem modela (Kartal i Ozveren, 2020.).

Princip rada umjetnih neuronskih mreža u određivanju donje ogrjevne vrijednosti

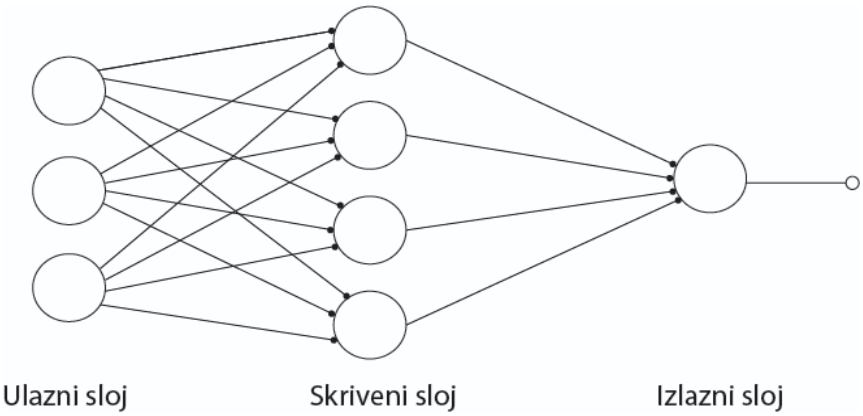
Za racionalno iskorištavanje biomase energetskih kultura potrebno je poznavati njihova energetska svojstva. Mjerenje donje ogrjevne vrijednosti je postupak koji zahtjeva posebnu pripremu uzoraka kako bi se smanjila mogućnost pogreške. Za predviđanje donje ogrjevne vrijednosti uglavnom se koriste već razvijeni matematički modeli. Odnos između varijabli korištenih za predviđanje donje ogrjevne vrijednosti sugerira nelinearne međusobne odnose, stoga se razvijanje nelinearnih modela smatra primjerenijim u predviđanju (Ghugare i sur., 2014.). Postoji međusobna povezanost između donje ogrjevne vrijednosti i razvijenih matematičkih modela temeljenih na elementnoj analizi organskih tvari te analizama gorivih i negorivih tvari biomase. Matematički modeli koji obuhvaćaju međusobnu povezanost

navedenih analiza i donje ogrjevne vrijednosti su jednostavni i pružaju brzo, ali ponekad i netočno predviđanje.
Umjetne neuronske mreže su sustavi koji obrađuju podatke na sličan način kao neuroni u ljudskom mozgu, gdje se umjetni neuroni povezuju različitim geometrijskim vezama (Staub i sur., 2015.). Osnovne značajke umjetnih neuronskih mreža su sposobnost samostalnog učenja, mogućnost prilagodbe sustava s obzirom na dostupne informacije te obrada podataka i složenih matematičkih operacija velikom brzinom. Pod učenjem umjetne neuronske mreže podrazumijeva se iterativni postupak podešavanja vrijednosti težinskih faktora na osnovu pogreške između proračunate vrijednosti modela i stvarne vrijednosti mjerne veličine (Andrijić i Bolf, 2019.). Umjetne neuronske mreže izračunavaju pomoću statističkih teorija učenja koje su metode prikladne za rješavanje izračuna nelinearnih modela te uspostavljanja odnosa između ulaznih varijabli i izlaznih podataka. Prema Ujević Andrijić i Bolf (2019.), umjetni neuroni primaju ulazne podatke koji su određeni težinskim koeficijentima nakon čega se podaci obrađuju pomoću unutarnjeg praga (*eng. bias).* Prijenosnom funkcijom dobiveni podaci se šalju prema izlazu i sljedećim neuronima.



Slika 1. Prikaz umjetnog neurona (shorturl.at/kAS18)

Postoji više različitih vrsta neuronskih mreža no u primjeni za izračunavanje nelinearnih modela je najučinkovitija višeslojna perceptron neuronska mreža (*eng. Multilayer Perceptron Neural Network)* (Pattanayak i sur., 2021.). Višeslojna mreža sastoji se od tri sloja: ulaznog, skrivenog i izlaznog sloja (Slika 2.) (Svaki sloj se sastoji od niza neurona a veze između neurona određene su pomoću težinskih koeficijenata. Optimizacijom težinskih koeficijenata među neuronima računa se krajnji željeni izlaz (Pattanayak i sur., 2021.). Osnovne značajke umjetnih neuronskih mreža opisani su u tablici 1.



Slika 2. Prikaz umjetne neuronske mreže (shorturl.at/deruJ)

Tablica 1. Značajke umjetnih neuronskih mreža (Staub i sur., 2015.)

Značajka	Opis
Nelinearni modeli	Mogućnost rješavanja složenih nelinearnih zadataka.
Tolerancija pogreške	Ravnomjerna raspodjela zadataka unutar sustava.
Trening sustava	Sposobnost prilagodbe u svrhu rješavanja problema.
Učenje	Prilagođavanje opterećenja pomoću algoritama.
Generalizacija	Stvaranje željenog odgovora tokom procesa učenja.

Osnovna jednadžba za računanje izlaznih podatak u neuronskoj mreži:

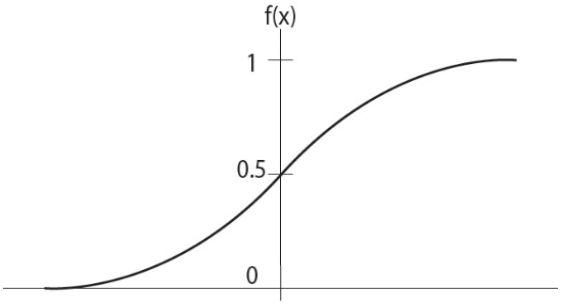
$$Y = f_1(W_2 \cdot f_2\ (W_1 \cdot X + B_1) + B_2$$

Y izlazni vektor; X ulazni vektor, W1 težinski vektor između skrivenog i ulaznog sloja; B1 vektor pristranosti (eng. bias) skrivenog sloja, W2 težinski vektor između izlaznog i skrivenog sloja, B2 vektor pristranosti skrivenog sloja.

Prema Bašić i sur. (2008.) najčešći oblik prijenosne funkcije jest sigmoidalna funkcija (slika 3). Sigmoidalna funkcija je derivabilna, što predstavlja bitnu prednost pri postupku učenja neuronske mreže. Sigmoidalna funkcija je definirana kao:

$$f(x) = \frac{1}{1 + e^{-x}}$$

U posljednje vrijeme umjetne neuronske mreže se sve više koriste kao alat za predviđanje jer modeli umjetnih neuronskih mreža mogu predvidjeti donju ogrjevnu vrijednost biomase sa velikom točnošću.



Slika 3. Sigmoidalna (logistička) funkcija (shorturl.at/kEQU6)

Predviđanje donje ogrjevne vrijednosti pomoću umjetnih neuronskih mreža

Çakman i sur. (2021.) su u istraživanju razvili model umjetnih neuronskih mreža za predviđanje donje ogrjevne vrijednosti biougljena. Usporedbom dobivenih podataka sa rezultatima dobivenim preko već razvijenih empirijskih modela, model umjetne neuronske mreže pokazao je veliku točnost. Također Estiati i sur. (2016.) prikazuju mogućnost korištenja modela umjetnih neuronskih mreža u procjeni donje ogrjevne vrijednosti. Dokazano je da model prikazuje zadovoljavajuću korelaciju i izlazne podatke. Za razliku od primijenjenih empirijskih modela, pojednostavljuju prikaz i strukturu podataka. Iako korišteni nelinearni modeli i modeli neuronskih mreža koriste iste principe podešavanja pojedinih parametara, učinak neuronskih mreža je bio viši.

Patel i sur. (2006.) navode da su postojeći modeli temeljeni na analizama elemenata ili gorivih tvari linearnog karaktera. Kroz istraživanje primjećuje se nelinearan odnos ulaznih podataka sa krajnjim rezultatom. Razvijeni modeli umjetnih neuronskih mreža za predviđanje ogrjevnih vrijednosti jasno prikazuju točnost pri predviđanju i generalizaciji

podataka. Uspoređujući navedeni modeli s tri linearna modela autori su dokazali da modeli neuronskih mreža s većom točnošću izračunavaju izlazne podatke.
Kartal i Ozveren (2020.) koriste model umjetnih neuronskih mreža za procjenu donje ogrjevne vrijednosti sintetskog plina dobivenog iz biomase. Kroz istraživanje dokazano je da modeli procjenjuju donju ogrjevnu vrijednost sa visokom toćnošću ukoliko model koristi dovoljan broj podataka za treniranje mreže.
Pandey i sur. (2016.) u istraživanju koriste modele umjetne neuronske mreže za predviđanje donje ogrjevne vrijednosti, gdje krajnji rezultati pokazuju da se prediktivna izvedba modela dobro slaže s eksperimentalnim skupovima podataka, čime se dokazuje da se neuronske mreže mogu koristiti kao alternativna metoda za modeliranje složenih procesa predviđanja.
U istraživanju koje su proveli Patel i sur. (2007.) razvijeno je sedam različitih nelinearnih modela neuronskih mreža. Rezultati navedenog istraživanja jasno prikazuju točnost pri predviđanju i simplifikaciji podataka. Također, usporedbom modela neuronskih mreža sa linearnim modelima koji koriste iste podatke, prikazana je veća preciznost i točnost modela neuronskih mreža.
Prema Ozveren (2016.) podaci dobiveni laboratorijskim analizama mogu se koristiti kao ulazne varijable za razvoj modela umjetnih neuronskih mreža. Model je pri predviđanju energetskih vrijednosti koristio ulazne podatke udjela fiksiranog ugljika, hlapive tvari i pepela te je pokazao zadovoljavajuće izlazne vrijednosti.
Postojeći empirijski modeli za procjenu uglavnom daju zadovoljavajuće rezultate te se koriste gdje je nemoguće eksperimentalno odrediti donju ogrjevnu vrijednost. No radi svoje stope pogreške nisu povoljni za točno određivanje vrijednosti. Najčešće se koristi umjetna neuronska mreža sa algoritmom unazadne propagacije radi jednostavne implementacije (Mousavian i sur., 2012.).

Zaključak

Ispitivanjem energetskih svojstava biomase određuje se mogućnost iskorištenja biomaterijala u smislu pretvorbe u energiju pri čemu su utvrđene razlike u sadržaju energije po jedinici mase za različitu biomasu. Razvojem sustava nelinearnih modela poput umjetnih neuronskih mreža izlazni podaci se mogu dobiti sa većom preciznošću. U usporedbi sa već korištenim metodama predviđanja, umjetne neuronske mreže mogu pojednostavljeno predvidjeti donju ogrjevnu vrijednost biomase. Najčešće se koriste u donošenju odluka, identifikaciji, kontroli pojedinih sustava i predviđanju. Među brojnim drugim prednostima, umjetne neuronske mreže mogu podnijeti obradu nepotpunih podataka. Nakon što model uspostavi obrazac, može obavljati složene podatke poput predviđanja, modeliranja i optimizacije. Modeli umjetne inteligencije u vidu neuronskih mreža radi svoje točnosti imaju potencijal zamijeniti postojeće empirijske modele razvijene za predviđanje donje ogrjevne vrijednosti.

Napomena

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Literatura

Bašić B. D., Čupić M., Šnajder J., (2008). Umjetne neuronske mreže, Umjetna inteligencija, Fakultet elektrotehnike i računarstva, Zagreb (2008).

Çakman G., Gheni S., Ceylan S. (2021). Prediction of higher heating value of biochars using proximate analysis by artificial neural network. Biomass Conversion and Biorefinery. 1-9.

Estiati I., Freire F., Freire J., Aguado R., Olazar M. (2016). Fitting performance of artificial neural networks and empirical correlations to estimate higher heating values of biomass. Fuel. 180: 377-383. j.fuel.2016.04.051.

Ghugare S.B., Tiwary S., Elangovan V., Tambe S.S. (2014). Prediction of Higher Heating Value of Solid Biomass Fuels Using Artificial Intelligence Formalisms. BioEnergy Research. 7: 681–692.

Izadifar M., Demneh F. (2006). Comparison between neural network and mathematical modeling of supercritical CO2 extraction of black pepper essential oil. The Journal of Supercritical Fluids. 35: 37.

Kartal F., Özveren U. (2020). A deep learning approach for prediction of syngas lower heating value from CFB gasifier in Aspen plus®. Energy, Elsevier, 209.

Mousavian, M. , Mofrad, M. , Vakili, M. , Ashouri, D. , Alizadeh, R. (2012), 'Investigation of Artificial Neural Networks Performance to Predict Net Heating Value of Crude Oil by Its Properties', International Journal of Chemical and Molecular Engineering, 6 (12): 1180-1184.

Ozveren U. (2017). An artificial intelligence approach to predict gross heathing value of lignocellulosic fuels. Journal of the Energy Institute. 90(3): 397-407.

Pandey D.S., Das S., Pan I., Leahy J.J., Kwapinski W. (2016). Artificial neural network based modelling approach for municipal solid waste gasification in a fluidized bed reactor. Waste Management. 58: 202-213.

Patel S., Kumar B., Badhe Y., Sharma B.K., Saha S., Biswas S., Chaudhury A., Tambe, S., Kulkarni B. (2007). Estimation of gross calorific value of coals using artificial neural networks. Fuel. 86: 334-344.

Pattanayak S., Loha C., Hauchhum L., Lalsangzela S. (2021). Application of MLP-ANN models for estimating the higher heating value of bamboo biomass. Biomass Conversion and Biorefinery. 11: 2499–2508.

Staub S., Karaman E., Kaya S., Karapınar H., Güven E. (2015). Artificial Neural Network and Agility. *Procedia - Social and Behavioral Sciences*. 195: 1477-1485.

Ujević-Andrijić Ž., Bolf N. (2019). Umjetne neuronske mreže. Kemija u industriji. 6(5- 6): 219-220.

Artificial neural networks as a statistical tool for predicting the net calorific value of biomass

Abstract

When using biomass as a raw material for the production of fuel and heat, it is necessary to determine its energy properties. By applying the model of artificial neural networks, it is possible to predict net calorific values of individual energy cultures with great accuracy, which makes neural networks good solution in finding the optimal values. The possibility of self-organization, optimized learning and high tolerance to errors, neural network allows reliable and accurate calculation of the lower calorific value of energy crops. With the correct network configuration and data collection, artificial neural network models can be used to predict the calorific value in real time.

Key words: biomass, energy crops, calorific value, artificial neural networks.

IZVORNI ZNANSTVENI RAD

Primjena niskoenergetskih lasera kao neinvazivnog predtretmana pri sušenju grožđa

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Sažetak
Cilj rada je bio utvrditi mogućnost primjene lasera kao predtretmana sušenju grožđa. Bobice grožđa tretirane su 100 i 200 mW laserima u trajanju od 60 i 120 s. Za analizu je korišteno svježe ubrano grožđe sorti Traminac i Muškat Hamburg s početnim udjelima vlage od 81,45 % i 77,25 %. Kod obe analizirane sorte dobivene krivulje sušenja se razlikuju ali su sve negativnog predznaka, što ukazuje na padajuću funkciju, a potvrđuje da se radi o procesu sušenja. Kod sorte Traminac primijećen je značajniji gubitak vode tijekom laserskog tretmana nego kod sorte Muškat Hamburg što se može pripisati razlikama u građi bobica među odabranim sortama. Iz rezultata je vidljivo da lasersko zračenje utječe na migraciju vode iz unutrašnjosti na površinu, te se kao takvo može koristiti kao učinkovit predtretman sušenju grožđa koji ujedno ne oštećuju biljni materijal.

Ključne riječi: laser, predtretman, grožđe, Traminac, Muškat Hamburg

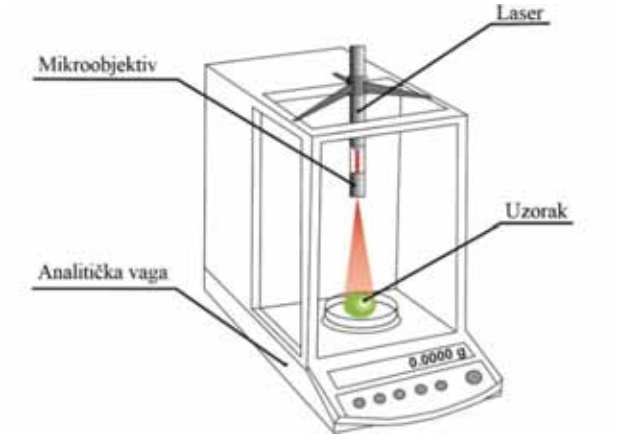
Uvod

Grožđe se uglavnom suši u svrhu proizvodnje grožđica te aromatiziranja i proizvodnje desertnih vina. Predtretmani sušenja grožđa su potrebni zbog specifičnog voštanog sloja kojim je prekrivena kožica ploda. Vosak na kožici grožđa služi kao zaštitna barijera protiv gljivičnih patogena te štiti grožđe od UV zračenja i fizičkih ozljeda. Međutim, prisutnost voska na kožici prepreka je sušenju i zbog toga ga je potrebno ukloniti (Wang i sur. 2016.). U tu svrhu koriste se različite vrste predtretmana poput toplinskih, kemijskih i abrazivnih. U posljednje vrijeme došlo je do porasta interesa za korištenje elektromagnetskog (EM) zračenja kao neinvazivne metode u preradi poljoprivrednih proizvoda. U tu svrhu se koristi EM zračenje u obliku mikrovalova, infracrvenog i koherentnog (laserskog) zračenja. Tri najčešća načina kombiniranja uporabe EM zračenja s konvencionalnim sušenjem su: predgrijavanje (pospješuje migraciju vode iz unutrašnjosti na površinu, odakle se ona uklanja sušenjem vrućim zrakom), dodatno sušenje (energija EM zračenja se koristi kada brzina konvencionalnog načina sušenja počne opadati) i završno sušenje (korištenjem EM zračenja na izlazu iz konvencionalne sušilice ubrzava se uklanjanje preostale vode). Za razliku od visokoenergetskih lasera, niskoenergetski laseri ne uzrokuju uništavanje biljnog materijala, već samo strukturne promjene gradivnih komponenti, što otvara mogućnost njihove široke primjene. Budući da se laserska zraka može fokusirati na vrlo malo područje, laser je uređaj koji može precizno dozirati dozračenu energiju. Kontroliranjem vremena izloženosti materijala laseru direktno možemo kontrolirati količinu energije koju materijal može primiti. Pri tome treba imati na umu da prodiranje i apsorpcija energije zračenja ovisi o vrsti biljnog materijala, njegovom sastavu te o valnoj duljini laserskog svijetla (Hernandez i sur., 2010.). Dosadašnja istraživanja na na laserima male izlazne snage pokazala su da njihova primjena izaziva više pozitivnih reakcija na biljnom materijalu, što im otvara mogućnost široke primjene (Nenadić i sur. 2007.; Galić i sur. 2017.). Pošto su pojedine fizikalne metode predobrade, poput primjene mikrovalnog zračenja, pulsirajućeg električnog

polja i ultrazvuka, kao i njihove kombinacije s kemijskim metodama pokazale dobre rezultate pri skraćivanju vremena sušenja biljnih materijala, cilj ovog rada bio je utvrditi mogućnost korištenja lasera kao predtretmana u procesu sušenja grožđa.

Materijal i metode

Za analizu je korišteno svježe ubrano grožđe sorti Traminac s početnim udjelom vode 81,45 % i Muškat Hamburg s početnim udjelom vode od 77,25%. Početna vlažnost materijala određena je standardnom AOAC metodom (1990.), sušenjem u laboratorijskoj sušilici (INKO ST40T) na 105 °C pri atmosferskom tlaku do konstantne mase. Prije laserskog tretmana, grožđe je oprano kako bi se uklonile prisutne nečistoće. Voštani premaz je uklonjen uranjanjem grožđa u 0,5% otopinu NaOH u trajanju od 60 sekundi nakon čega su bobice isprane destiliranom vodom i otrgnute sa peteljki. Uklonjene su pljesnive i oštećene bobice, a za analizu su odabrane bobice ujednačenog oblika i veličine. Bobice su podvrgnute laserskom zračenju izlazne snage 100 mW (HLM1845) i 200 mW (HLP18130) oba u crvenom dijelu spektra vidljive svjetlosti (λ = 650 nm). Korištene su različite izlazne snage lasera kako bi se utvrdio njihov utjecaj na proces uklanjanja vode. Dva glavna elementa opreme za provođenje laserskog tretmana su izvor koherentne svjetlosti i mikroobjektiv sastavljen od dvije optičke leće kojim se postiže širenje laserske zrake. Širenje laserske zrake je provedeno kontrolirano unutar izračunatog radijusa snopa s ciljem da se njome obuhvati pojedina bobica pri čemu se pazilo da ono ne bude pre veliko jer bi se time smanjila izlazna snaga zračenja (Pliestić, 2007). Trajanje tretmana mjereno je elektroničkim kronometrom. Tijekom tretmana bobice grožđa postavljene su na laboratorijsku analitičku vagu Sartorius BP 221S (Göttingen, Njemačka) s razredom točnosti mjerenja I i podjeljkom ljestvice od 0,1 mg. Mjerenjem promjene mase tijekom tretmana određena je količina isparene vode. Svi rezultati prikazani su kao srednje vrijednosti od 30 ponavljanja (n=30). Za vrijeme trajanja tretmana relativna vlažnost zraka iznosila je 77 %, a atmosferski tlak oko 1013 hPa.



Slika 1. Postupak laboratorijskog provođenja laserskog tretmana

Pojedinačne bobice grožđa postavljene su unutar osvijetljenog područja promjera 15 mm. Bobice su tretirane samo s jedne strane u kontroliranim vremenskim intervalima od 60 i 120 s. Površina osvijetljene površine izračunata je pomoću sljedeće jednadžbe:

$$A = r^2\pi \tag{1}$$

Izračun predane energije unutar osvijetljenog područja proveden je pomoću jednadžbe:

$$Intenzitet = \frac{E_{lasera}(W)}{A\ (m^2)} \times trajanje\ tretmana\ (s) \tag{2}$$

Energija pojedinačnog fotona izračunata je pomoću jednadžbe:

$$E_{fotona} = h\upsilon = \frac{hc}{\lambda} \tag{3}$$

gdje su: h – Planckova konstanta (6,626 × 10^{-34} Js); υ – frekvencija fotona = 4,61538 × 10^{15} s^{-1}; c – brzina svijetlosti = 2,99 × 10^{8} ms^{-1}; λ – valna duljina (650 nm).

Broj fotona emitiranih od strane lasera određen je pomoću jednadžbe:

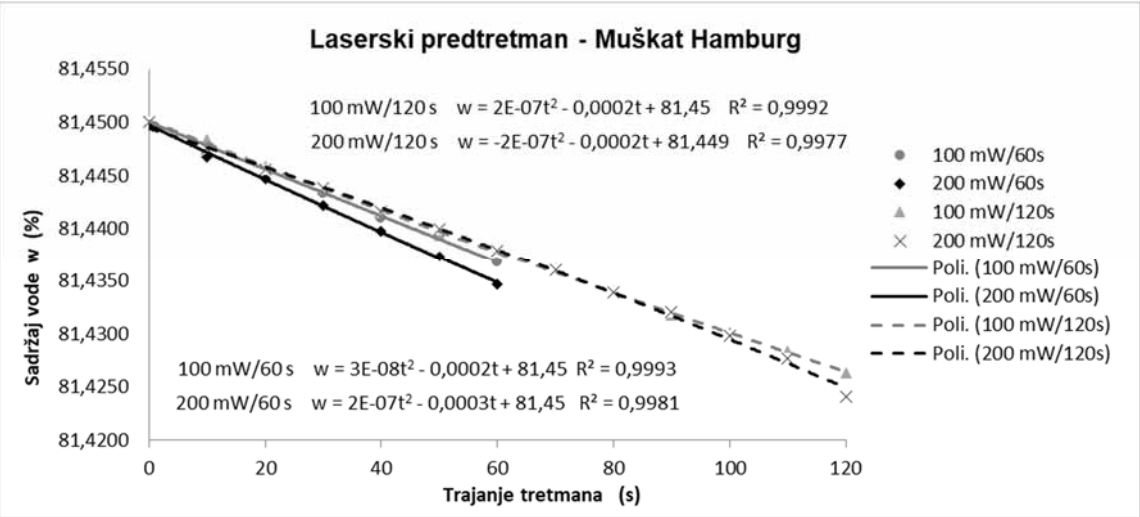
$$n_{fotona} = \frac{E_{laser}}{E_{foton}} \tag{4}$$

Ukupna energija izvora laserske svjetlosti tijekom tretmana izračunata je pomoću jednadžbe:

$$E_{lasera} = E_{fotona} \times n_{fotona} \times trajanje\ tretmana \tag{5}$$

Rezultati i rasprava

Prema jednadžbi (1), osvijetljena površina iznosila je 1,766 ×10^{-4} m^2. Intenzitet laserskog zračenja iznosio je 566,17 Wsm^{-2} za laser od 100 mW i 1132,34 Wsm^{-2} za laser od 200 mW. Energija pojedinačnog fotona izračunata jednadžbom (3) iznosila je 3,06 × 10^{-18} J. Broj fotona iz pojedinačnih laserskih izvora izračunat iz jednadžbe (4) iznosio je 3,27 × 10^{16} fotona s^{-1} za laser od 100 mW i 6,54 × 10^{16} foton s^{-1} za laser od 200 mW. Ukupna energija koju su laseri isporučili tijekom 60 s bila je 6 J za 100 mW laser i 12 J za 200 mW laser, dok je tijekom 120 s iznosila 12 J za 100 mW laser i 24 J za 200 mW laser. Iz rezultata se može vidjeti da su količine energije koje emitiraju laseri relativno male. Sukladno tome toplinski utjecaj lasera je zanemariv, pa se može zaključiti da je efekt laserskog djelovanja na bobice grožđa isključivo posljedica biostimulacije. Lasersko tretiranje sorte Muškat Hamburg u trajanju od 60 s rezultiralo je smanjenjem sadržaja vode od 0,0132 % kod 100 mW lasera i 0,0153 % kod 200 mW lasera. Tijekom tretmana od 120 s, smanjenje sadržaja vode iznosilo je 0,0236 % kod 100 mW lasera i 0,0259 % kod 200 mW lasera. Lasersko tretiranje sorte Traminac u trajanju od 60 s rezultiralo je smanjenjem sadržaja vode od 0,0241 % kod 100 mW lasera i 0,0382 % kod 200 mW lasera. Tijekom tretmana od 120 s, smanjenje sadržaja vode iznosilo je 0,0562 % kod 100 mW lasera i 0,0626 % kod 200 mW lasera. Iz rezultata je vidljivo da tijekom tretmana sorta Traminac ispušta više vode od sorte Muškat Hamburg što se može pripisati razlici u građi njihovih bobica. Analiza brzine otpuštanja vode prikazana je grafikonima 1 i 2 a krivulje sušenja u tablicama 1 i 2.



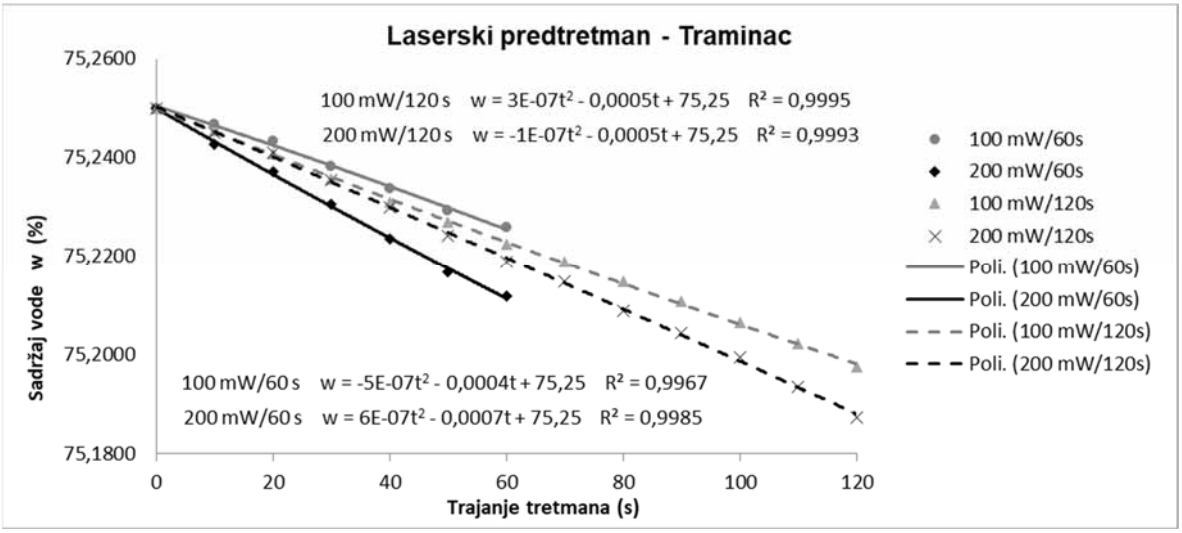
Grafikon 1. Gubitak sadržaja vode (%) sorte Muškat Hamburg tijekom laserskog tretmana

Da bi se dobivene krivulje sušenja mogle uspoređivati, odnosno da bi se utvrdili njihovi međusobni odnosi nagiba, provedeno je njihovo deriviranje *dw/dt* (tablice 1 i 2).

Tablica 1. Derivacije polinomnih jednadžbi II. stupnja - krivulje sušenja sorte Muškat Hamburg tijekom laserskog tretmana

	Izlazna snaga lasera / trajanje tretmana	Derivacije polinomnih jednadžbi II. stupnja
Muškat Hamburg	100 mW/60 s	$dw/dt = -0{,}0002 + 6\times10^{-8}t$
	200 mW/60 s	$dw/dt = -0{,}0003 + 4\times10^{-7}t$
	100 mW/120 s	$dw/dt = -0{,}0002 + 4\times10^{-7}t$
	200 mW/120 s	$dw/dt = -0{,}0002 + 4\times10^{-7}t$

Kod sorte Muškat Hamburg rezultati sve jednadžbe su negativnog predznaka, što ukazuje na padajuću funkciju, a potvrđuje da se radi o procesu sušenja materijala. Kod tretmana u trajanju od 60 s vidljivo je da se koeficijenti nagiba dobivenih jednadžbi razlikuju. Koeficijent nagiba je veći kod uzorka tretiranog 200 mW laserom što pokazuje da je brzina uklanjanja vode pri navedenom tretmanu intenzivnija. Kod tretmana u trajanju od 120 s, vidljivo je da su koeficijenti nagiba dobivenih jednadžbi jednaki, odnosno da nema značajne razlike u brzini uklanjanja vode među laserima od 100 i 200 mW.



Grafikon 2. Gubitak sadržaja vode (%) sorte Traminac tijekom laserskog tretmana

Tablica 2. Derivacije polinomnih jednadžbi II. stupnja - krivulje sušenja sorte Traminac tijekom laserskog tretmana

	Izlazna snaga lasera / trajanje tretmana	Derivacije polinomnih jednadžbi II. stupnja
Traminac	100 mW/60 s	$dw/dt = -0{,}0004 - 10\times10^{-7}t$
	200 mW/60 s	$dw/dt = -0{,}0007 + 12\times10^{-7}t$
	100 mW/120 s	$dw/dt = -0{,}0005 + 6\times10^{-7}t$
	200 mW/120 s	$dw/dt = -0{,}0005 - 2\times10^{-7}t$

I kod sorte Traminac dobivene jednadžbe su negativnog predznaka, što ukazuje na padajuću funkciju. Kod tretmana u trajanju od 60 s, smanjenje sadržaja vode je bilo intenzivnije kod

tretiranja 200 mW laserom, dok je kod tretmana u trajanju od 120 s smanjenje sadržaja vode je bilo intenzivnije kod primjene 100 mW lasera. Iz rezultata je vidljivo da lasersko zračenje pospješuje migraciju vode iz unutrašnjosti na površinu, te se kao takvo može koristiti kao učinkoviti predtretmani sušenju grožđa koji ujedno ne oštećuju biljni materijal i nije štetan za okoliš. Međutim, za praktičnu primjenu potrebno je razviti uređaje s integriranom mrežom laserskih dioda kojima bi se mogao vršiti predtretman veće količine grožđa.

Zaključak

Tretmani niskoenergetskim laserima tijekom 60 i 120 s uzrokovali su smanjenje sadržaja vode u svim analiziranim uzorcima. Razine smanjenja sadržaja vode vrlo su niske i kreću se u rasponu od 0,0132 do 0,0626 %. Rezultati navode na to da postoji indicija utjecaja laserskog zračenja na kinetiku sušenja, no kako bi se potvrdili takvi rezultati potrebno je provesti daljnja istraživanja u koja je potrebno uključiti i lasere drugih izlaznih snaga i valnih duljina.

Literatura

AOAC Official methods of Analysis (16th ed.). (1995). AOAC standard, Association of Official Analytical Chemists. Washington, USA.

Galić A., Pliestić S., Dobričević N., Voća S., Šic Žlabur J. (2017). Predsušenje lista stevije (Stevia Rebaudiana) kao dodatka za krmne smjese uporabom nisko energetskog laserskog zračenja. Agronomski glasnik: Glasilo Hrvatskog agronomskog društva. 79(1-2): 3-14.

Hernandez A.C., Dominguez P.A., Cruz O.A., Ivanov R., Carballo C.A., Zepeda B.R. (2010). Laser in agriculture. International Agrophysics. 24: 407–422.

Nenadić K., Popović R., Jović F. (2007). Preconditions for Automatic Laser Treatment of Wheat Grain. *Proceedings Agricontrol - 2nd IFAC international conference on modeling and design of control systems in agriculture*, Osijek, Hrvatska.

Pliestić S. (2007). Projekt MZOS: Identifikacija samooporavljivih mehanizama u biološkim materijalima“

Wang J., Mujumdar A.S., Mu W., Feng J. Zhang X., Zhang Q., Fang X. M., Gao Z. J., Xiao H. W. (2016). Grape Drying: Current Status and Future Trends, Grape and Wine Biotechnology. IntechOpen. DOI: 10.5772/64662.

Application of low energy lasers as a non-invasive pretreatment for grape drying

Abstract

The aim of this study was to determine the possibility of using a laser as a pretreatment for drying grapes. Grape berries were treated with lasers of 100 and 200 mW for 60 and 120 s, respectively. Freshly harvested Traminac and Muscat Hamburg grapes with initial moisture content of 81.45% and 77.25% were used for the analysis. For both varieties analyzed, the drying curves obtained differ but all have a negative sign, indicating a decreasing function and confirming that it is a drying process. In the Traminac variety, a greater water loss was observed during the pretreatment than in the Muscat Hamburg variety, which can be attributed to differences in the structure of the berries. The results show that laser radiation affects the migration of water from the interior to the surface and can thus be used as an effective pretreatment for drying grapes, which also does not damage the plant material.

Key words: laser, pre-treatment, grapes, Traminer, Muscat Hamburg

PREGLEDNI RAD

Primjena nanotehnologije kod proizvodnje biogoriva

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Sažetak
Još uvijek je prisutna značajna ovisnost o fosilnim gorivima, što tjera svijet u potragu za alternativnim izvorima energije i novim tehnologijama za njihovo bolje iskorištavanje. Prije svega, glavni problem kod iskorištavanja biomase jest dostupnost učinkovite biotehnologije za iskorištavanje biomase na što isplativiji način na komercijalnoj razini. Kao potencijalno rješenje ovog problema može biti upotreba nanotehnologije u postupcima pretvorbe biomase u biogoriva, stoga cilj ovog rada je dati uvid u mogućnost primjene nanomaterijala u iskorištavanju biomase za bioenergiju.

Ključne riječi: biomasa, nanotehnologija, pretvorba, biogoriva

Uvod

Cilj nanotehnologije je manipuliranje materijalima na atomskoj i molekularnoj razini za stvaranje novih molekularnih struktura poznati kao „nanomaterijali“. Tako izmijenjeni novi materijali imaju drugačije karakteristike od prvobitnog materijala. Važno je naglasiti da se na razini nanočestica mogu dogoditi promijene električnih, kemijskih, magnetskih, mehaničkih ili bioloških svojstva koje ih razlikuju od prvobitnog materijala, iako nije zamijećena promijene u kemijskom sastavu (Srivastava, i sur., 2018.).

Nanotehnologija se pokazala kao obećavajuća prekretnica u proizvodnji biogoriva time što ima potencijal rješavanja problema koje nalazimo u dosad korištenim metodama. Dosadašnje metode predtretmana lignocelulozne mase zahtijevaju puno vremena, opasne kemikalije, velike količine energije i vode da bi se celuloza, hemiceluloza i lignin transformirali iz jednog oblika u dugi, a ostavlja za sobom opasne otpadne tvari koje se moraju pravilno zbrinuti da ne predstavljaju opasnost za okoliš. Jedna od metoda zahtjeva enzimatsku razgrađuju celuloze na jednostavne šećere, gdje kasnije ti šećeri prolaze proces fermentacije u etanol koristeći etanogene mikroorganizme. Tu dolazi nenotehnologija kao zamjena klasičnoj metodi zbog potencijala izgradnje materijala promijenjenog poretka atoma u nanostrukture raznih tipova. To je moguće zbog toga što lignocelulozna masa je napravljena od gradivnih nanočestica koje daju karakteristična svojstva materijalu, stoga nanočestice mogu imobilizirati slojeve skupih enzima koji se mogu iznova koristiti za razgradnju dugog lanca polimera celuloze u jednostavnije oblike šećere podložnih fermentaciji za proizvodnju etanola (LTU, 2009.). Primjena nanotehnologije ima potencijal povećanja učinkovitosti razgradnje sirovina te poboljšati proizvodnju biogoriva i njegovu efikasnost, što bi doprinijelo nižim cijenama novim oblicima biogoriva.

Unatoč obećavajućim prednostima, nanotehnologija je izazvala zabrinutost vezanu za potencijalna okolišna i gospodarska pitanja (Srivastava i sur., 2018.) zbog nestabilnosti materijala na nanoskali. Jedna od velikih prepreka za bolje implementiranje i provođenje ispitivanja nanotehnologije čini nepostojanje regulativa i zakona vezanih za korištenje i istraživanje nanotehnologije (Engelmann i Von Hohendorff, 2019.).

Slijedom navedenog cilj rada je dati pregled mogućnost primjene nanotehnologije u proizvodnji biogoriva.

Budućnost sektora biogoriva uz nanotehnologiju

Različiti vrste nanomaterijala zanimljive su u ulozi nosača u tehnici imobilizacije enzima zbog toga što imaju veliku površinu u odnosu na druge materijale koje se primjenjuju u imobilizaciji, a istovremeno enzimi se mogu koristiti u više ciklusa sa pojačanim enzimatskim djelovanjem. Svojstva nanomaterijala su: velika površina, visoki stupanj kristaličnosti, katalitička aktivnost, stabilnost, visoki adsorpcijski kapacitet, trajnost, učinkovito skladištenje te mogućnost za oporabu, ponovnu upotrebu i recikliranje (Anand i sur., 2020.). Upravo zbog dobrih karakteristika nanomaterijala, primjenom istih može se doprinjeti optimizaciji proizvodnje energije iz biomase.

Osim što se nanomaterijali koriste kao katalizatori, pokazali su se korisni za imobilizaciju enzima koji se upotrebljavaju u procesima biokemijske pretvorbe biomase u biogoriva. To je važno iz razloga što se enzim može izolirati pomoću nanočestica te ponovno upotrebljavati za sljedeće tehnološke procese. Od nanomaterijala najčešće se u imobilizaciji koriste: nanočestice, nanocjevčice, nanovlaknaste membrane.

Komercijalno važni enzimi su lipaze i celulaze prisutni i u proizvodnji biogoriva. Ekstrahirana lipaza i određeni mikroorganizmi sa sposobnošću sinteze lipaze su imobilizirani u biomasi pomoćnih čestica i koristi se kao katalitički sloj za postizanje njihove trajne uporabe (Srivastava i sur., 2018.). Uvođenje imobilizirane lipaze putem nanočestica u proizvodne procese izuzetno je važno kad se nastoji prevesti takva tehnologija na industrijsku razinu. Primjenom imobilizirane lipaze u bioreaktorima ostvaruje se veća količina enzima, mogućnost višekratne upotrebe u procesima proizvodnje biogoriva kao i veća otpornost na enzimatsku denaturaciju (Yücel i sur., 2012.), čime se povećava životni vijek enzima.

Uloga nanomaterijala u proizvodnji bioulja i krutog biogoriva

Nanotehnologija se može primijeniti u proizvodnji biogoriva na različite načine koji uključuju: manipulaciju struktura staničnih stijenki unutar različitih tipova biomase radi postizanja lakše razgradnje, izravnu primjena nanokatalizatora za razgradnju celuloze te primjenu enzima ili sustava enzima (npr. glikol hidrolaze, enzimi koji razgrađuju lignin) za poboljšanje učinkovitosti pretvorbe celuloze u šećere i razvoj novih simbiotskih bioloških sustava kojima bi se omogućila proizvodnja biogoriva (Engelmann i Von Hohendorff, 2019.).

Proizvodnja bioulja i biogoriva iz lignocelulozne biomase

Enzimatska hidroliza celuloze i hemiceluloze u jednostavnije šećere pomoću celulaze, hemicelulaze i lipaze je glavni korak u proizvodnji bioetanola (Binhayeeding i sur., 2020.). U korištenju lignocelulozne biomase kao sirovine za proizvodnju biogoriva postoji prepreka, a to je primjena predtretmana kojim se postiže bolja dostupnost šećera za pretvorbu u bioetanol. Kada je riječ o korištenju lignocelulozne sirovine važno je istaknuti visoke troškove predtretmana u proizvodnom procesu koji su neophodni za uspješnu konverziju biomase. Postoji više vrsta predtretmana poput fizikalnih, kemijskih, fizikalno kemijskih, toplinskih, bioloških, a danas se istražuje isplativost i efikasnost korištenja nanočestica u predtretmanu proizvodnih procesa (Arora i sur., 2020.).

U studiji Ali i sur. (2020.) koristila se kao polazna sirovina za proces uplinjavanja biomasa različitih vrsta korova. U istraživanju korištene su nanočestice od kobalta i nikla, koje su imale ulogu katalizatora tijekom uplinjavanja. Termokemijskom pretvorbom uzorka dobiveno je 23,75 % bioplina, 57,5 % bioulja i 18,75 % biougljena. Nastalo bioulje poslužilo je kao sirovina za dobivanje biodizela. Obično se smatra pogodnim gorivom za upotrebu

kada je sadržaj estera 15–20 %. Ovim istraživanjem je procesom transesterifikacije dobiveno 40,79 % sadržaja estera, čime se potvrdilo da je korištena biomasa korova dobar izvor metilnih estera (Ali i sur., 2020.).
Iz dobivenih rezultata se može uočiti da su nanokatalizatori utjecali na povećanje kvalitete nastalih produkta, učinkovitost samog procesa te su pokazali važnu ulogu u smanjenju sadržaja katrana. Nanočestice su utjecali na proces rasplinjavanja na način da su omogućili provođenje procesa na nižoj temperaturi na 650 °C u odnosu na proces bez njihova dodatka i to sa relativno visokom efikasnošću (Ali i sur., 2020.).
Zbog ponovne mogućnosti upotrebe uvjetovane magnetskim svojstvima u proizvodnji goriva zanimljivi su magnetski nanomaterijali. Magnetski nanokatalizatori mogu se vrlo lako i učinkovito odvojiti iz reakcijskih smjesa primjenom vanjskog magnetskog polja (Sanusi i sur., 2021.). Tako je u istraživanjima Manasa i sur. (2017.) provedena imobilizacija celulaze na nanočestice cinkovog ferita radi provođenja hidrolize biomase indijske konoplje. Utvrđeno je oko 74 % učinkovitosti vezanja enzima pri pH 5 i temperaturi od 60 °C dodatkom glutaraldehida. Enzim je ponovno korišten tijekom tri ciklusa, a imobilizacija je povećala njegovu toplinsku stabilnost. Chang i sur., (2011.) istraživali su imobilizaciju celulaze na pore nanočestica SiO_2 preko kovalentne veze, kao i transformaciju celuloze u glukozu pomoću ovog sustava dok su Zhang i sur. (2015.) zabilježili o imobilizaciji celulaze na magnetsku nanosferu Fe_3O_4 koja ne samo da je poboljšala stabilnost enzima, nego je enzim zadržao aktivno djelovanje od oko 87 %.
Biodizel ima veću viskoznost i gustoću u usporedbi sa dizelom, stoga za rješenje ovog problema, nanomaterijali su korišteni kao aditivi u gorivu kako bi se smanjila viskoznost i gustoća smjese goriva (Chacko i Jeyaseelan, 2020.). Alves i sur. (2014.) koristili su kombinaciju magnetskih nanočestica željeza/kadmija i željeza/kositra oksida u transesterifikaciji sojinog ulja. Također je zabilježeno da su ostvarili najveći prinos biodizela od 84 % kada su koristili nanočestice željeznog/kositrenog oksida.

Proizvodnja biogoriva treće generacije

Mikroalge imaju veću stopu proizvodnje biomase u usporedbi sa kopnenim usjevima. Mnogi znanstvenici koji su koristili lipazu, imobiliziranu na funkcionalni nanomaterijal u proizvodnji biodizela, ostvario se veći prinos biodizela na kraju procesa sa manjim zagađenjem, nižom cijenom i pojednostavljenim postupakom. Stoga imobilizacija enzima ima veliki utjecaj na stabilnost i učinkovitost djelovanja lipaze (Anand i sur., 2020.).
Pattarkine i Pattarkine (2012.) navode da su prepreke u proizvodnji biogoriva na bazi mikroalgi; manjak industrijskih pogona za uzgoj mikroalgi, visoki troškovi uzgoja i sakupljanja mikroalgi, energetski zahtjevni tretmani ekstrakcije lipida te upućuju da bi nanotehnologija mogla pomoći kod smanjenja postojećih zapreka u uzgoju i korištenju mikroalgi primjenom nanočestica serbra za bolju fotokonverziju, nanokristala kalcijevog oksida za transesterifikaciju i mezoporozne čestice u separaciji biogoriva. Nova tehnologija „milking algae" do 70 dana može povećati učinkovitost i olakšati vađenje ulja iz algi te za razliku od konvencionalnih metoda ne dolazi do uništavanja stanične strukture (Chaudry i sur., 2016.).

Primjena nanotehnologije u proizvodnji bioplina

Proces nastanka bioplina zahtjeva organski materijal podložan razgradnji, vodu, i metanogene organizme koji imaju mogućnost obavljanja procesa anaerobne fermentacije. Organski ostatci su bogati ugljikom i dušikom, a energija oslobođena u procesu fermentacije ovisi o C:N odnosu (Feng i sur., 2014.).
Istraživanja su pokazala da dodavanjem određenih metalnih iona u tragovima povećava aktivnost metanogenih bakterija, samim time oni imaju ulogu katalizatora te reakcije. Pošto metanogete bakterije zahtijevaju malu količinu željeza, kobalta i nikla za uspješnu

anaerobnu digestiju, istraživanja su pokazala da korištenje nanomaterijala umjesto glomaznih materijala bolje doprinosit uspješnoj reakciji (Feng i sur., 2010.). Magnetske nanočestice imaju snažno paramagnetsko svojstvo i veliku silu, pa se stoga mogu koristiti u proces metanogeneze (Yang i sur. 2015.).
Istraživanjima su otkrili da nanočestice kobalta i nikla povećavaju proizvodnju metana (Abdelsalam i sur., 2016.). Usporedili su aktivnost nanočestica željeza sa nanočesticama željeznog oksida i zaključili da je potonji pokazao veću aktivnost u usporedbi sa nanočesticama željeza. Abdelsalam i sur., (2017.) su u istraživanju došli do zaključka da je učinak različitih nanočestice poput Fe, Fe_3O_4, nikla (Ni) i kobalta (Co) doprinosi najvišom proizvodnjom bioplina anaerobnom digestijom goveđe balege. Casals i sur., (2014.) navode da je prilikom anaerobne digestije korišten Fe_3O_4 s organskim ostatkom te je zamječeno ubrzano raspadanje i povećanje prinosa bioplina.

Zaključak

Metode proizvodnje biogoriva imaju veliki potencijal za napretkom, a nanotehnologija tu može uvelike ubrzati određene reakcije, povećati učinkovitost goriva i procesa. Trenutno najveća uporaba nanotehnologije je imobilizacija enzima zbog velike profitabilnosti, s obzirom da se imobilizacijom omogućava njihovo ponovno korištenje. Jedna od najvećih prepreka pri uporabi nanotehnologije u proizvodnji biogoriva je visoka cijena nanomaterijala. Prema iznesenim činjenicama vidljivo je da uporaba nanočestica u proizvodnji i korištenju biogoriva ima velikih potencijala, ali nema dovoljno provedenih istraživanja na tu temu da bi se moglo reći koliko je sigurno njihovo korištenje.

Napomena

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Literatura

Abdelsalam E., Samer M., Attia Y. A., Abdel-Hadi M. A., Hassan H. E., Badr Y. (2016). Comparison of nanoparticles effects on biogas and methane production from anaerobic digestion of cattle dung slurry. Renewable Energy. 87: 592-598.

Abdelsalam E., Samer M., Attia Y. A., Abdel-Hadi M. A., Hassan H. E., Badr Y. (2017). Effects of Co and Ni nanoparticles on biogas and methane production from anaerobic digestion of slurry. Energy Conversion and Management. 141: 108-119.

Ali S., Shafique O., Mahmood S., Mahmood T., Khan B. A., Ahmad I. (2020). Biofuels production from weed biomass using nanocatalyst technology. Biomass and Bioenergy. 139: 105595.

Alves M. B., Medeiros F., Sousa M. H., Rubim J. C., Suarez P. A. (2014). Cadmium and tin magnetic nanocatalysts useful for biodiesel production. Journal of the Brazilian Chemical Society. 25: 2304-2313.

Anand A., Rajchakit U., Sarojini V. (2020). Detection and removal of biological contaminants in water: the role of nanotechnology. In *Nanomaterials for the Detection and Removal of Wastewater Pollutants*, 69-110. Elsevier.

Arora A., Nandal P., Singh J., Verma M. L. (2020). Nanobiotechnological advancements in lignocellulosic biomass pretreatment. Materials Science for Energy Technologies. 3: 308-318.

Binhayeeding N., Klomklao S., Prasertsan P., Sangkharak K. (2020). Improvement of biodiesel production using waste cooking oil and applying single and mixed immobilised lipases on polyhydroxyalkanoate. Renewable Energy. 162: 1819-1827.

Casals E., Barrena R., García A., González E., Delgado L., Busquets-Fité M., Puntes V. (2014). Programmed iron oxide nanoparticles disintegration in anaerobic digesters boosts biogas production. Small. 10 (14): 2801-2808.

Chacko N., Jeyaseelan T. (2020). Comparative evaluation of graphene oxide and graphene nanoplatelets as fuel additives on the combustion and emission characteristics of a diesel engine fuelled with diesel and biodiesel blend. Fuel Processing Technology. 204: 106406.

Chang, R. H. Y., Jang, J., Wu, K. C. W. (2011). Cellulase immobilized mesoporous silica nanocatalysts for efficient cellulose-to-glucose conversion. Green Chemistry. 13(10): 2844-2850.

Chaudry S., Bahri P. A., Moheimani N. R. (2016). Selection of an Energetically More Feasible Route for Hydrocarbon Extraction from Microalgae–Milking of B. braunii as a Case Study. In Computer Aided Chemical Engineering. 38: 1545-1550.

Engelmann W., Von Hohendorff R. (2019). Regulatory challenges in nanotechnology for sustainable production of biofuel in Brazil. In Sustainable Bioenergy. 367-381.

Feng J.H., Xiong L., Ren X.F., Ma Z.H. (2014). Silica supported perfluorobutylsulfonyl imide catalyzed hydrolysis of cellulose. Journal of Wuhan University of Technology-Mater. Sci. 5:9–14.

Louisiana Tech University (LTU) (2009). Nanotechnology used in biofuel process to save money, environment. Science Daily.

Manasa P., Saroj P., Korrapati N. (2017). Immobilization of cellulase enzyme on zinc ferrite nanoparticles in increasing enzymatic hydrolysis on ultrasound-assisted alkaline pretreated Crotalaria juncea biomass. Indian Journal of Science and Technology. 10(24): 1-7.

Pattarkine M. V., Pattarkine V. M. (2012). Nanotechnology for algal biofuels. In The science of algal fuels. 147-163. Springer, Dordrecht.

Sanusi I. A., Sewsynker-Sukai Y., Gueguim-Kana E. B. (2021). Nanotechnology in Bioprocess Development: Applications of Nanoparticles in the Generation of Biofuels. In Microbial Nanobiotechnology. 165-184. Springer, Singapore.

Srivastava N., Srivastava M., Pandey H., Mishra P. K., Ramteke P. W. (Eds.). (2018). In *Green nanotechnology for biofuel production*, Srivastava, Srivastava, Pandey, P.K. Mishra, Ramteke. Springer Cham.

Yang Z., Huang R., Qi W., Tong L., Su R., He Z. (2015). Hydrolysis of cellulose by sulfonated magnetic reduced graphene oxide. Chemical Engineering Journal. 280: 90-98.

Yücel S., Terzioğlu P., Özçimen D. (2012). Lipase applications in biodiesel production. Biodiesel-Feedstocks, Production and Applications. IntechOpen, London. 209-250.

Zhang W., Qiu J., Feng H., Zang L., Sakai E. (2015). Increase in stability of cellulase immobilized on functionalized magnetic nanospheres. Journal of Magnetism and Magnetic Materials. 375: 117-123.

Application of nanotechnology in biofuel production

Abstract

Excessive dependence on fossil fuels is still present, forcing the world to search for alternative energy sources and new technologies for their better utilization. First of all, the main problem with biomass utilization is the availability of efficient biotechnology to exploit biomass in the most cost-effective way on a commercial level. As a potential solution to this problem may be the use of nanotechnology in the processes of biomass conversion into biofuels, so the aim of this paper is to provide insight into the possibility of nanomaterials using in the biomass utilizatiuon for bioenergy.

Key words: biomass, nanotechnology, conversion, biofuels

IZVORNI ZNANSTVENI RAD

Proizvodnja bioplina iz miskantusa u ovisnosti o veličini čestica sirovine

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Sažetak
Većina bioplinskih postrojenja u EU koristi kukuruznu silažu kao sirovinu u proizvodnji bioplina. Iako je proizvodnja bioplina iz kukuruzne silaže najučinkovitija i tehnički najnaprednija opcija, mogla bi rezultirati ozbiljnom konkurencijom između opskrbe energijom i hranom, što dugoročno nije poželjno. Imajući u vidu nove smjernice EU kroz program REPowerEU, proizvodnja bioplina mora postati nekonkurentna prehrambenom lancu, odnosno održiva s pozicije sirovine. Energetske kulture, poput miskantusa, predstavljaju zadovoljavajuće rješenje, kako u tehnološkom i ekološkom, tako i u ekonomskom smislu. Cilj ovog rada bio je utvrditi biorazgradivost miskantusa tijekom procesa anaerobne digestije, ovisno o veličini ulazne čestice sirovine. Dobiveni rezultati pokazali su da veličina frakcija ulaznog supstrata utječe na proizvodnju bioplina i biometana.
Ključne riječi: miskantus, bioplin, veličina čestica

Uvod

Poljoprivredni ostaci i energetske kulture važan su izvor biomase koji može biti supstrat u procesu anaerobne digestije. Energetske kulture, u koje se ubraja i trava miskantus, pripadaju skupini sirovina druge generacije, a takva je biomasa potencijalno visokovrijedan izvor za proizvodnju obnovljive energije (Grubor i sur., 2021.). Danas je miskantus vodeća višegodišnja energetska trava u EU zbog visokog prinosa suhe tvari i sposobnosti da raste pri različitim klimatskim uvjetima.

Uslijed prijeteće energetske krize, Europska komisija je u ožujku 2022. godine objavila dokument REPowerEU (COM, 2022.) 108), u kojem se potiče povećana proizvodnja bioplina i biometana u državama članicama EU, s ciljem povećanja njihove energetske neovisnosti. Međutim, danas se u većini bioplinskih postrojenja kao primarna sirovina koristi kukuruzna silaža, čija je tržišna cijena porasla za 30 % u posljednjem kvartalu, na taj način ozbiljno konkurirajući i narušavajući sektor stočarske proizvodnje, budući je kukuruzna silaža prije svega visokonutritivna hrana u stočarstvu. Energetske kulture stoga predstavljaju optimalno rješenje, budući se mogu uzgajati na marginalnim tlima, odnosno tlima koja nisu pogodna ili ne mogu ostvariti ekonomsku isplativost uzgoja konvencionalnih poljoprivrednih kultura.

Cilj ovog rada bio je utvrditi biorazgradivost i proizvodnju bioplina i biometana energetske kulture miskantus u procesu anaerobne digestije u ovisnosti o veličini čestice ulazne sirovine te utvrditi optimalnu veličinu čestica za postizanje maksimalnog stupnja biorazgradivosti.

Materijal i metode

U istraživanju je korištena biomasa energetske kulture miskantus požeta u jesenskom roku žetve (studeni 2020. godine), s uzgojne parcele H2020 projekta GRACE na Pokušalištu Šašinovec. Uzorak je nakon žetve osušen prirodnim putem te usitnjen na laboratorijskom mlinu (IKA). U uzorku su standardnim metodama određeni sadržaji vode (HRN EN 18134-2:2015), pepela (HRN EN ISO 18122:2015) te ugljika, vodika, dušika (HRN EN ISO

16948:2015) i sumpora (HRN ISO 16994:2015). Sadržaj kisika utvrđen je računski. Za potrebe anaerobne digestije, uzorci su frakcionirani na laboratorijskoj sito-tresilici (Retsch), a tri frakcije miskantusa, i to (i) 1,25 mm, (ii) 500 µm te (iii) <500 µm uzete su za daljnji proces. Anaerobna digestija provedena je u laboratorijskom bioreaktoru (CROTEH, Hrvatska) tijekom 15 dana pri temperaturi od 35°C pri čemu je praćena produkcija bioplina i biometana (mL).

Rezultati i rasprava

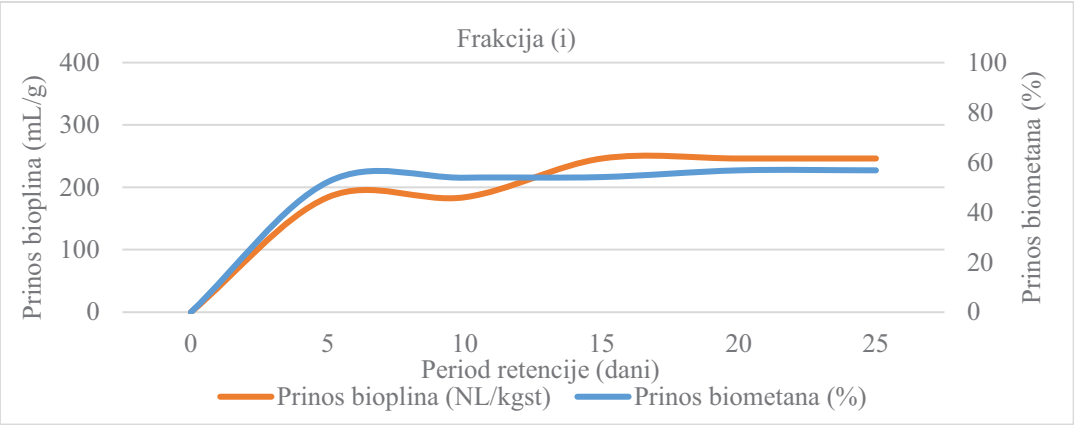
U Tablici 1. prikazan je sastav miskantusa kao ulazne sirovine u procesu anaerobne digestije. Anaerobna digestija provedena je s miskantusom kao monosupstratom uz dodatak inokuluma.

Tablica 1. Svojstva ulazne sirovine

Veličina čestice	Voda, svježi uzorak (%)	KPK (g kg^{-1})	Pepeo (%)	Ugljik (%)	Vodik (%)	Dušik (%)	Sumpor (%)	Kisik (%)
Uzorak prije frakcioniranja	57,62	947,40						
1,25 mm			1,4	48,87	5,86	1,00	0,02	44,25
500 µm			1,61	44,51	5,43	1,43	0,02	48,60
<500 µm			2,45	52,46	6,06	0,63	0,14	40,71

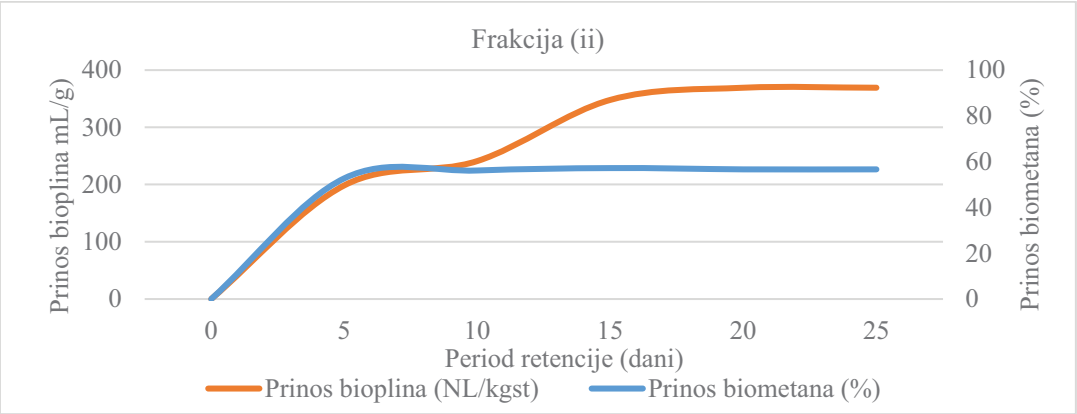
U uzorku miskantusa utvrđen je sadržaj vode nakon žetve, koji je iznosio 57,62 %, što je očekivana viša vrijednost vode s obzirom da je žetva provedena u kasnom jesenskom roku. Sličan podatak dobili su Bilandžija i sur. (2018.), u čijem istraživanju je udio vode miskantusa požetog u jesen bio 53,28 %. Kemijska potrošnja kisika (KPK) iznosila je 947,40 g kg^{-1} s.tv., što je u skladu s literaturom (Nges i sur., 2016.). Udio pepela iznosio je 1,4 % za frakciju (i), nešto veći udio pepela je imala frakcija (ii) s prosjekom od 1,61 % te frakcija (iii) s prosjekom od 2,45 %. Francescato i sur. (2008.) navode da se sadržaj pepela u poljoprivrednoj biomasi kreće od 2 % do 25 %. U istraživanju Grubor i sur. (2021.), u ispitivanim kulturama, bio je 2 % za miskantus i 4 % za stabljiku kukuruza. Ugljik u anaerobnoj digestiji služi kao izvor energije za mikroorganizme, stoga je njegova količina poželjna dokle god je optimalan C/N omjer. Prosječni udio u frakciji (i) iznosio je 48,87 %, za (ii) 44,51 % te za (iii) 52,46 %. U istraživanju Grubor i sur. (2021.), sadržaj ugljika bio je oko 47 % za miskantus, dok je u stabljici kukuruza bio oko 46 %. Prema Jenkins (1998.), udio vodika u biomasi je približno 6 %. U provedenom istraživanju, udio vodika iznosio je za frakciju (i) 5,86 %, za (ii) 5,44 %, te za (iii) 6,07 %, što je u skladu s očekivanim. Prosječni udio kisika u istraživanim frakcijama iznosio je za frakciju (i) 44,25 %, za (ii) 48,59 % te za (iii) 40,70 %. Slične vrijednosti u posliježetvenim ostacima, od 40,09 % za kukuruz i 50,05 % za pšenicu prikazuje Kramar (2018.). Sumpor je od svih prethodno navedenih elemenata, najmanje zastupljen u sastavu biomase. Količine sumpora u biomasi su najčešće niže od 0,2 % ali u nekim slučajevima ga može biti od 0,5 % do 0,7 % (Demirbas, 2004.). U istraživanim frakcijama, sumpor je bio prisutan u udjelima od (i) 0,0215 %, (ii) 0,0235 % te (iii) 0,14 %, iz čega je vidljivo da su utvrđeni udjeli sumpora zadovoljavajuće niski. Uz sumpor, dušik je također nepoželjan element (Matin i sur., 2013.). Ako je u optimalnom omjeru s ugljikom, poželjan je budući da djeluje pozitivno na razvoj anaerobnih bakterija. Ako ga je previše, višak se pretvara u amonijak koji djeluje inhibirajuće. Ukoliko ga je premalo, neće biti moguća razgradnja organskog dijela te će i samim time proizvodnja bioplina biti manja. Udio dušika u pojedinim frakcijama u ovom istraživanju iznosio je kod (i) 0,9995 %, za (ii) 1,43 % te za (iii) 0,627 %. Grubor i sur. (2021.) utvrđuju sadržaj dušika oko 0,5 % za miskantus te 0,9 % za stabljiku kukuruza.

Udio proizvedenog bioplina ovisi o brojnim čimbenicima, poput veličine i vrste supstrata, vremenu berbe, klimatskim uvjetima, sastavu lignina, hemiceluloze i celuloze, C/N omjeru, temperaturi radnog procesa, kao i periodu hidrauličke retencije. Biometan je najvažnija komponenta bioplina jer ima najveću gustoću energije u odnosu na ostale komponente bioplina. Period hidrauličke retencije u ovom istraživanju bio je 25 dana, u mezofilnim uvjetima rasta pri temperaturi od ~37 °C. Grafikon 1. prikazuje krivulju proizvodnje bioplina i biometana za frakciju (i) 1,25 mm.



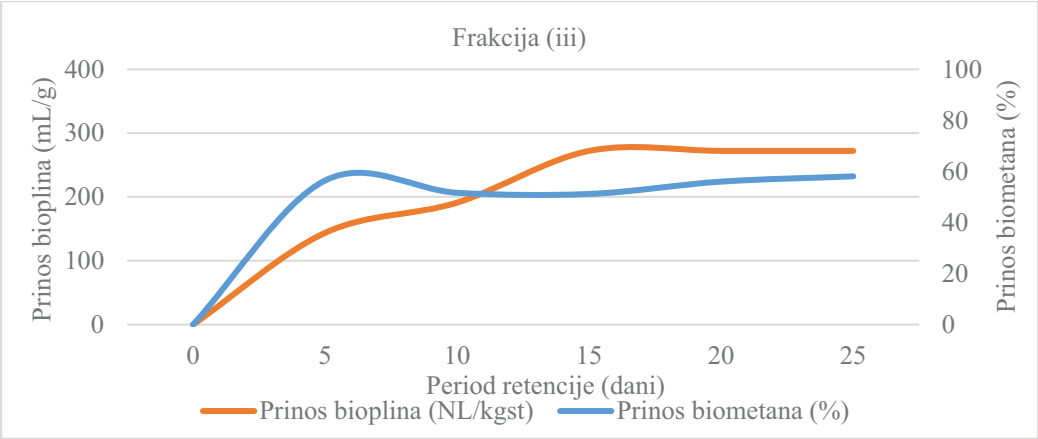
Grafikon 1. Proizvodnja bioplina i biometana po danima za frakciju (i) 1,25 mm

Iz Grafikona 1. vidljivo je da je unutar prvih 5 dana količina bioplina iznosila 184,15 mL g^{-1}, nakon čega je stagnirala do 15. dana, kada postiže svoj maksimum od 246,2 mL g^{-1}. Udio biometana proizvedenog unutar prvih 5 dana iznosio je 52,19 %. U narednim danima, prinos je bio oko 1,53 %, dok nije dosegao svoj maksimum 20. dan koji je iznosio 56,79 %. Grafikon 2. prikazuje krivulju proizvodnje bioplina i biometana za frakciju (ii) 500 µm.



Grafikon 2. Proizvodnja bioplina i biometana po danima za frakciju (ii) 500 µm

Iz Grafikona 2. može se uočiti da je od početka retencijskog perioda očigledan kontinurani rast u proizvodnji bioplina. U prvih 5 dana prinos bioplina iznosio je 198,66 mL g^{-1}. Narednih 10 dana prinos bioplina naglo je porastao do 347,98 mL g^{-1}. Nakon toga, uslijedio je usporeni rast, dok nije dosegao maksimum 20. dan u iznosu od 369,06 mL g^{-1}. To je ujedno i najveći dobiveni udio među istraživanim frakcijama, dok je najveći udio biometana dobiven 15. dan i iznosio je 57,21 %. Proizvodnja bioplina i biometana kod frakcije (iii) <500 µm prikazan je u Grafikonu 3.



Grafikon 3. Proizvodnja bioplina i biometana po danima za frakciju (iii) <500 µm

Iz Grafikona 3. vidljivo je da je u prvih 5 dana prinos bioplina iznosio je 143,62 mL g^{-1} te je kontinuirano rastao (u prosjeku za 65 mL g^{-1}), sve dok nije dosegao maksimum proizvodnje 15. dan (272,15 mL g^{-1}). S druge strane, udio biometana u prvih 5 dana iznosio je 56,46 %, nakon čega je uslijedio period smanjenja (u prosjeku od 2,94 %) do 20. dana. Najveći udio biometana postignut 25. dan (58,06 %), što je i najveći udio od svih istraživanih frakcija.
Rukavina (2019.) u svojem istraživanju uspoređuje proizvodnju bioplina svježe goveđe gnojovke (kontrolna skupina) te smjese svježe goveđe gnojovke i svježe biomase miskantusa (eksperimentalna skupina). Količina proizvedenog bioplina za kontrolnu skupinu iznosila je 16,18 mL g^{-1} dok je za eksperimentalnu iznosila 146 mL g^{-1}, što je niže od vrijednosti bioplina dobivenih u ovom istraživanju. Slične rezultate dobivaju Al Seadi i sur. (2008) za ukupnu količinu bioplina iz kukuruzne silaže koja je bila 202 mL g^{-1}, u odnosu na miskantus, kod kojeg je količina proizvedenog bioplina bila 146,04 mL g^{-1}. Biometan je u kukuruznoj silaži bio zastupljen s 52 % dok je u miskantusu bio s 51,67 %. Nges i sur. (2016.) u svom istraživanju predtretiraju *M. lutarioriparius* procesom usitnjavanja. Autori su zaključili da je smanjenje veličine čestice biomase dovelo do značajnog poboljšanja u prinosu metana i stopi proizvodnje metana te da predtretmani mogu pospješiti učinkovitost anaerobne digestije do 71 % teoretskog prinosa metana *M. lutarioriparius*.

Zaključak

Na temelju provedenog istraživanja, može se zaključiti da se učinkovitost procesa mijenja ovisno o veličini čestice miskantusa. U provedenom istraživanju, biorazgradivost miskantusa je bila veća kod ulaznih čestica manje veličine, što je moguće objasniti činjenicom da je mehaničkim usitnjavanjem došlo do bolje dostupnosti složenih struktura supstrata – lignina, celuloze i hemiceluloze u procesu anaerobne digestije.

Napomena

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Literatura

Al Seadi T., Rutz D., Prassl H., Köttner M., Finsterwalder T., Volk S., Rainer J. (2008). Priručnik za bioplin. Biogas for Eastern Europe. Nacionalni dodatak EIHP.

Amon T., Amon B., Kryvoruchko V., Machmüller A., Hopfner-Sixt K., Bodiroza V., Hrbek R., Friedel J., Pötsch E., Wagentristl H., Schreiner M., Zollitsch W. (2007). Methane

production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresour Technology. 98: 3204-3212.

Angelidaki I., Ahring B.K. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water science and technology. 41:189-194.

Bilandžija N., Voća N., Leto J., Jurišić V., Grubor M., Matin A., Geršić A., Krička T. (2018). Yield and biomass composition of *Miscanthus x giganteus* in the mountain area of Croatia. Transactions FAMENA. 42(SI-1): 51-60.

Demirbas A. (2004). Combustion characteristics of different biomass fuels. Progress in energy and combustion science. 30(2): 219-230.

COM (2022) 108 final (2022). REPowerEU: Joint European Action for more affordable, secure and sustainable energy. European Commision, Brussels

Francescato V., Antonini E., Zuccoli Bergomi L., Metschina C., Schnedl C., Krajnc N., Stranieri S. (2008). Priručnik o gorivima iz drvne biomase. REGEA, (ur.) V. Šegon, Zagreb, Hrvatska, 1-84.

Grubor M., Matin A., Bilandžija N., Bischof S., Jurišić V., Kontek M., Krička T. (2021). Miscanthus and maize stalk as source for green energy production. *Zbornik radova. 48th International Symposium Actual Tasks on Agricultural Engineering*. 455-461.

Jenkins B.M., Baxter L.L., Miles T.R. (1998). Combustion properties of biomass. Fuel Processing Technology. 54: 17-46.

Kramar, B. (2018). Iskoristivost posliježetvenih ostataka kukuruza i pšenice za energetske svrhe. Diplomski rad. Sveučilište u Zagrebu Agronomski fakultet.

Nges I. A., Li C., Wang B., Xiao L., Yi Z., Liu J. (2016). Physio-chemical pretreatments for improved methane potential of Miscanthus lutarioriparius. Fuel. 166: 29-35.

Rukavina J. (2019). Mogućnost proizvodnje bioplina iz miskantusa (*Miscanthus x giganteus*). Diplomski rad. Sveučilište Josip Juraj Strossmayer u Osijeku, Fakultet agrobiotehničkih znanosti Osijek.

Biogas production from miscanthus in dependence of the particle size of the raw material

Abstract

Majority of the biogas plants in the EU use corn silage as a co-substrate in the biogas production. Although the production from corn silage is the most efficient and technically advanced option, it could result in a serious competition between energy supply and food, which is not desirable in the long run. Having in mind the new EU guidelines through the REPowerEU programme (March 2022), the biogas production has to become non-competitive towards the food sector, as well as sustainable from the raw material point of view. For this reason, energy crops, such as miscanthus, are an ideal solution, both environmentally and economically. The objective of this study was to determine the biodegradability of miscanthus during the anaerobic digestion, depending on the particle size of the raw material. The obtained results support the claim that the size of the input substrate fraction affects biogas and biomethane production.

Key words: miscanthus, biogas, particle size

IZVORNI ZNANSTVENI RAD

Teoretski energetski potencijal novih hibrida roda *Miscanthus* u punom prinosu

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Sažetak
Većina istraživanja provedenih na miskantusu, provedena je na prirodnom sterilnom hibridu *Miscanthus* x *giganteus*. Nemogućnost generativne propagacije otežava veću komercijalnu primjenu. Zbog toga, kroz nekoliko europskih uzgojnih programa razvijeni su novi hibridi koji se trenutno intenzivno istražuju.
Cilj ovog rada bio je odrediti energetska svojstva biomase novih hibrida roda *Miscanthus*, nakon čega je izračunat njihov ukupan potencijal. U istraživanju je korištena biomasa ukupno 13 novih hibrida roda *Miscanthus*, te *M. giganteus* kao kontrolni hibrid.
Rezultati analiza upućuju na to da su energetska svojstva biomase novih hibrida usporediva s svojstvima uobičajenih energetskih kultura, pa tako i kontrolnim hibridom. No međutim, ukoliko se uspoređuje ukupan teoretski energetski potencijal, između istraživanih hibrida postoje značajne razlike.

Ključne riječi: energetska svojstva, miskantus, novi hibridi

Uvod

Biomasa energetske kulture miskantus već više desetljeća privlači istraživački i industrijski interes diljem svijeta, no prvenstveno u Europi kao rezultat ekološki orijentiranih politika i strategija. Miskantus je moguće uzgajati na marginalnim površinama te tako izbjeći koliziju s prehrambenim kulturama, što uvelike omogućava iskoristivost zapuštenih površina bez negativnih utjecaja na poljoprivrednu proizvodnju.

Trenutna istraživanja usmjerena su na identifikaciju novih hibrida, prilagođenih različitim agroklimatskim uvjetima te koji posjeduju veću produktivnost i stabilnost proizvodnje biomase (Kontek i sur., 2019.). Ključni dugoročni cilj tih programa je uzgoj hibrida s visokim prinosom biomase na marginalnim tlima. To su tla koja su svojim sastavom nepovoljna za poljoprivrednu proizvodnju zbog različitih ograničenja, primjerice ekstremnih pH vrijednosti, nepovoljnih vodnih režima i dr. No međutim, visok prinos biomase ne uvjetuje nužno i visok energetski potencijal, već je on rezultat različitih svojstava biomase koja su izravno povezana s procesom nekog oblika energetskog iskorištavanja (Lewandowski i sur., 2016.: Jurišić i sur., 2017.).

Cilj ovog rada bio je utvrditi osnovne energetske karakteristike biomase novih hibrida roda *Miscanthus*, kao i kontrolnog hibrida *M.x giganteus* te izračunati njihov ukupan teoretski energetski potencijal u punom prinosu.

Materijal i metode

U ovom istraživanju analizirano je 13 novih hibrida roda *Miscanthus* te kontrolni *M.* giganteus (GRC 9). Oznake GRC 1-GRC 8 odnose se na hibride *M.sinensis x M.sinensis*, dok se ostale oznake odnose na hibride *M.sinensis x M.sacchariflorus*. Novi hibridi su fertilni te sađeni u obliku presadnica. Nasad je uspostavljen u svibnju 2018. godine na

pokusnom polju Pokušališta Šašinovec (45°50'59.1"N 16°11'25.8"E) na tlu lošije kvalitete, pH 5,5 - 6,0, bez korištenja gnojiva. Nasad je postavljen u 4 repeticije. Sirovina biomase je uzorkovana u kasnoj zimskoj žetvi treće vegetacijske sezone, u ožujku 2021. godine, što podrazumijeva žetvu nakon dostizanja punog prinosa nasada.
Nakon prikupljanja i određivanja prinosa (ručno uzorkovanje po pet biljaka u svakoj od repeticija te vaganje), biomasa je usitnjena (IKA Analysentechnik GmbH, Njemačka) i homogenizirana te joj je određen sadržaj suhe tvari (HRN EN ISO 18134-2:2017). Laboratorijske analize prikupljene biomase provedene su u tri ponavljanja, a svi podaci su iskazani na suhu tvar.
Standardnim metodama određen je sadržaj pepela (HRN EN ISO 18122:2015), koksa (EN 15148:2009), fiksiranog ugljika (EN 15148:2009), hlapivih tvari (EN 15148:2009), gornje ogrjevne vrijednosti IKA C200 kalorimetrom (IKA Analysentechnik GmbH, Heitersheim, Njemačka) (EN ISO 18125:2017) te donje računski. Na dobivenim rezultatima provedena je analiza varijance ANOVA, dok su razlike između srednjih vrijednosti testirane Tukey post-hoc testom (R Studio, 2019). Naposljetku, temeljem prinosa te energetskih svojstava, izračunat je ukupan teoretski energetski potencijal novih hibrida u punom prinosu.

Rezultati i rasprava

Određivanjem osnovnih energetskih svojstava moguće je odrediti mogućnost primjene pojedinih hibrida u procesu neposrednog izgaranja, kao i usporediti nove sa kontrolnim hibridom. U tablici 1, prikazan je sadržaj pepela, koksa, fiksiranog ugljika te hlapivih tvari istraživanih hibrida, kao i pripadajući rezultati analize varijance (ANOVA) te Tukey post-hoc testa.

Tablica 1. Sadržaj određivanih svojstava u biomasi istraživanih hibrida

Hibrid	Pepeo (%)	Koks (%)	Cfix (%)	HT (%)
GRC 1	$1{,}84^{ab} \pm 0{,}10$	$9{,}33^{ab} \pm 0{,}51$	$8{,}03^{b} \pm 1{,}20$	$84{,}95 \pm 0{,}58$
GRC 2	$2{,}87^{ef} \pm 0{,}11$	$10{,}73^{bc} \pm 0{,}89$	$7{,}87^{b} \pm 0{,}98$	$83{,}37 \pm 0{,}69$
GRC 3	$1{,}50^{a} \pm 0{,}12$	$10{,}25^{bc} \pm 1{,}28$	$8{,}75^{bc} \pm 1{,}17$	$82{,}16 \pm 3{,}63$
GRC 4	$2{,}08^{bc} \pm 0{,}07$	$10{,}38^{bc} \pm 0{,}75$	$8{,}30^{bc} \pm 0{,}77$	$84{,}67 \pm 1{,}01$
GRC 5	$2{,}17^{bc} \pm 0{,}12$	$10{,}60^{bc} \pm 0{,}17$	$8{,}43^{bc} \pm 0{,}16$	$85{,}01 \pm 1{,}09$
GRC 6	$2{,}59^{de} \pm 0{,}05$	$10{,}57^{bc} \pm 0{,}34$	$7{,}97^{b} \pm 0{,}30$	$83{,}77 \pm 0{,}21$
GRC 7	$3{,}67^{h} \pm 0{,}20$	$11{,}45^{c} \pm 0{,}23$	$7{,}94^{b} \pm 0{,}30$	$84{,}42 \pm 2{,}22$
GRC 8	$2{,}31^{cd} \pm 0{,}18$	$10{,}61^{bc} \pm 0{,}61$	$8{,}30^{bc} \pm 0{,}71$	$83{,}88 \pm 0{,}75$
GRC 9	$1{,}90^{ab} \pm 0{,}11$	$11{,}18^{bc} \pm 0{,}63$	$9{,}28^{bc} \pm 0{,}66$	$84{,}01 \pm 0{,}71$
GRC 10	$3{,}42^{gh} \pm 0{,}11$	$11{,}12^{bc} \pm 0{,}38$	$7{,}71^{b} \pm 0{,}44$	$82{,}21 \pm 2{,}94$
GRC 11	$3{,}00^{f} \pm 0{,}26$	$10{,}34^{bc} \pm 0{,}35$	$7{,}34^{b} \pm 0{,}42$	$83{,}01 \pm 1{,}96$
GRC 13	$3{,}01^{f} \pm 0{,}12$	$7{,}71^{a} \pm 0{,}85$	$4{,}70^{a} \pm 0{,}89$	$84{,}68 \pm 0{,}93$
GRC 14	$3{,}09^{fg} \pm 0{,}07$	$10{,}99^{bc} \pm 0{,}26$	$7{,}90^{b} \pm 0{,}32$	$84{,}89 \pm 1{,}82$
GRC 15	$1{,}63^{a} \pm 0{,}08$	$11{,}93^{c} \pm 0{,}44$	$10{,}3^{c} \pm 0{,}50$	$84{,}30 \pm 2{,}19$
p	**<0,005**	**<0,005**	**<0,005**	0,113

**svi parametri su izraženi na suhu tvar. Legenda: Cfix – fiksirani ugljik; HT – hlapive tvari.*

Signifikantna razlika zabilježena je kod svih promatranih svojstava izuzev hlapivih tvari. Pepeo je jedno od najčešće istraživanih svojstava biomase te je nepoželjan u sustavima za neposredno izgaranje. Sadržaj pepela kontrolnog hibrida GRC 9 iznosio je 1,90 %, što je uspredivo s dosadašnjim istraživanja provedenim pri usporedivim agroklimatskim uvjetima. U tim istraživanjima je biomasa sadržavala između 1,20 % (Krička i sur., 2017.) i 1,65 %

pepela (Jurišić i sur., 2014.). S druge strane, samo su tri nova hibrida imala niži sadržaj (GRC 1, 3 i 15), dok su svi zadovoljavali normativne vrijednosti od minimalnih 1,00 % do 6,00 % (HR EN ISO 17225-1).
Sadržaj koksa povoljno je svojstvo jer on predstavlja sekundarni ugljen koji nastaje pri višim temperaturama (Mohan i sur., 2006.; Baboulos, 2010.). Biomasa kontrolnog hibrida sadržavala je 11,18 % koksa, što je također usporedivo s dosadašnjim istraživanjima, gdje je sadržaj varirao između 11,24 % (Krička i sur., 2017.) te 12,14 % (Voća i sur., 2021.). Najvišu vrijednost koksa sadržavao je GRC 15 (11,93 %).
Fiksirani ugljik odnosi se na čvrstu frakciju koja ostaje nakon isparavanja hlapivih tvari. Uglavnom se sastoji od ugljika, ali i određene količine vodika, kisika, sumpora i dušika (Sarkar, 2015.). Kontrolni hibrid GRC 9 sadržavao je 9,28 % fiksiranog ugljika, što je nešto niže nego podaci dostupni o sadržaju u biomasi miskantusa uzgojenog pri usporedivim agroekološkim uvjetima. U tim je istraživanjima sadržaj fiksiranog ugljika varirao između 10,01 % (Krička i sur., 2017.) te 10,42 % (Bilandžija i sur., 2018.). Najveći sadržaj je imao GRC 15 (11,93 %), dok je najniži GRC 13 (7,71 %).
Prilikom izgaranja biomase, nastaju hlapivi plinovi i čvrsti ostatak. Za biomasu je karakteristično da imaju veći sadržaj hlapivih tvari, najčešće iznad 80 %, a takva goriva imaju manju energetsku vrijednost (Quaak i sur., 1999.). Svi istraživani hibridi sadržavali su između 82,16 % (GRC 3) i 84,95 % (GRC 1) hlapivih tvari. U dosadašnjim je istraživanjima biomase miskantusa uzgojenog pri usporednim agroekološkim uvjetima utvrđen sadržaj hlapivih tvari između 81,46 % (Voća i sur., 2021.) te 88,57 % (Bilandžija i sur., 2017.).

U tablici 2, prikazan je prinos pojedinih istraživanih hibrida kao i pripadajuće gornje ogrjevne vrijednosti. Također, daljnjim izračunom određenje ukupan teoretski energetski potencijal pojedinog hibrida po jedinici površine.

Tablica 2. Teoretski energetski potencijal istraživanih hibrida

Hibrid	HHV (MJ kg^{-1})	Prinos (t st ha^{-1})	GJ ha^{-1}
GRC 1	17,83^{d} ± 0,06	8,64^{ab} ± 1,60	154,00^{a-c} ± 28,42
GRC 2	17,41^{a-d} ± 0,03	9,54^{ab} ± 0,62	166,07^{a-c} ± 10,57
GRC 3	17,58^{b-d} ± 0,05	9,46^{ab} ± 1,54	166,35^{a-c} ± 27,48
GRC 4	17,56^{b-d} ± 0,09	8,44^{ab} ± 0,22	148,33^{a-c} ± 4,52
GRC 5	17,41^{a-d} ± 0,05	8,37^{ab} ± 2,78	145,71^{ab} ± 48,06
GRC 6	17,61^{b-d} ± 0,13	7,39^{a} ± 1,03	130,10^{a} ± 18,10
GRC 7	17,35^{a-c} ± 0,14	8,87^{ab} ± 1,17	153,81^{a-c} ± 20,33
GRC 8	17,65^{b-d} ± 0,13	9,72^{ab} ± 1,35	171,58^{a-c} ± 23,92
GRC 9	17,75^{cd} ± 0,09	8,95^{ab} ± 2,19	159,03^{a-c} ± 39,64
GRC 10	17,30^{a-c} ± 0,02	14,11^{b} ± 2,81	244,19^{bc} ± 48,77
GRC 11	17,03^{a} ± 0,35	6,75^{a} ± 2,17	114,59^{a} ± 35,39
GRC 13	17,27^{ab} ± 0,08	10,03^{ab} ± 3,72	173,39^{a-c} ± 65,01
GRC 14	17,48^{a-d} ± 0,05	8,64^{ab} ± 1,23	150,88^{a-c} ± 21,15
GRC 15	17,56^{b-d} ± 0,10	14,36^{b} ± 2,52	252,28^{c} ± 45,21
p	**<0,005**	**<0,005**	**<0,005**

**svi parametri su izraženi na suhu tvar. Legenda: HHV – gornja ogrjevna vrijednost.*

Gornja ogrjevna vrijednost kontrolnog hibrida iznosila je 17,75 MJ kg^{-1}. Prema normativnim vrijednostima, gornja ogrjevna vrijednost biomase miskantusa varira između 17,00 MJ kg^{-1} te 20,00 MJ kg^{-1} (HR EN ISO 17225-1). Usporedive rezultate objavili su i Jurišić i sur. (2014.) gdje je biomasa miskantusa imala gornju ogrjevnu vrijednost od 17,88 MJ kg^{-1}, te

Voća i sur. (2021.) gdje je vrijednost iznosila 17,64 MJ kg^{-1}. Krička i sur. (2017.), Bilandžija i sur. (2017.) te Bilandžija i sur. (2018.) objavili su nešto više gornje ogrjevne vrijednosti, odnosno 18,15 MJ kg^{-1}, 18,57 MJ kg^{-1} te 18,75 MJ kg^{-1}.
S druge strane, prinos suhe tvari biomase kontroliranog hibrida iznosio je 8,97 t st ha^{-1}, što je značajno niže nego što je uobičajeno za *M. x giganteus*. Najveći prinos zabilježen je kod hibrida GRC 6 (7,39 t st ha^{-1}), GRC 10 (14,11 t st ha^{-1}) te GRC 15 (14,36 t st ha^{-1}), dok je najniži kod hibrida GRC 5 (8,37 t st ha^{-1}) te GRC 4 (8,44 t st ha^{-1}). Ukoliko se izračuna ukupan teoretski energetski potencijal nasada pojedinog hibrida, najveći potencijal imaju hibridi GRC 10 te GRC 15. Njihovim uzgojem na marginalnom tlu moguće je ostvariti ukupan prinos energije od 244,19 GJ ha^{-1}, odnosno 252,28 GJ ha^{-1}. GRC 9, kao kontrolni hibrid sadrži ukupan teoretski energetski potencijal od 159,03 GJ ha^{-1}, dok najnižu vrijednost sadrži GRC 6 (130,10 GJ ha^{-1}).

Zaključak

Ovim istraživanjem dan je uvid u mogućnost iskorištenja novih hibrida roda *Miscanthus* za potrebe energetskog iskorištenja neposrednim izgaranjem. Hibridi GRC 3, 9 te 15 imaju najpovoljniji sadržaj određivanih svojstava koji su povezani s energetskim potencijalom. S druge strane, najnepovoljnija svojstva općenito ima hibrid GRC 13. Najveći ukupan teoretski energetski potencijal sadrži GRC 15. Uzevši u obzir navedene činjenice, svi hibridi sadrže značajan teoretski energetski potencijal te poželjna energetska svojstva. No međutim, GRC 15 predstavlja hibrid s najvećim energetskim potencijalom po jedinici površine te najpovoljnijim svojstvima biomase.

Napomena

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Literatura

Bilandžija N., Jurišić V., Voća N., Leto J., Matin A., Sito S., Krička T. (2017). Combustion properties of *Miscanthus x giganteus* biomass – Optimization of harvest time. Journal of the Energy Institute. 90(4): 528-533.

Bilandžija N., Voća N., Leto J., Jurišić V., Grubor M., Matin A., Geršić A., Krička T. (2018). Yield and Biomass Composition of *Miscanthus x Giganteus* in the Mountain Area of Croatia. Transactions FAMENA. 42(SI-1): 51-60.

Boboulos M. (2010). Biomass Properties and Fire Prediction Tools. Bookboon.

Jurišić V., Bilandžija N., Krička T., Leto J., Matin A., Kuže I. (2014). Fuel properties' comparison of allochthonous *Miscanthus x giganteus* and autochthonous *Arundo donax* L.: a study case in Croatia. Agriculturae Conspectus Scientificus. 79(1): 7-11.

Jurišić V., Voća N., Bilandžija N., Krička T., Antonović A., Grubor M., Matin A., Kontek M. (2017). Pyrolysis properties of major agricultural energy crops. *Zbornik radova 52. hrvatskog i 12. međunarodnog simpozija agronoma*. 651-655. Dubrovnik, Hrvatska.

Kontek M., Krička T., Matin A., Grubor M., Bilandžija N., Jurišić V. (2019). Energy properties of new *Miscanthus* genotypes in autumn harvest. *Zbornik radova 54. hrvatskog i 14. međunarodnog simpozija agronoma.* 578-582. Vodice, Hrvatska.

Krička T., Matin A., Bilandžija N., Jurišić V., Antonović A., Voća N., Grubor M. (2017). Biomass valorisation of *Arundo donax* L., *Miscanthus x giganteus* and *Sida hermaphrodita* for biofuel production. International Agrophysics. 31(4): 575.

Lewandowski I., Clifton-Brown J., Trindade L. M., van der Linden G. C., Schwarz K. U., Müller-Sämann K. i sur. (2016). Progress on optimizing miscanthus biomass production

for the European bioeconomy: Results of the EU FP7 project OPTIMISC. Frontiers in plant science. 7: 1620.
Mohan D., Pittman C.U., Steele P.H. (2006). Pyrolysis of wood/biomass for bio-oil: a critical review. Energy Fuels. 20(3): 848-889.
Sarkar D. K. (2015). Chapter 3–Fuels and combustion. Thermal Power Plant: Design and Operation, Elsevier, 91-137.
Quaak P., Knoef H., Stassen H. (1999). Energy from biomass: a review of combustion and gasification technologies. World Bank.
Voća N., Leto J., Karažija T., Bilandžija N., Peter A., Kutnjak H., Šurić J., Poljak M. (2021). Energy Properties and Biomass Yield of *Miscanthus x Giganteus* Fertilized by Municipal Sewage Sludge. Molecules. 26(14): 4371.

Theoretical energetic potential of new Miscanthus hybrids in full yield

Abstract
Most research on miscanthus has been conducted on the natural sterile hybrid *Miscanthus* x *giganteus*. The impossibility of generative propagation makes major commercial application difficult. Therefore, new hybrids have been developed through several European breeding programs and are currently being intensively researched. These hybrids can be established by seed or rhizomes, which will facilitate planting in large areas in the future. In addition, the development of a larger number of hybrids will allow careful selection of those that produce better yields under specific agroclimatic conditions. In order to collect appropriate data and calculate the total potential of each hybrid, the energetic characteristics must be determined after full crop yield has been reached, i.e., after the third year of growth.
The objective of this study was to determine the biomass energetic properties of new *Miscanthus* genus hybrids and then calculate their total potential considering dry matter yield. A total of 14 hybrids of the *Miscanthus* genus were used for the study, including 8 hybrids *M. sinensis* x *M. sinensis*, 5 hybrids *M. sinensis* x *M. sacchariflorus*, and *M. giganteus* as a control hybrid. The results of the analyzes indicate that the biomass characteristics of the new hybrids are consistent with the important energy crops, including the control hybrid, and that there are significant differences among them when the total theoretical energy potential is compared.

Key words: energy characteristics, miscanthus, new hybrids

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PRETHODNO PRIOPĆENJE

Utjecaj poljoprivredne mehanizacije na degradaciju i destrukciju tala

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Sažetak

Poljoprivreda štetno djeluje na okoliš, u istom intenzitetu kao i uporaba fosilnih goriva. To je potvrđeno i u Izvješću UN-a iz 2010., koje je izrađeno povodom Svjetskog dana zaštite okoliša. Pri ulasku Republike Hrvatske u EU, preuzete su i pravne stečevine EU te su inkorporirane u nacionalno zakonodavstvo na području okoliša. Republika Hrvatska preuzela je i obvezu ispunjavanja standarda zaštite okoliša sustavom izdavanja dozvola za sva postrojenja na koja se primjenjuje Direktiva 96/61/EC o integriranom sprječavanju i kontroli onečišćenja**.** Poljoprivreda snažno utječe na okoliš u smislu potencijalnih onečišćenja tla i voda, te doprinosi globalnom zagrijavanju zbog emisije stakleničkih plinova. Globalno gledajući, zbog rastućih potreba za proizvodnjom hrane, šire se poljoprivredne površine, intenzivira proizvodnja, povećava se uporaba pesticida, a sve to neizbježno dovodi do povećanog opterećenja okoliša. Intenzivna eksploatacija tla uz neizbježnu uporabu mehanizacije pospješuje degradaciju tla, što ima višestruke i složene učinke na globalno okruženje kroz niz izravnih i neizravnih procesa, koji utječu na veliki broj funkcija i usluga ekosustava. Posljedice degradacije očituju se kroz: gubitak organske tvari, smanjenje plodnosti tla, smanjenje biološke raznolikosti, kvalitete zraka i vode, onečišćenje, prekrivanje i prenamjenu tla te klimatske promjene. Destrukcija tla u vidu prekomjernog sabijanja, te pojedini procesi degradacije tla imaju prirodne uzroke, ali se njihovo napredovanje višestruko ubrzava antropogenim utjecajem.

Ključne riječi: poljoprivreda, mehanizacija, degradacija i destrukcija tla

Uvod

"Kada šume više ne mogu njegovati izvore vode koji opskrbljuju vitalnost, tada obradivo zemljište nizvodno ne može izgraditi napon u tlu koji je neophodan za održavanje ravnoteže parazitskih bakterija" (Schauberger, 1999.). Onečišćenje je svaka kvalitativna i kvantitativna promjena fizikalnih, kemijskih i bioloških svojstava okoliša, tj. zraka, vode, tla, hrane, nastala unošenjem u okoliš kemijskih ili fizikalnih čimbenika koji na bilo koji način ugrožavaju ekosustave i njihovu dinamiku (Fanuko, 2005.). Degradacija zemljišta je posljedica višestrukih procesa koji izravno i neizravno smanjuju njegovu uporabljivost. To je složeni pojam kojim se opisuje kako se pojedini resursi ili više njih mijenjaju u smjeru pogoršanja odnosno smanjenja kvalitete (Stocking i Murnaghan, 2000.). Agroekološki pristupom treba se usredotočiti na interakcije između biljaka, životinja, organizama u tlu, ljudi i okoliša. Tako je moguće optimizirati upotrebu prirodnih resursa, poboljšati biološke procese u tlu i poboljšati cikluse biomase, hranjivih sastojaka, ugljika i vode (Kraljević i sur., 2021.). Od tla se očekuje: da bude: siguran filter za gnojiva, herbicide, insekticide i fungicide, da onemogući dospijevanje štetnih tvari u vodu za piće, zadrži, veže i po mogućnosti razgradi štetne tvari koje s oborinama, prašinom ili aerosolom dospijevaju na/u tlo, omogući ekološko kruženje biogenih elemenata, u okviru zaštite prirode omogući nesmetan razvitak i trajni opstanak određenih biljnih vrsta i zajednica, bude siguran temelj za gradnju kuća, cesta, prometnica (Šoštarić i Marković, 2011.). Dakle, obrada tla se

uglavnom povezuje s jednom od najstarijih tehnologija obrade, s oranjem. Naime, znanstvenici su davnih godina dokazali kako je obrada tla plugom izuzetno degradirajući proces. Njime se iznimno jako uništava tlo, energično i često puta, kroz duže razdoblje, nepovratno. Ukratko, oranjem se uništava fizikalni, kemijski i biološki kompleks tla. U tlu žive mikro, makro i mezo organizmi, fauna i flora. Kad se prođe plugom i "okrene" tlo, dolazi do poremećaja njihovog prirodnog staništa. (Jug i sur., 2015.). Kako se i kod ostalih obrada tla (npr. podrivačima, tanjuračama, sjetvo spremačima itd.) nastavlja s degradacijom i destrukcijom tala, nužno je posljedice istih minimalizirati. Budući je uloga poljoprivredne mehanizacije iznimno velika u cijelom tehnološkom lancu obrade tla, posljedice njezine učestalosti se mogu reflektirati na cijeli okoliš, kao i na neposredne rukovaoce i sudionike.

Pregled literature

Jedna od temeljnih karakteristika tla je plodnost. Plodnost tla je stanje koje označava njegovu sposobnost da biljkama kontinuirano osigurava odgovarajuće toplinske uvjete, povoljne vodno zračne odnose i opskrbu biogenim (neophodnim) hranjivima. Plodnost tla je najbolje definirati vrednovanjem njegovih specifičnih funkcija koje kvantificiraju biljnu proizvodnju (Vukadinović i Vukadinović, 2011.). Linearna korelacijska analiza ukazuje kako se između koncentracije humusa, potrebe za kalcizacijom, potencijalom N-mineralizacije, pH i relativnom pogodnosti, uspostavlja vrlo čvrste, signifikantne i pozitivne veze. Interpolacijom navedenih parametara, moguće je iskazati i međusobnu korelaciju prikazanu u tablici 1.

Tablica 1. Korelacije između interpoliranih vrijednosti

	Humus (%)	**CaO (kg ha^{-1})**	**pNmin**	**pH-KCl**	**RP (%)**
Humus (%)	1				
CaO (kg ha^{-1})	0,838^{++}	1			
pNmin	0,978^{++}	0,719^{+}	1		
pH-KCl	0,985^{++}	0,889^{++}	0,952^{++}	1	
RP (%)	0,899^{++}	0,990^{++}	0,793^{+}	0,930^{++}	1

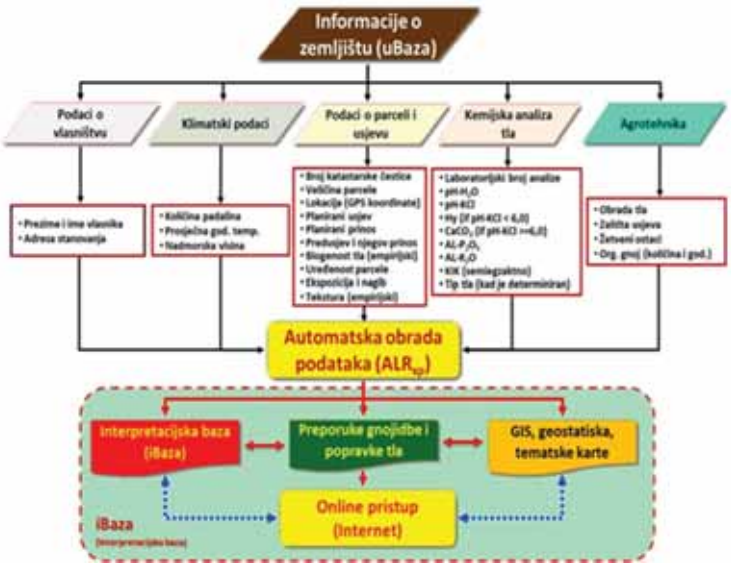
Relativna pogodnost tla za usjeve procjenjuje se sukladno *Liebsherovom zakonu optimuma*. Prirodna plodnost dolazi do izražaja nakon iskorištenja primarne plodnosti, a javlja se kao rezultat prirodnih značajki svakog pojedinog tipa tla: dubina, tekstura, dreniranost, itd. Ona je glavni pokazatelj sposobnosti poljoprivrednih tala da budu supstrat za biljnu proizvodnju. Tradicionalna plodnost tla predstavlja vrhunac plodnosti nekog tla pri tradicionalnoj agrotehnici, odnosno uz primjenu krutog stajskog gnojiva, pliću obradu i plodored u koji su uključene leguminoze. Polazna potencijalna plodnost (ukupna plodnost – definirana je konstelacijom svih čimbenika tla kao što su geološko porijeklo, tekstura, tip tla, reljef, klima, vodni režim, itd.) i efektivnu plodnost (produktivnost tla – definirana je intenzitetom svih vrijednosti edafskih i vegetacijskih čimbenika kao što su sadržaj i kvaliteta humusa, kationski izmjenjivački kapacitet, sadržaj pristupačnih biljnih hranjiva, itd.). Efektivna plodnost je rezultat čovjekovog ulaganja i djelovanja. Tehnološka plodnost tla bazira se na prirodnoj plodnosti ili se od neplodnih tala pod utjecajem antropogenizacije stvaraju antropogena tla (rigosol, vitisol, itd.). Tehnološka plodnost je rezultat radikalnih i kompleksnih, znanstveno utemeljenih zahvata koji uvelike ovise o svojstvima tla (biološkim, kemijskim i fizikalnim) (Kisić, 2012.). Prosječna kiselost najplodnijih hrvatskih tala, onih istočne Slavonije, svega 5,6 pH, što je izuzetno nisko i znatno je ispod optimalne vrijednosti za neke poljoprivredne kulture. Kiselosti tla doprinosi i razmjerno nizak sadržaj organske

tvari tla. Organska tvar je temelj plodnosti svakog tla. Izvor je mikroorganizama, opskrbljuje biljke hranjivima i tvarima koje povećavaju otpornost na biljne bolesti i štetnike, povećava kapacitet tla za vodu i zrak, stvara mrvičastu strukturu, olakšava obradu, i dr. U svijetlu sve učestalijih suša u Hrvatskoj te rasta pedološke suše (manjka korisne vode u tlu), prisustvo organske tvari u tlu je od još većeg značenja. Nažalost, prosječan sadržaj organske tvari najplodnijih hrvatskih poljoprivrednih tala, onih istočne Slavonije, iznosi svega 1,9 %. Zbog neracionalnog gospodarenja, u posljednjih pedesetak godina, sadržaj organske tvari hrvatskih poljoprivrednih tala je u stalnom opadanju. Procjenjuje se da je izgubljeno 50-70 % ishodišne organske tvari tla (Znaor i Karoglan-Todorović, 2011.). Sveukupni povijesni tehnološki razvoj, pa tako i razvoj poljoprivredne mehanizacije, tradicionalno osim velikih benefita, nažalost ostavlja i neke neželjene posljedice. One se najviše uočavaju u degradaciji i destrukciji tla i narušavanju okolišne ravnoteže. Zbog navedenog, nameću se nove zadaće u cijelom tehnološkom lancu, što određuju i novi propisi kao što su Direktiva 96/61/EC o zaštiti okoliša, te o integriranom sprječavanju i kontroli onečišćenja. Sve to stavlja nove zadaće pred proizvođače i korisnike poljoprivredne mehanizacije. Jedna od najzahtjevnijih ljudskih aktivnosti je obrada tla. Što se događa s tlom prilikom obrade plugom? Npr. dubokim oranjem miješaju se gornji slojevi tla s donjim slojevima, čime se osiromašuje gornji sloj tla organskom tvari. Do sabijanja tla kod upotrebe mehanizacije dolazi uslijed pritiska kotača na tlu (Jug i sur., 2017.). Kretanje pogonskih kotača narušava strukturne agregate i istovremeno dolazi do sabijanja tla. Posebice je opasno uobičajeno oranje traktorom pri čemu se kotači kreću po dnu otvorene brazde, gdje tlo uslijed djelovanja većeg sadržaja vlage i nižeg sadržaja organskih tvari podložnije sabijanju nego na površini (Sekulić i sur., 2003.). Za očuvanje organske tvari preporuča se sljedeće: izbjegavanje nepotrebne obrade tla i dubokog oranja, gnojidba stajskim gnojem, zaoravanje žetvenih ostataka, a ne spaljivanje, uvođenje u plodored među usjeva za zelenu gnojidbu. (Vukadinović, 2011.) Zbijanje (kompaktnost) tla nastaje kada se čestice tla tlače uz smanjivanje prostora pora između njih. Stoga raste masa tla po jedinici volumena (tzv. volumna gustoća tla) uz pogoršavanje strukture tla. Zbijanje tla najčešće je posljedica obrade teškim strojevima kada je tlo mokro ili vlažno, ali uzrok može biti i berba/žetva ili ispaša. Suho tlo mnogo je otpornije na zbijanje u odnosu na vlažno ili mokro tlo. Istraživanja su pokazala da su strukturalna oštećenja tla na dubini od 25 – 40 cm nastala obradom tla teškom mehanizacijom vidljiva i nakon 14 godina od trenutka nastanka zbijanja tla, što znači da su negativne promjene – trajne. Zbijanje tla smanjuje optimalne uvjete proizvodnje hrane. Poljoprivredno tlo je podložno negativnim efektima težine i veličine mehanizacije koja se značajno povećavala posljednjih desetak godina. Sabijanje površinskog dijela tla (0 – 25 cm) može smanjiti urod od 5 do 15 %, što može biti privremeno, ako se tlu posveti dovoljno pažnje. No ako se sabijanje tla pojavi u zoni ispod 50 cm ispod površine, gubitak prinosa će se nastaviti, i ostat će u naslijeđu slijedećih generacija. Slaba struktura tla smanjuje i usvajanje dušika, što uzrokuje gubitak ispiranjem dušika koji završava u vodotocima. Primjena mehanizacije u poljoprivrednoj proizvodnji ima negativne posljedice u pogledu zbijanja tla (do 60 cm) čime se smanjuje poroznost tla. Nadalje, homogenizira se gornji sloj i narušava struktura tla, uslijed čega se smanjuje stabilnost strukturnih agregata. U prirodnim se tlima u gornjih 10 cm tla nalazi 70-90 % organske tvari, u preoranim tlima, u tome sloju preostaje < 20 %, a u dubini 15-25 cm, nalazi se 60 % ukupne organske tvari u tlu (Đikić i sur., 2001.). Zahvat u okoliš je svako trajno ili privremeno djelovanje čovjeka koje može narušiti ekološku stabilnost i može nepovoljno utjecati na okoliš. Onečišćavanje okoliša je promjena stanja okoliša koja je posljedica štetnoga djelovanja, ispuštanja, unošenja, odlaganja štetnih tvari i ispuštanja energije i utjecaja i pojava nepovoljnih po okoliš. Šteta u okolišu je oštećenje ili gubitak prirodne funkcije sastavnih dijelova okoliša, prouzročena gubitkom pojedinih sastavnih dijelova i/ili unutarnjim poremećajem odnosa i prirodnog

tijeka, nastalog zbog ljudskog djelovanja. Rabljeno motorno ulje ekološki je problem. Različiti su načini postupanja s rabljenim motornim uljem, kao i uljem iz transmisije. Gospodarsta direktno onečišćuju okoliš i to ispuštanjem u tlo, zagađuju podzemne vode, spaljivanjem onečišćuju atmosferu. Štetni utjecaj na okoliš imaju i gospodarstva koja nepravilno postupaju s plastičnom ambalažom od ulja, kao i sa zamijenjenim pročistačem. Iako nemaju sva gospodarstva utjecaja na onečišćenje, još uvijek se može primijetit kršenje zakonskih normi. Npr. ako se ne popunjava prijavne listove o količinama prikupljenog rabljenog ulja, tzv. Očevidnike, ili ne skuplja dio rabljenog ulja prema koeficijentu skupljanja, što je propisano zakonom. Miješanje ulja iz motora i ulja iz transmisije, dakle dvije različite kategorije ulja, je zabranjeno. Postupanje s otpadnim gorivom: otpadno gorivo se ne smije odlagati u odvodne kanale, kanalizaciju i vodotoke jer će se time onečistiti voda i okoliš, otpadna goriva koja se ne mogu upotrijebiti moraju se odlagati u za to namijenjene sabirne centre. Otpadna maziva ulja iz poljoprivredne proizvodnje opasan su otpad i nepravilnim odlaganjem otpadnih ulja zagađuje se okoliš (Kiš i sur., 2007.). Koncentracijom od samo 1-2 mg/l vode, voda postaje nepitka. Pravilnikom o gospodarenju otpadnim uljima (N.N. 178/04 i 111/06), i Uredbom o kategorijama, vrstama i klasifikaciji otpada s katalogom otpada i listom opasnog otpada (N.N. 50/05) uređuje se način gospodarenja otpadnim uljima, kojemu je cilj uspostavljanje sustava skupljanja otpadnih ulja radi uporabe i/ili zbrinjavanja zaštite i okoliša i zdravlja ljudi.

Rezultati i rasprava

Sve zahvate u okolišu treba planirati i izvesti tako da ne onečišćuju okoliš. Ukoliko se koriste opasne tvari, tzv. zagađivači okoliša, strogo treba postupati s njima, jer onečišćivač snosi troškove nastale onečišćavanjem okoliša (članak 16. Zakona o zaštiti okoliša, NN 82./94.). S obzirom na ekološke norme i zaštitu okoliša, razvijena je nova metoda odlaganja i zbrinjavanja opasnog otpada, tzv. BSU metoda: baliranje, spaljivanje, utiskivanje (NN 27./96.). Zbog navedenog je poželjan stalni monitoring o stanju tla. Tako npr. model Kontrole plodnosti tla Osječko-baranjske županije obuhvaća pet grupa ulaznih podataka (Slika 1.), odnosno: podaci o proizvođaču, klima, podaci o parceli i usjevu, kemijska analiza tla i agrotehnika. Bez razvoja sustava periodičnog prikupljanja informacija o tlu, nemoguće je pravovremeno uočiti negativne promjene koje dovode do degradacije kemijskih, fizikalnih i/ili bioloških svojstava tla.



Slika 1. Shematski prikaz modela kontrole plodnosti Osječko-baranjske županije (Vukadinović, 2013.)

Model je podržan originalnim računalnim programom (ALRxp) (Vukadinović, 2013.) za utvrđivanje relativne pogodnosti tla za usjeve, potrebe za kalcifikacijom, popravkom tla i kreiranje gnojidbenih preporuka za konkretnu parcelu i usjev u konvencionalnoj, integriranoj ili ekološkoj proizvodnji. Mineralna maziva teško su razgradiva i jako nepovoljno utječu na zagađenje okoliša (litra min. ulja može zagaditi milijun litara vode). Dobro održavani traktori, kombajni i ostali strojevi osnova su čišćega okoliša. Mehanizaciju treba redovito održavati da bi se smanjilo neželjeno ispuštanje ulja i tako spriječilo onečišćenje tla i vodotoka (Katalinić i sur., 2009.). Ne treba očekivati da će se stanje u pogledu zbrinjavanja rabljenog ulja u skoroj budućnosti popraviti i doći samo od sebe. Stoga treba provoditi redovite kontrole na obiteljskim gospodarstvima te educirati poljoprivredne proizvođače.

Zaključak

Ekološka kriza na svjetskoj razini priznata je tek devedesetih godina prošlog stoljeća kada se sve više počelo uočavati da neodrživo gospodarenje prirodnim resursima nema dugoročno pozitivan učinak te da donosi brojne negativne posljedice za zdravlje čovjeka, ali i za njegov okoliš. Stoga su izrađeni brojni programi, pravilnici, propisi i druge mjere kojima se nastojalo i još uvijek se nastoji djelovati na smanjenje onečišćenja okoliša te održivo gospodarenje njime. Svim tim mjerama cilj je povećati kvalitetu života na način da se rješavaju ekološki problemi i višestoljetno neodrživo gospodarenje prirodom. Potrebe za promjenama uočene su i na razini gospodarenja poljoprivrednim zemljištem. Naime, sve više se uočava da neadekvatnim postupanjem s poljoprivrednim zemljištem dolazi do negativnih posljedica, kako u smislu njegove produktivnosti, tako i na razini kvalitete proizvoda. U pogledu fizikalno-mehaničkih svojstava (koji su prvi na "udaru" negativnih promjena u i na tlu), na uporabu teških poljoprivrednih traktora i strojeva, tlo reagira promjenama u tvrdoći, plastičnosti, ljepljivosti, vodenoj fazi tla, otporu tla rastezanju, tlačenju, uvijanju i trenju, te promjenama u specifičnom vučnom otporu tla pri obradi i otporu kretanju strojeva i vozila površinom tla, povećavaju se troškovi proizvodnje, dolazi do pojačane erozije i gubitka hranjiva, pogoršava se kvaliteta uroda i struktura tla, smanjuje se sposobnost infiltracije, propusnosti tla za zrak, sadržaj fiziološki aktivne vode, itd. Negativni učinci zbijanja tla posebno su istaknuti frazom "pretjerano zbijanje tla teškom mehanizacijom iziskuje pretjeranu obradu još težom mehanizacijom", te kroz svoj nepovoljan utjecaj na rast i razvoj biljaka, u ranim fazama, postaje sve veći agroekološki problem. Iz prethodno rečenog proizlazi da se navedeni problemi trebaju rješavati i uskladiti sa proizvodnim zahtjevima današnje poljoprivredne proizvodnje. Uslijed brojnih prohoda u primarnoj i sekundarnoj obradi teškim strojevima i oruđima, te stalne ili podjednake dubine primarne obrade tla, gotovo redovno dolazi do pojave stvaranja tabana tanjurače i tabana pluga. Reduciranjem zahvata obrade tla, direktnom sjetvom bez obrade tla, dolazi do smanjenja pokrivenosti površine tla prohodima pneumatika strojeva i oruđa. Uslijed intenzivnog korištenja te obrade tla, vlada stalni trend narušavanja svih fizikalnih, kemijskih i bioloških svojstava tla. Ove negativne procese destrukcije tla treba obavezno prekinuti i to smanjivanjem broja prohoda teškim strojevima i oruđima, a posebice pri nepovoljnom stanju vlažnosti.

Literatura

Direktiva IPPC 2008/1/EC - prenesena u hrvatsko zakonodavstvo preko Zakona o zaštiti okoliša (NN 110/2007).

Direktiva 96/61/EC- Council Directive 96/61/EEC of 24 September 1996 concerning the integrated pollution prevention and control.

Đikić D., Glavač H., Glavač V., Hršak V., Jelavić V., Njegač D., Simončić V., Springer O., Tomašković I., Vojvodić V. (2001). Ekološki leksikon.

Fanuko N. (2005.). Ekologija: udžbenik za stručne studije vinarstva i mediteranske poljoprivrede, Poreč-Rijeka, Hrvatska; Veleučilište u Rijeci.

Jug D., Birkás M., Kisić I. (2015): Obrada tla u agroekološkim okvirima. Sveučilišni udžbenik. Hrvatsko društvo za proučavanje obrade tala (HDPOT), 275. Osijek, Hrvatska.

Jug D., Jug I., Vukadinović V., Đurđević B., Stipešević B., Brozović B.(2017): Konzervacijska obrada tla kao mjera ublažavanja klimatskih promjena. Sveučilišni priručnik. Hrvatsko društvo za proučavanje obrade tala (HDPOT), 176. Osijek, Hrvatska.

Katalinić, I., Krnić, S., Brstilo, M., Poljak, F., Rakić, M., Buković Šošić, B., Lukšić, M., Pavlović, D., Bičak, L., Danjek, I., Jukić, I., Pejaković, D., Zagorec, D. (2009.): Načela dobre poljoprivredne prakse. Ministarstvo poljoprivrede, ribarstva i ruralnog razvoja. 78. Zagreb.

Kisić, I. (2012.): Sanacija onečišćenog tla, Agronomski fakultet Sveučilišta u Zagrebu, Zagreb.

Kiš, D., Plašćak, I., Voća, N. i Arežina, M. (2007). Motorno ulje – opasan otpad? Poljoprivreda. 13(2): 53-58.

Kraljević, D., Šumanovac, L., Baličević, P., Zimmer, D. (2021). Primjena precizne poljoprivrede i tehnoloških traka u optimalizaciji poljoprivredne proizvodnje, *56. hrvatski i 16. međunarodni simpozij agronoma*. 331-332.Vodice, Hrvatska.

Narodne novine (1994). Zakon o zaštiti okoliša, broj 82./94., Zagreb

Narodne novine (1996). Pravilnik o vrstama otpada, broj 27./96., Zagreb

Narodne novine (2005). Strategija gospodarenja otpadom, broj 130./05, Zagreb

Schauberger V. (1999). Život i učenje od prirode, Priručnik-Popularna znanost, AGM.

Sekulić P., Kastori R., Hadžić V. (2003). Zaštita zemljišta od degradacije. Naučni institut za ratarstvo i povrtarstvo, Novi Sad, 230

Stocking M., Murnaghan N. (2000). Land Degradation – Guidelines For Field Assessment, cofunded by UNEP, UN University. http://www.unu.edu/env/plec/l-degrade/index-toc.html, pristup: 30.04.2016.

Šoštarić J., Marković M. (2011). Zaštita tla i voda, Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet Osijek.

Šumanovac L., Bukvić Ž., Jurić T., Jurišić M., Knežević D. (2000). Utjecaj različitih varijanti obrade tla na kvalitetu raspodjele sjemena po površini i dubini. Poljoprivreda. 2: 39-43.

Vukadinović V**.** i Vukadinović V. **(**2011). Ishrana bilja, Poljoprivredni fakultet Osijek.

Vukadinović V. (2013). Prijedlog sustava kvalitete plodnosti poljoprivrednog zemljišta RH, Osijek.

Znaor D., Karoglan-Todorović S. (2011). Priručnik za provođenje mjera zaštite okoliša na SAPARD i IPARD projektima za poljoprivredu i prehrambenu industriju.

Impact of agricultural machinery on soil degradation and destruction

Abstract
Agriculture has a detrimental effect on the environment, at the same intensity as the use of fossil fuels. This was confirmed in the 2010 UN Report, produced on the occasion of World Environment Day. When the Republic of Croatia joined the EU, the same acquis was also taken into national environmental legislation. The Republic of Croatia has also undertaken to meet environmental standards through a licensing system for all installations subject to Directive 96/61/EC on integrated pollution prevention and control. Agriculture has a strong impact on the environment in terms of potential soil and water pollution, and contributes to global warming due to greenhouse gas emissions. Globally, due to growing food production needs, agricultural areas are expanding, production is intensifying, the use of protection products is increasing, all of which inevitably leads to an increased environmental burden. Intensive soil exploitation with the inevitable use of mechanization promotes soil degradation, which has multiple and complex effects on the global environment through a series of direct and indirect processes, affecting a large number of ecosystem functions and services. The consequences of degradation are manifested through the loss of organic matter, the reduction of soil fertility, the reduction of biodiversity, air and water quality, pollution, soil cover and conversion, and climate change. Soil destruction in the form of excessive compaction, and certain processes of soil degradation have natural causes, but their progression is accelerated multiple times by anthropogenic influence.

Key words: agriculture, mechanization, degradation and destruction of the soil

IZVORNI ZNANSTVENI RAD

Sabirno logistički centri za biomasu u procesu proizvodnje bioplina

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Sažetak
Globalni pristup energiji je neodrživ te se počinju korisitit obnovljivi izvori energije. Najveći se doprinos očekuje od biomase, jer osim što je ona sama obnovljiva i njeni su produkti obnovljivi te dovoljno slični fosilnim gorivima što omogućuje njihovu zamjenu. Uklanjanje ostataka nakon žetve ili berbe može se povećati prihod poljoprivrednika, osiguravajući sirovine koje bi se mogle koristiti u industrijske i energetske svrhe. Postupci manipulacijom biomasom obuhvaćeni su u sabirno logističkim centrima. Stoga je cilj ovog rada opisati sabirno logističke centrare za biomasu uz opis proizvodnje bioplina.

Ključne riječi: obnovljivi izvori energije, biomasa, sabirno logistički centri za biomasu, bioplin

Uvod

Europskim zelenim planom utvrđuje se cilj da se do 2050. postigne klimatska neutralnost, a kako bi se to postiglo potrebno je postići smanjenje emisija stakleničkih plinova za 55 % do 2030. Za to su pak potrebni znatno veći udjeli OIE u integriranom energetskom sustavu. Trenutačni cilj EU-a od najmanje 32 % energije iz obnovljivih izvora do 2030., utvrđen u Direktivi o promicanju uporabe energije iz obnovljivih izvora (RED II) nije dovoljan te ga je u skladu s Planom za postizanje klimatskog cilja (COM/2021/557 final) potrebno povećati na 38–40 %. Kao jedno od mogućih rješenja primjena je biomase kao zamjena za fosilna goriva u proizvodnji energije. Međutim, energetski lanac vrijednosti zahtijeva isporuku sirovine odnosno biomase od proizvodnih mjesta do centara za korištenje. Na tom putu biomasa prolazi kroz objekte i procese koji su činili takozvani lanac opskrbe biomasom. Svaki korak opskrbnog lanca zahtijeva posebnu stručnost, tehnologiju i operacije uključujući proizvodnju biomase, transport sirovina, preradu i potrošnju (Ghaderi i sur., 2016.).

Biomasa

Biomasa je jedini obnovljiv izvor energije koji se može koristiti neograničeno, bilo za korištenje u svrhu proizvodnje električne i toplinske energije ili za proizvodnju biogoriva. Izvori biomase se nalaze u poljoprivredi, šumarstvu i drvnoj industriji, energetskim nasadima, kod biljaka se velikom količinom ulja i šećera te u kućnom i industrijskom organskom otpadu i ostacima (Iličković, 2014.).

Kao što je već poznato, moguće je izravno zamijeniti fosilna goriva biomasom, jer je velika sličnost biomase i njenih produkata nakon prerade s fosilnim gorivima (Krička i sur., 2007.).

Najčešće se koristi poljoprivredna biomasa koja uključuje ostatke godišnjih kultura (slama, kukuruzovina, stabljike) i energetske kulture (Schjønning i sur., 2009.; Powlson i sur., 2011.) uz preduvjet očuvanja kvalitete tla i vraćanja organske tvari u tlo (Matin i sur., 2019.).

Željeni oblik energije, kvaliteta i vrsta sirovine su čimbenici koji ponajviše utječu na postupak pretvorbe u energiju, te na izbor procesa i tehnike pretvorbe biomase. Uz to, potrebno je voditi računa također i o načinu upotrebe, okolišnim normama i ekonomskim mogućnostima (McKendry, 2002.).

Biogoriva

Prema obliku krajnjeg korištenja biogoriva su podijeljena na kruta, tekuća i plinovita (Krička i sur., 2017.). Najčešće korištena kruta goriva su sječka, peleti i briketi; tekuća su biodizel i bioetanol te plinovito bioplin. Ovisno o tehnologiji koja se primjenjuje, iz biomase je moguća proizvodnja toplinske i električne energije kao i derivata iz kojih se dobiva korisna energija. Korištenje biogoriva je vrlo važno jer se povećava vlastita opskrbljenost energentima što znači da se smanjuje uvoz fosilnih goriva, smanjuje se emisija stakleničkih plinova, usvajaju nove tehnologije i otvaraju novi proizvodni pogoni, povećava se lokalna i regionalna gospodarska aktivnost i uspostavljaju novčani tijekovi u lokalnoj zajednici, odnosno omogućava se zapošljavanje.

Bioplin

Upravo energetska i klimatska politika u EU za promicanje korištenja obnovljivih izvora potaknuli su razvoj bioplinskih postrojenja za proizvodnju energije. Proizvodnja energije iz bioplina obuhvaća ekološki manje štetan način dobivanja energije smanjenjem emisije CO_2 i emisije staklenika (Scarlat i sur, 2010.). Pritom anaerobna digestija pruža mogućnosti da se bioplin koristi za proizvodnju energije, kao što su električna energija, toplina i gorivo, uz dodatne ekonomske, ekološke i klimatske prednosti smanjivanjem emisija CH_4 (Moeller i sur., 2004; Thompson i sur., 2013.). Digestori su uglavnom spojeni na plinske motore za proizvodnju topline i električne energije s električnim kapacitetom od nekoliko desetaka kWe do nekoliko MWe. Proizvedena toplina može se koristiti za zadovoljavanje lokalnih potreba za toplinom u zatvorenom krugu ili se može isporučiti vanjskim korisnicima. Bioplin se može nadograditi u biometan i ubrizgati u mrežu prirodnog plina ili ga koristiti u transportnim vozilima, uz odgovarajuće pročišćavanje za uklanjanje plinova u tragovima kao što su H_2S, voda i CO_2.

Sabirno logistički centri

Sabirno logistički centri obuhvaćaju sve postupke manipulacijom biomasom te predstavljaju jedan od osnovnih pristupa za brzi prelazak s fosilnih goriva na obnovljive izvore energije na održiv način. Podrazumijevaju regionalna ili lokalna mjesta za gorivo iz biomase vrhunske kvalitete gdje se uz optimalnu potporu energenti stavljaju na tržište s garantiranom kakvoćom (Loibnegger i sur., 2010.). Uspostavom sabirno logističkog centra za biomasu prihvaća se izazov opskrbe energijom u budućnosti koji se odnosi na to da je energija, a time i gorivo, dostupno u svako vrijeme, da je ekološki prihvatljivo te da je osigurano od strane lokalnih proizvođača neovisnih o međunarodnim industrijama nafte i plina. Tako je i glavni argument sabirno logističkih centara korištenje lokalno dostupne biomase za proizvodnju energije, a koristi nastale u poslovanju distribuiraju se od vlasnika biomase do proizvođača energije, odnosno na cijeli lanac opskrbe. Prilikom osnivanja sabirno logističkih centara treba uzeti u obzir trošak logističkih operacija i lanaca opskrbe. Pritom, sve veći zahtjevi za sirovinama i lanaci opskrbe naglašavaju važnost za globalno upravljanje sustavom nabave biomase. Svaki lanac opskrbe sirovinama treba različite karakteristike biomase na temelju zahtjeva za energijom, sustavima pretvorbe energije, korištenjem proizvedene energije i vrsti logistike (Iakovou i sur., 2010.). Stoga su financijske prepreke koje su obuhvaćene u sabirno logističkim centrima kao što su skupljanje, transport, rukovanje i skladištenje, prerada biomase te konačna distribucija gotovih proizvoda kupcima uvelike utjecale na ekonomsku izvedivost korištenja nusproizvoda (Yazan i sur., 2016.; Carriquiry i sur., 2011.).

Postupci u sabirno logističkim centrima počinju sa dobavom koja uključuje skupljanje biomase s polja ili mjesta prikupljanja. Kako bi se smanjila volumninoznost biomasa se

može odmah usitniti. Pritom se koriste kombajni za žetvu, strojevi namijenjeni za prikupljanje slame i određene balirke za baliranje slame, samoutovarne prikolice ili samohodni teleskopski utovarivači za utovar te kamioni ili traktori s prikladnim prikolicama za transport (Wieser i Miljić, 2017.). Bale nakon baliranja mogu biti različitih izvedbi, male ili velike, valjkaste ili četvrtaste. Međutim, najefikasnije je koristiti prešanu biomasu u energetske svrhe blizu mjesta njenog prikupljanja (u radijusu od 10 do 15 km) zbog visoke cijene transporta na veće udaljenosti (Šišić i sur., 2013.). Transport poljoprivredne biomase se može obavljati traktorima s prikolicama, kamionima, vlakovima i brodovima, a ovisno o udaljenosti i obliku same biomase, bira se najprikladnije prijevozno sredstvo. Traktori s prikolicama koriste se za prijevoz biomase na manje udaljenosti, do 10 km, dok se kamioni upotrebljavaju za prijevoz na srednje i veće udaljenosti. Ukoliko biomasa nakon žetve ili berbe ima povečanu vlažnost mora se prije skladištenja osušiti. Sušenje biomase na tlu obavlja se strujanjem zraka kroz biomasu, okretanjem ili sušenjem na suncu. Sušenje prirodnom ventilacijom (cirkulacijom okolnog zraka) postiže se postupkom samozagrijavanja (Matin i sur., 2019.). Sušenjem biomase poboljšava se efikasnost sagorijevanja, povećava se proizvodnja pare, smanjuje se emisija u okolinu te se poboljšava rad uređaja za sagorijevanje. Postupak sušenja biomase nije jeftin te bi stoga trebao biti što jednostavniji jer značajno utječe na cijenu i kvalitetu biomase (Umer i sur., 2020.). Osim toga, sušenje smanjuje cijenu transporta, te je takva biomasa manje podložna mikrobiološkoj razgradnji. Kako se biomasa sakuplja jednom godišnje, a za korištenje je potrebna kroz cijelu godinu, poljoprivrednu biomasu je potrebno skladištiti. Skladištenje je neizostavan postupak za premošćivanje vremenskog razdoblja od nastanka biomase do njezinog korištenja (Matin i sur., 2019.). Skladištenje biomase u skladišnim objektima pruža povećanu zaštitu od utjecaja vremenskih prilika. Plan skladištenja za pojedinačne sirovine od velike je važnosti za ispravan i učinkovit rad u sabirno logističkom centru (Gaber i sur., 2014.).

U pravilu, sabirno logistički centar može djelovati kao pružatelj usluga za energetske konzultacije, diverzifikaciju poslovanja za lokalne poljoprivrednike, organizaciju i podršku s mogućnostima financiranja u vezi s obnovljivim izvorima energije i temama okoliša i klime (Epp, 2011.). Sveukupno, može odigrati odlučujuću ulogu u kontekstu ekoloških i energetskih pitanja, posebno ako u regiji još nema takve organizacije. Ovisno o opsegu svojih aktivnosti, sabirno logistički centar može biti infrastrukture. No, također može posjedovati ili upravljati logističkim sustavom, organizacijom prikupljanja biomase ili može imati vlastite proizvodne pogone koji dorađuju i modificiraju biomasu u različite vrste goriva (obično više kvalitete).

U pravilu, uspostava sabirno logističkih centara je projekt koji zahtjeva pomno planiranje prije samog ulaska u investiciju, te kao takav zahtjeva osnovna pitanja koja treba postaviti i na njih dati odgovore, a najvažnija je analiza tržišta (Keene i sur., 2015.).

Pitanja su vezana za količinu i potencijal biomase, kvalitetu biomase, tko je dobavljač biomase i koja je njegova lokacija, tko su klijenti, postoje li u blizini toplane na biomasu ili kogeneracijska postrojenja na biomasu, koji su konkukentski sektori i slično.

Odgovori na ova pitanja pomažu u razumijevanju ponude i potražnje budućeg novog sabirno logističkog centra. Da bi projekt uspostave sabirno logističkog centra bio uspješan, svi pozitivni i mogući negativni utjecaji bi se trebali uzeti u obzir i projekt bi trebao biti razvijen zajedno s lokalnim dionicima. Ideja se sastoji od izgradnje kolektivnog ruralnog kanala za plasiranje goriva iz drvne biomase i energetskih usluga na tržište država Europske unije. Centri za biomasu stavljaju na tržište sve vrste goriva iz biomase koje proizvođači dobavljaju. U prodajni asortiman mogu se uključiti i ostale vrste goriva iz biomase, npr. peleti posliježetvenih ostataka čije se peletiranje može izvesti izravno na polju ili u centru za biomasu. Slijedom svega navedenog glavni ciljevi sabirno logističkog centra za biomasu

su uspostavljanje regionalnih sabirno logističkih centara za biomasu u državama EU koji nude gorivo iz biomase, ostala goriva iz biomase i energetske usluge, marketing pod standardiziranim zaštitnim znakom (riječ/slika) koji kod klijenata treba izazvati asocijacije poput sigurnosti, zaštite, pouzdanosti, kvalitete, očuvanje sigurnosti nabave, jamčenje dosljednih standarda kvalitete, promoviranje usluga poput dostave goriva, stručno savjetovanje o svim vezanim temama, uključenost u projekte ugovorne prodajne topline (Loibnegger i sur., 2010.).

Zahtjevi za kvalitetom razlikuju se ovisno o tome da li je riječ o potražnji goriva visoke kvalitete ili o potražnji goriva koje može biti bilo koje kvalitete, a osiguranje kvalitete za cilj ima izgradnju sigurnosti i povjerenja u proizvođača krutih biogoriva te jamčenje sigurne kupnje krutog goriva koje je u skladu sa zahtjevima koje potrošač postavlja. Kvaliteta i kontrola kvalitete goriva u lancu nabave osigurava sljedivost i daje klijentima sigurnost da su u lancu nabave svi procesi pod kontrolom. Sustav osiguranja kvalitete treba biti jednostavan i osiguravati potrošačima potvrde o podrijetlu i sigurnoj kontroli faktora koji utječu na kvalitetu te na taj način doprinositi stvaranju povjerenja među krajnjim korisnicima (Gaber i sur., 2014.). Prednost uvođenja sustava osiguranja/kontrole kvalitete jest organizirana i isplativa trgovina krutim biogorivima koja također jamči kupnju biogoriva u skladu sa zahtjevima na tržištu. Lanac nabave za kruta biogoriva sastoji se od procesa nabave sirovine, obrade i distribucije.

Zaključak

Opskrba energijom ubuduće temeljit će se velikim dijelom na principu regionalne opskrbe. Sabirno logistički centri za biomasu pokazuju jedan od osnovnih pristupa za brzi prelazak s fosilnih goriva na obnovljive izvore energije na održiv način. Izvrstan su primjer pozitivnog poslovnog modela koji može doprinijeti boljem poslovanju i razvoju, dok istovremeno, s aspekta proizvodnje bioplina, ovaj način poslovanja osigurava temelje kružnog biogospodarstva i ruralnog razvoja u RH.

Literatura

Carriquiry M.A., Du X., Timilsina, G.R. (2011). Second generation biofuels: Economics and policies. Energy Policy. 39: 4222–4234.

COM/2021/557 final - Directive of the European Parliament and of the Council

Epp C. (2011). Biomass Trading Centre Applicability in the target regions. Regional Networks for the development of a Sustainable Market for Bioenergy in Europe.

Gaber M., Handlos M., Metschina Ch. (2014). Priručnik o biomasi. Sustavi osiguranja kvalitete i Mjere kontrole kvalitete. Poljoprivredna komora Štajerske. Graz.

Ghaderi H., Pishvaee M.S., Moini A. (2016). Biomass supply chain network design: an optimization-oriented review and analysis. Industrial crops and products. 94: 972-1000.

Iakovou E., Karagiannidis A., Vlachos D., Toka A., Malamakis A. (2010). Waste biomass-to-energy supply chain management: A critical synthesis. Waste Management. 30: 1860–1870.

Iličković, Z. (2014). Biogoriva. Univerzitet u Tuzli.Tuzla.

Krička T., Voća N., Tomić F., Janušić, V. (2007). Experience in production and utilization of renewable energy sources in EU and Croatia. *Conference Proceedings*. Sibiu, Rumunjska, Politechnica Faculty Temisoara, 203 – 210.

Krička T., Leto J., Bilandžija N., Jurišić V., Matin A., Voća N., Horvat I. (2017). Technology of growing, processing and storage of energy crop Sida hermaphrodita (L.) Rusby, Sveučilište u Zagrebu Agronomski fakultet.

Loibnegger T., Metschina C., Solar T. (2010). Regionale Biomassehöfe, 3 Schritte zu Einer Erfolgreichen Projectrealisierung. Landwirtschaftskammer Steiermark, Report.
Matin A., Krička T., Grubor M., Leto J., Bilandžija N., Voća N., Jurišić V. (2019). Iskoristivost posliježetvenih ostataka za proizvodnju energije, Sveučilište J. J. Strossmayera u Osijeku Fakultet agrobiotehnički znanosti Osijek.
McKendry P. (2002). Energy production from biomass (part 3): gasification technologies. Bioresource Technology. 83: 55 - 63.
Møller H.B., Sommer S.G., Ahring B.K. (2004). Methane productivity of manure, straw and solid fractions of manure. Biomass and bioenergy. 26 (5): 485-495.
Powlson D.S., Glendining M.J., Coleman K., Whitmore A.P. (2011). Implications for soil properties of removing cereal straw: results from long-term studies. Agronomy Journal. 103(1): 279-287.
Scarlat N., Martinov M., Dallemand J.F. (2010). Assessment of the Availability of Agricultural Crop Residues in the European Union: Potential and Limitations for Bioenergy Use. Anaerobic Digestion (AD) of Solid Waste Anaerobic Digestion. 30(10): 1889–97.
Schjønning P., Heckrath G., Christensen B.T. (2009). Threats to soil quality in Denmark. In: A review of existing knowledge in the context of the EU soil thematic strategy. Tjele DK: Faculty of Agricultural Sciences. Aarhus University.124 p. Report Plant Science No. 143.
Šišić I., Ćehajić A., Rekanović S. (2013). Istraživanje optimalnih rješenja valorizacije poljoprivredne biomase u energetske i druge svrhe. *9th International Scientific Conference on Production Engineering development and modernization of production,* Tehnički fakultet Univerziteta u Bihaću, Bihać, Bosna i Hercegovina.
Thompson E., Wang Q., Li M. (2013). Anaerobic digester systems (ADS) for multiple dairy farms: A GIS analysis for optimal site selection. Energy Policy. 61: 114-124.
Umar H.A., Sulaiman S.A., Ahmad R.K., Tamili S.N. (2020). Characterisation of oil palm trunk and frond as fuel for biomass thermochemical. In *IOP Conference Series: Materials Science and Engineering*. 863(1): 01201.
Yazan D.M., van Duren I., Mes M., Kersten S., Clancy J., Zijm H. (2016). Design of sustainable second-generation biomass supply chains. Biomass Bioenergy. 94: 173–186.
Wieser H., Milijić V. (2017). Study on Agro biomassAvailability in Serbia. GIZ DKTI

Collection and logistics centers for biomass in the process of biogas production

Abstract
Global access to energy is unsustainable and RES is beginning to be exploited. The greatest contribution is expected from biomass, since its products are not only renewable, but also sufficiently similar to fossil fuels so that they can be directly replaced. Postharvest residue disposal can increase farmers' income by providing raw materials that can be used for industrial and energy purposes. Biomass processing is included in the collection and logistics centers. Therefore, the objective of this article is to describe the collective logistics centers for biomass with a description of biogas production.

Key words: renewable energy sources, biomass, collection and logistics centers for biomass, biogas

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STRUČNI RAD

Potencijal biorazgradivog otpada kao sirovine za proizvodnju bioplina i digestata

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Sažetak
Kontinuiranim povećanjem broja stanovnika, kao i poboljšanjem standarda dolazi i do povećanja proizvodnje otpada. Stoga ne čudi da sve veće količine otpada uzrokuju zabrinutost zbog ekonomske i ekološke održivosti trenutnog načina gospodarenja komunalnim otpadom. Gospodarenje biorazgradivim komunalnim otpadom od posebnog je značenja zbog njegovog potencijala u proizvodnji komposta ili kao sirovine u bioplinskim postrojenjima za proizvodnju energije i digestata. Izgradnjom bioplinskih postrojenja osigurali bi se uvjeti za provedbu odvojenog prikupljanja biorazgradivog otpada, povećala bi se proizvodnja energije iz obnovljivih izvora i posljedično smanjila količina odloženog otpada.

Ključne riječi: komunalni otpad, biorazgradivi otpad, bioplin, količine

Uvod

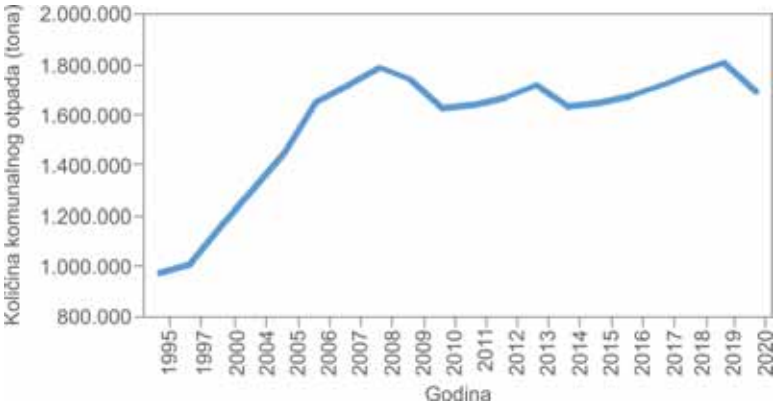
Zakon o gospodarenju otpadom definira otpad kao „svaku tvar ili predmet koje posjednik odbacuje, namjerava ili mora odbaciti“ (NN 84/21.). Otpad i njegova proizvodnja neizbježni su rezultat ljudskog postojanja, a opasnost od lošeg upravljanja otpadom ozbiljan je globalni problem. S povećavanjem broja stanovništva povećava se i količina nastalog otpada te istodobno dolazi do veće proizvodnje otpada po jedinici površine čime se smanjuje udio zemljišta dostupnog za njegovo odlaganje (Barles, 2014.). Gospodarenje otpadom označava bilo koju aktivnost nad otpadom, kao što su sakupljanje otpada, prijevoz, oporaba i zbrinjavanje otpada. U današnje vrijeme posebna pozornost usmjerena je na gospodarenje otpadom, a glavni ciljevi gospodarenja otpadom su zaštita ljudi i okoliša te očuvanje resursa (Brunner i Fellner, 2007.).

Današnja politika Europske unije (EU) odražava prestanak shvaćanja otpada kao problema i prelazak na razmišljanje i iskorištavanje otpada kao resursa. Navedeno se provodi poštivajući red prvenstva u gospodarenju otpadom. Red prvenstva predstavlja koncept koji izražava principe minimiziranja štete koju uzrokuje gospodarenje otpadom na okoliš gdje se prednost daje recikliranju i ponovnoj upotrebi otpada. Najmanje poželjna opcija u ovom konceptu je zbrinjavanje otpada, zatim slijede drugi postupci oporabe u obliku termičke obrade materijala u kombinaciji s energetskom oporabom, nakon čega slijedi recikliranje. Recikliranje se odnosi na transformacije rastavljanjem, sortiranjem i cirkulacijom materijalima, kako bi se omogućio njihov ponovni ulazak u industrijske i biološke proizvodne procese. Sljedeći korak u redu prvenstva je priprema za ponovnu uporabu, a najpoželjnija opcija je sprječavanje da određena tvar ili materijal postanu otpad. (Hultman i Corvellec, 2012.; HAOP, 2018.).

Proizvodnja mješanog komunalnog otpada

Prema zadnje raspoloživim podacima, u 2019. godini u EU je nastalo gotovo 225 milijuna tona komunalnog otpada. Podijeljeno po stanovnicima, to odgovara količini od 502 kilograma komunalnog otpada po stanovniku. Riječ je o malom porastu u usporedbi s 2018. godinom kada je nastalo 220 milijuna tona komunalnog otpada odnosno 495 kilograma po stanovniku (Eurostat, 2020., 2021a). Iako su količine u 2019. godini bile u blagom porastu u usporedbi s 2018. godinom, nisu nadmašene najveće količine komunalnog otpada od 518 kilograma po osobi koje su zabilježene u 2008. godini (Eurostat, 2020.). Na prostoru EU, količina komunalnog otpada razlikuje se od države do države krečući se od 280 kilograma po stanovniku u Rumunjskoj do 844 kilograma po stanovniku u Danskoj. Zemlje koje su generirale najviše otpada i to više od 600 kilograma po stanovniku bile su Luksemburg (791 kilograma), Malta (694 kilograma), Cipar (642 kilogramag) i Njemačka (609 kilograma). Na drugom kraju ljestvice, uz Rumunjsku su još samo tri države članice EU stvorile manje od 400 kilogama po osobi i to Poljska (336 kilograma), Estonija (369 kilograma) i Mađarska (387 kilograma) (Eurostat, 2021a.; Eurostat, 2021b.). Varijacije u količinama nastalog komunalnog otpada u državama članicama EU odražavaju razlike u obrascima potrošnje i ekonomskom bogatstvu, ali ovise i o načinu prikupljanja i upravljanja komunalnim otpadom (Namlis i Komilis, 2019.). U zemljama s nižim BDP-om stanovnici uglavnom troše manje robe i generiraju manje otpada nego što to čine razvijene zemlje (Kumar i sur, 2011.)

Prvi podaci o nastalom komunalnom otpadu u Republici Hrvatskoj (RH) odnose se na podatke iz 1995. godine, dok je kontinuirano praćenje nastalog komunalnog otpada započelo od 2004. godine nadalje, iako su se podaci do 2005. godine u najvećoj mjeri temeljili na procjenama. Gledajući količine nastalog komunalnog otpada u RH od 1995. godine (slika1.) primjećuje se porast ukupnih količina nastalog komunalnog otpada do 2008. godine. Od 2008. do 2010. godine dolazi do smanjenja količina nastalog komunalnog otpada što je povezano s gospodarskom krizom 2008. godine i njezinim posljedicama. Od 2010. do 2019. godine količine nastalog komunalnog otpada su u stalnom porastu. Nakon dugogodišnjeg porasta, u 2020. godini dolazi do pada ukupno nastalog komunalnog otpada u RH, što je većim dijelom posljedica pandemije uzrokovane korona virusom (Ministarstvo gospodarstva i održivog razvoja, 2021.).



Grafikon 1. Količine ukupno nastalog komunalnog otpada u RH od 1995. do 2020. godine Izvor: MINGOR, 2021.).

Količine biorazgradivog komunalnog otpada

Velik dio komunalnog otpada čini biorazgradivi komunalni otpad. Biorazgradivi otpad je svaki otpad koji je moguće anaerobno ili aerobno razgraditi poput papira i kartona, biootpada, tekstila, drva i dr. Biorazgradivi otpad je širi pojam dok je biootpad vrsta otpada koja spada u biorazgradivi otpad te predstavlja uži pojam koji označava „biološki razgradiv

otpad iz vrtova i parkova, hranu i kuhinjski otpad iz kućanstva, restorana, ugostiteljskih i maloprodajnih objekata i sličan otpad iz proizvodnje prehrambenih proizvoda“ (NN 84/21). Ovisno o lokalnim uvjetima, klimi, izvoru energije, stupnju industrijalizacije i navikama potrošača u pogledu konzumacije hrane i pića, u pravilu se u EU između 60 i 85 % komunalnog otpada sastoji se od biorazgradivog komunalnog otpada (Garcia i sur., 2005.; Vergara i Tchobanoglous, 2012.). Prema procijenjenom sastavu biorazgradivi dio čini oko 65 % udjela u miješanom komunalnom otpadu u RH (NN 84/21.). S obzirom da se velike količine biorazgradivog otpada i dalje odlažu na odlagalištima te imajući na umu da navedena praksa ima negativan utjecaj na okoliš, neophodno je žurno primijeniti alternativne metode gospodarenja ovom vrstom otpada (Garcia i sur., 2005.). Prema Zakonu o gospodarenju otpadom „najveća dopuštena masa biorazgradivog komunalnog otpada, čije odlaganje u kalendarskoj godini se može dopustiti svim dozvolama za gospodarenje otpadom u Republici Hrvatskoj, je 264.661 tona.“ Navedeni iznos čini 35 % mase biorazgradivog komunalnog otpada proizvedenog u 1997. godini. Također, na odlagališta otpada se do 2035. godine može odložiti najviše 10 % mase ukupno proizvedenog komunalnog otpada. Spomenutim mjerama želi se smanjiti količina odloženog biorazgradivog komunalnog otpada na odlagalištima te indirektno smanjiti proizvodnju stakleničkih plinova na odlagalištima koji nastaje uslijed razgradnje organske tvari i spriječiti potencijalno onečišćenje vode, tla i zraka.

Tablica 1. Nastali i odloženi biorazgradivi komunalni otpad (BKO) u RH, 1997.-2020. (MINGOR, 2021.)

Godina	Nastali BKO (tona)	Odloženi BKO (tona)
2020.	1.058.703	596.013
2019.	1.132.614	679.080
2018.	1.109.011	744.506
2017.	1.091.066	801.238
2016.	1.072.439	831.977
2015.	1.070.783	828.564
2010.	1.012.651	963.889
2005.	971.085	952.969
2000.	873.538	863.538
1997.	756.175	756.175

Potencijal biorazgradivog komunalnog otpada u proizvodnji bioplina i digestata

O važnosti pravilnog gospodarenja biootpadom kao i o drugim sastavnicama biorazgradivog otpada govori i činjenica da na području EU nastane oko 60 milijuna tona biootpada koji bi se mogao obraditi postupcima kompostiranja i anaerobne digestije (HAOP, 2018.). Koji način obrade biootpada je najpogodniji ovisi o vrsti i karakteristikama otpada koji ulazi u proces kompostiranja ili digestije. Tako u bioplinskim postrojenjima nije uputno koristiti materijal koji sadrži lignin (npr. drvo ili slama) jer lignin može značajno otežati proces anaerobne digestije. To znači da u bioplinska postrojenja ne bi trebalo upućivati vrtni (zeleni) otpad, već bi se isti trebao upućivati u kompostane. Izračuni govore da se iz svake tone otpada podvrgnutog anaerobnoj biološkoj obradi može dobiti od 100 do 200 m^3 bioplina, a svaka tona biootpada ujedno potencijalno predstavlja potencijal od 350 do 400 kilograma digestata (Commission of the European Communities, 2008.). Energetska vrijednost bioplina ovisi o količini metana koju sadrži i obrnuto je proporcionalna količini ugljikovog dioksida. Metan je zapaljiv plin i njegov sadržaj u bioplinu je normalno 55-70 %. Jedan kubični metar metana je ekvivalentan oko deset kilovat sati (9,97 kWh) energije. Dakle, ako bioplin sadrži 60 % metana, energetska vrijednost jednog kubičnog metra takvog

bioplina iznosi oko 6 kWh. U slučaju razgradnje biootpada ogrjevna vrijednost kubičnog metra bioplina je oko 0,6 litara lož ulja.

Kada bi se 60 milijuna tona biootpada koji nastane na području EU uistinu obradilo postupcima kompostiranja i anaerobne digestije, umjesto da se odlaže na odlagališta, procjenjuje se da bi došlo do uštede od 1 milijuna tona dušika i 20 milijuna tona organskog ugljika (European Parliament, 2017). Kao i kompost, i digestirani ostatak anaerobne razgradnje može se sigurno upotrebljavati kao gnojivo kod ekološke poljoprivrede (Voća i sur., 2004.). Korištenjem digestata kao materijala za poboljšanje kvalitete tla zatvorio bi se cijeli krug kružnog gospodastva, iako to na žalost nije uvijek tako. Porazni su podaci koji prikazuju da se 52 % ili 1,3 milijuna tona cjelokupnog digestata anaerobne digestije i dalje odlaže na odlagalište (European Parliament, 2017.). Procjenjuje se kako je u 2020. godini u RH 326.668 tona biootpada odloženo na odlagališta otpada, što znači da se godišnje u RH na odlagalištima odloži između 115 i 130 tisuća tona potencijalnog komposta (MINGOR, 2021.).

U RH je poljoprivreda jedna od najvažnijih gospodarskih grana koja se temelji na prirodnim i komparativnim prednostima - plodnom tlu i povoljnim klimatskim uvjetima. Problem je u tome što poljoprivrednici u RH nemaju običaj koristiti ovu vrstu organskog supstrata dobivenog iz biootpada. Stoga će biti potrebno provesti dodatne aktivnosti na doradi digestata kako bi se komercijalizirao i stvorila mogućnost njegovog daljnjeg korištenja. Nedostatak znanja ključni je problem za korištenje digestata iz biootpada u uzgoju ratarskih ili travnatih kultura. Svakako da bi na početku ciljna skupina korisnika digestata trebala ostati poljoprivredna proizvodnja za neprehrambeni lanac (energetski usjevi, ukrasno bilje, duhan) te travnjaci i parkovi. Treba uzeti u obzir i cijenu korištenja digestata, koja u konačnici mora biti prihvatljiva za poljoprivrednike, odnosno njegova cijena nesumnjivo mora biti niža od cijene organskih gnojiva dobivenih iz poljoprivrede.

Zaključak

U ovom radu prikazan je značajan potencijal održivog gospodarenja biorazgradivim otpadom s ciljem uspostavljanja promijena u svijesti ljudi i načinu gospodarenja s navedenom vrtom otpada s potencijalom za proizvodnju obnovljive energije i korisnog digestata. Ključna korist poljoprivrednih proizvođača ogleda se u uštedi mineralnih gnojiva kao skupe komponente poljoprivredne proizvodnje. Primjerice, na travnjacima, kao glavnoj ciljnoj skupini za primjenu digestata, njegova primjena ima biološke i ekonomske učinke jer može smanjiti iznos ulaganja u proizvodnju. To znači da je moguće ostvariti značajne uštede u troškovima nabave mineralnih gnojiva, čije su cijene nepredvidive i rastu svake godine te ovise i o cijeni prirodnog plina. Uštede na mineralnim gnojivima i ekonomske koristi od korištenja digestata trebale bi biti primarni motiv za njegovo uvođenje u poljoprivrednu proizvodnju u RH. Ujedno bi se riješio problem gospodarenja biorazgradivim otpadom što bi posljedično imalo pozitivne pomake u povećanju stopa odvojenog sakupljanja otpada, smanjenja stopa odloženog otpada i povećanje iskorištavanja prirodnih sirovina.

Napomena

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Literatura

Barles S. (2014). History of Waste Management and the Social and Cultural Representations of Waste. The Basic Environmental History. Environmental History, vol 4. Springer, Cham. https://doi.org/10.1007/978-3-319-09180-8_7.

Brunner H.P., Fellner J. (2007). Setting Priorities for Waste Management in Developing Countries. Waste Management and Research. 25: 234-240.

Commission of the European Communities (2008). Green paper on the management of bio-waste in the European Union, Brussels. https://op.europa.eu/en/publication-detail/-/publication/b2341160-6612-4d97-a147-93308c9c8f5e/language-en, 15.11.2021
European Parliament. (2017). Towards a circular economy - Waste management in the EU. Scientific Foresight Unit.
Eurostat (2020). 492 kg of municipal waste generated per person in the EU. https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20200318-1, 24.5.2021.
Eurostat (2021a). Half a tonne of municipal waste generated per person in the EU. https://ec.europa.eu/eurostat/en/web/products-eurostat-news/-/ddn-20210216-1, 24.5.2021.
Eurostat (2021b). Municipal waste statistics. https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Municipal_waste_statistics#Municipal_waste_generation, 24.5.2021.
Garcia A.J., Esteban M.B., Ma´rquez M.C., Ramos P. (2005). Biodegradable municipal solid waste: Characterization and potential use as animal feedstuffs. Waste Management. 25: 780–787.
Hrvatska agencija za okoliš i prirodu. (2018). Unaprjeđenje sustava za prikupljanje podataka o biootpadu i otpadu od hrane. Zagreb
Hultman J., Corvellec H. (2012). The European Waste Hierarchy: from the sociomateriality of waste to a politics of consumption. Environment and Planning. 44: 2413-2427.
Narodne novine 84 (2021): Zakon o gospodarenju otpadom https://narodne-novine.nn.hr/clanci/sluzbeni/2021_07_84_1554.html, 25.8.2021.
Kumar J.S., Venkata Subbaiah K., Prasada Rao.P.V.V. (2011). Prediction of Municipal Solid Waste with RBF NetWork- A Case Study of Eluru, A.P, India. International Journal of Innovation, Management and Technology. 2 (3): 238-243.
Ministarstvo gospodarstva i održivog razvoja (2021). Izvješće o komunalnom otpadu za 2020. godinu. Zagreb.
Namlis K-G., Komilis D. (2019). Influence of four socioeconomic indices and the impact of economic. Waste Management. 89: 190-200.
Vergara S.E., Tchobanoglous G. (2012). Municipal Solid Waste and the Environment: A Global Perspective. Annual Review of Environment and Resources. 37: 277–309.
Voća N., Krička T., Ćosić T., Rupić V., Jukić Ž., Kalambura S. (2004). Kakvoća digestiranog ostatka nakon anaerobne digestije pilećeg gnoja. Krmiva. 47(2): 65-72.

Potential of biodegradable waste as a raw material for biogas production

Abstract
With the continuous increase in the number of inhabitants, waste generation is also increasing. Likewise, with economic growth and the associated improvement in living standards, there has also been an increase in waste production. It is therefore not surprising that the increasing amount of waste generated each year raises legitimate concerns about the economic viability and environmental sustainability of the current way of managing waste. The study of biodegradable municipal waste is of particular interest as this type of waste has great potential for its relatively simple and cheap use in the form of raw material for compost suitable for further use in agriculture or as feedstock in biogas plants for electricity generation and thermal energy.

Key words: Municipal waste, biodegradable waste, biogas, quantities

IZVORNI ZNANSTVENI RAD

Fizikalno-kemijska svojstva organskog ostatka od jabuke

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Sažetak
Jabučna komina predstavlja organski ostatak ploda jabuke nakon prerade koji se klasificira kao biootpad te ga je kao takvog potrebno adekvatno zbrinuti. Osim već postojećih metoda zbrinjavanja jabučne komine, zbog njenog značajnog nutritivnog sastava, mogućnosti dodatnog iskorištenja, odnosno oporabe su velike. Cilj ovog rada bio je utvrditi fizikalno-kemijska svojstva jabučne komine te mogućnosti njene oporabe. Analizirani uzorci jabučnih komina imali su visok sadržaj ukupne suhe tvari (20 %), topljive suhe tvari (13,5 %) te nizak sadržaj ukupnih kiselina (0,65 %), a zbog čega se može smatrati vrijednim nusproizvodom za daljnju oporabu od prehrambene industrije do biotehnologije, a tek onda klasificirati kao otpad i takav zbrinjavati.

Ključne riječi: oporaba, biootpad, suha tvar, šećeri, ukupne kiseline

Uvod

Prema podacima FAOSTAT-a (2021.a) tijekom 2019. godine u svijetu je proizvedeno oko 87,236.221 t ploda jabuka. Velika količina ploda jabuke plasira se u svježem stanju, ali i prerađuje u sok. Posljedično povećanju proizvodnje procjenjuje se kako u svijetu godišnje kao nusproizvod prerade zaostane oko 4 milijuna t organskog ostatka iste (Lyu i sur., 2020.). Jabučna komina predstavlja izravni nusproizvod koji čini otprilike 20-30 % mase ploda jabuke i to u vidu kožice, peteljke, pulpe, sjemene lože i sjemenki. Spomenuti dijelovi bogati su važnim hranjivim tvarima poput ugljikohidrata, vlakana, vitamina i brojnih drugih antioksidansa (posebice polifenola), a zbog čega je i njezin potencijal daljnjeg korištenja i oporabe velik (Kalinowska i sur., 2020.). Zbog visokog sadržaja ugljikohidrata, organski ostatak jabuke podložan je fermentaciji te ukoliko nije adekvatno zbrinut predstavlja značajnu opasnost za zagađenje okoliša (Skinner i sur., 2018.). Također, hrpe jabučne komine koja se najčešće odlaže izvan ili u blizini pogona za preradu, uz kršenje norma kontrole zagađenja okoliša, može imati i značajne rizike po ljudsko zdravlje (Bhushan i sur., 2008.). Veliki problem predstavlja i činjenica da se veći dio takvog organskog ostatka još uvijek definira otpadom, u najboljem slučaju zbrinjava na odlagalištu, te u konačnici ne bude uopće iskorišten usprkos značajnim mogućnostima. Prema literaturnim podacima samo 14 % biootpada u RH prosljeđuje se na oporabu i to najčešće postupcima kompostiranja ili anaerobnom digestijom, dok ostatak završi na odlagalištima otpada (Puntarić i sur., 2020.). Također, valja naglasiti kako zbrinjavanje nusproizvoda iz poljoprivredne i prehrambene proizvodnje predstavlja i značajne financijske troškove što je u konačnici dovelo do potrebe za procjenom njihove dodatne vrijednosti i pronalaskom potencijalnih načina ponovnog korištenja od prehrambene, farmaceutske industrije, proizvodnje hrane za životinje do biotehnologije (O'Shea i sur., 2012.). Iz svega navedenog treba istaknuti kako se danas u svim koracima prehrambene i poljoprivredne proizvodnje, kao i zbrinjavaju otpada treba voditi načelima održivosti, prilikom čega se daje prednost onim praksama koje pokazuju manji otisak na okoliš, primijenjujući model cirkularne ekonomije čime se stvara dodana vrijednost uz korištenje manjih količina resursa i generiranje manjih količina otpada

(Fernandes i sur., 2019.). Stoga je i glavni cilj ovog rada bio utvrditi fizikalno-kemijska svojstva jabučne komine, te temeljem toga istaknuti i mogućnosti njene oporabe u svrhu dobivanja proizvoda dodane vrijednosti, a time i posljedično utjecati na smanjenje količine biootpada za zbrinjavanje na odlagalištu.

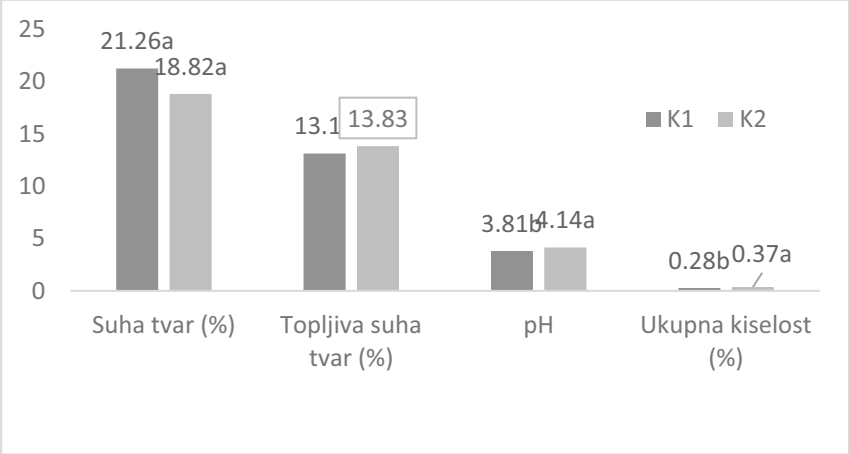
Materijal i metode

Analizirana su dva uzorka jabučne komine prikupljena s pogona za proizvodnju soka od jabuke s područja Zagrebačke županije. Prvi uzorak prikupljen je u pogonu Proizvodnja Jurišić d.o.o. (uzorak K1), a koji proizvodi jabučni sok postupkom prešanja od dviju sorti jabuka: 'Granny Smith' i 'Golden Delicious' s radmanom dobivenog soka od 70 %. Drugi uzorak (uzorak K2) prikupljen je u pogonu tvrtke Fragaria d.o.o. koji također jabučni sok proizvodi postupkom prešanja od mješavine slijedećih sorti jabuka: 'Gala', 'Cripps Pink', 'Idared' te u najmanjem udjelu 'Granny Smith' s randman dobivenog soka između 55 i 60 %. Preostali organski ostatak jabučne komine zbrinjavaju kao biootpad preko posrednika koji za to posjeduje važeću dozvolu. Odmah nakon prikupljanja s navedenih pogona uzorci jabučne komine transportirani su u laboratorij Zavoda za poljoprivrednu tehnologiju, skladištenje i transport Sveučilišta u Zagrebu Agronomskog fakulteta gdje su čuvani u hlađenom prostoru pri prosječnoj temperaturi zraka od 7 °C (prosječne vlage zraka od 78 %), kako bi se spriječila fermentacija, do namijenjenih kemijskih analiza. Za potrebe određivanja osnovnih fizikalno-kemijskih svojstava provedene su slijedeće analize prema standardnim laboratorijskim metodama: određivanje ukupne suhe tvari (%) sušenjem na 105 °C do konstantne mase (AOAC, 1995.), određivanje topljive suhe tvari (% Brix) izravnim očitanjem s digitalnog refraktometra (Refracto 30 PX, Mettler-Toledo, Švicarska), određivnaje sadržaja ukupnih kiselina (%) potenciometrijskom titracijom (AOAC, 1995.), određivanjem pH- vrijednosti pomoću digitalnog pH metra (SevenMulti, Mettler Toledo, Švicarska) te određivanje kromatskih parametara boje (L*, a*, b*, C i h°) pomoću kolorimetra (ColorTec PCM+, Kina) CIElab metodom. Dobiveni rezultati statistički su obrađeni u programskom paketu SAS, verzija 9.4 (SAS, 2010.). Korišten je Duncanov test signifikantnosti razlika (1 %).

Rezultati i rasprava

U Grafikonu 1 prikazani su rezultati osnovnih fizikalno-kemijskih svojstava analiziranih uzoraka jabučne komine. Prema provedenoj statističkoj analizi, uzorci komine značajno se ne razlikuju u sadržaju ukupne suhe tvari i topljive suhe tvari, dok su siginifikantne razlike utvrđene u sadržaju ukupnih kiselina i pH- vrijednosti. Naime, bez obzira na to što statističkom analizom nije utvrđena značajna razlika u sadržaju suhe tvari između uzoraka K1 i K2 primijetan je nešto veći sadržaj iste u uzorku jabučne komine K1, a što se može povezati s randmanom, odnosno iskoristivosti dobivanja soka prilikom prerade jabuke. Naime, prilikom proizvodnje soka na pogonu Proizvodnje Jurišić d.o.o. randman za dobivanje soka je nešto veći (oko 70 %), u usporedbi s radmanom koji se dobiva na pogonu Fragaria d.o.o. (55-60 %), a posljedično tome organski ostatak (uzorak K1) nakon prerade u sok imao je manji sadržaj vode, odnosno veći sadržaj ukupne suhe tvari. Uzorak komine s većim sadržajem suhe tvari (K1) može se smatrati i nutritivno kvalitetnijim s obzirom na činjenicu kako u suhoj tvari zaostaju i vrijedne fitokemikalije poput vitamina, minerala, polifenola i ostalih (Katalinić, 2006.). Također, važno je naglasiti kako će i jabučna komina s manjim sadržajem vode biti i povoljnija za daljnje postupke oporabe, a prvenstveno zato što je sirovina s manjim inicijalnim sadržajem vode manje podložna razvoju mikroorganizama, time i kvarenju, a olakšana je i daljnja manipulacija njome u smislu primjene pojedinih predtretmana koji se najčešće primijenjuju u obliku sušenja, a kako bi se zaustavili neželjeni procesi poput fermentacije, enzimske aktivnosti, ubrzanih metaboličkih

procesa i u konačnici degradacije nutritivne vrijednosti sirovine. Sirovina s većim udjelom suhe tvari zahtjevat će kraće vrijeme sušenja čime se značajno štedi na energiji i povećava efikasnost procesa. Također, uzorci se po sadržaju topljive suhe tvari značajno ne razlikuju te ona prosječno iznosi 13,48 %. Prema drugim literaturnim navodima, a koja su se primarno odnosila na određivanje topljive suhe tvari u pojedinim sortama jabuka zastupljenim u ovdje istraživanim kominama, može se utvrditi kako se dobivena prosječna vrijednost topljive suhe tvari u sklopu ovog istraživanja poklapa s onima iz drugih (Šic Žlabur i sur., 2013.; Dan i sur., 2015.; Michailidis i sur., 2021.). Uzroci analiziranih komina značajno se razlikuju u sadržaju ukupnih kiselina, a prilikom čega je veći sadržaj (0.37 %) utvrđen u uzorku K2. Dobiveni rezultati su očekivani s obzirom na to kako sorte zastupljene u komini K2 sadržavaju veći udio onih sorti izraženijeg sadržaja kiselina poput 'Cripps Pink' i ' Granny Smith' (Ghafir i sur., 2009.; Šic Žlabur i sur., 2013.; Eisenstecken i sur., 2015.). Također, uzorci komina razlikuju se i u pH vrijednosti, a prilikom čega za kominu koja sadrži veći udio sorte 'Granny Smith', uzorak K1, utvrđen je i značajno niži pH. Naime, prema drugim literaturnim navodima (Šic Žlabur i sur., 2013.; Mezey i Serralegri, 2017.) spomenuta sorta u prosjeku ima pH vrijednost od 3,2 te je stoga niži pH u uzorku K1 i očekivan. Oba uzorka komine imaju utvrđen pH u području kiselog te se stoga mogu i smatrati pogodnim za daljnje postupke oporabe, posebice zbog mogućnosti lakšeg održavanja stabilnosti takve sirovine.



K1- uzorak komine iz pogona Jurišić; K2- uzorak komine iz pogona Fragaria. Različita slova prikazuju značajne statističke razlike između srednjih vrijednosti pri p≤0,0001.

Grafikon 1. Fizikalno-kemijska svojstva jabučnih komina

Kromatski parametri (L*, a* b*, C i h°), odnosno parametri boje analiziranih uzoraka jabučne komine prikazani su u Tablici 1. Prema CIElab sustavu, L* vrijednost označava intenzitet svjetla ili tame, odnosno ukoliko vrijednost L* = 0 tada nema refleksije svjetlosti što upućuje na prisutnost crne boje, dok kod L* = 100 refleksija je najveća što sugerira na bijelu boju. Vrijednost a* označava intenzitet crvene ili zelene boje, te negativne vrijednosti (-a*) ukazuju na prisutnost zelene boje, a pozitivne vrijednosti (+a*) ukazuju na prisutnost crvene boje. Nadalje, vrijednost b* označava intenzitet žute ili plave boje; negativne vrijednosti (-b*) ukazuju na prisutnost plave boje, a pozitivne vrijednosti (+b*) ukazuju na prisutnost žute boje. Prema statističkoj analizi, uzorci komine međusobno se ne razlikuju prema L* i a* vrijednostima, dok se ostali kromatski parametri značajno razlikuju. Uzorak komine K1 ima veću b* vrijednost što ukazuje na veći udio žute boje u odnosu na uzorak K2, a što je i očekivano s obzirom na to kako je to obojenje karakteristično za sorte 'Granny Smith' i 'Golden Delicious' koje su značajno zastupljene u uzorku komine K1 (Drogoudi i sur., 2007.). Vrijednosti C i h° (zasićenost boje i nijansa boje) također se statistički značajno

razlikuju, što se može objasniti različitošću boja sorti obuhvaćenih u oba uzorka (sorta 'Grany Smith' je zelena i svjetlija, 'Golden Delicious' je žućkasta, 'Cripps Pink' i 'Idared' su crvenkaste sorte, a 'Gala' je svjetla, prošarana) (Henríquez i sur., 2010.).

Tablica 1. Kromatski parametri uzoraka jabučne komine

Uzorak	L*	a*	b*	C	h°
K1	40,73±3,19	10,77±1,06	$26,35^{a}$±0,78	$28,47^{a}$±0,99	$67,78^{a}$±1,78
K2	38,62±0,99	12,67±0,09	$21,98^{b}$±0,64	$25,37^{b}$±0,59	$60,03^{b}$±0,59
ANOVA	p≤0,3364	p≤0,0568	p≤0,0017	p≤0,0095	≤0,0020

L*- refleksija; a*- intenzitet crvene ili zelene boje; b*- intenzitet žute ili plave boje; C- zasićenost boje; h°- nijansa boje.

Zaključak

Temeljem dobivenih rezultata, odnosno visokih utvrđenih vrijednosti fizikalno-kemijskih svojstava i to suhe tvari, topljive suhe tvari i ukupnih kiselina, oba uzorka jabučne komine mogu se smatrati važnom sirovinom, a koja pokazuje veliki potencijal nusproizvoda za daljnju oporabu u različitim industrijama. Zbog visokog sadržaja suhe tvari uzorci jabučne komine mogu se podvrgnuti predtretmanima poput sušenja i koristiti kao „brašno“, pulver, u prehrambenoj industriji, ali i kao dodatak prehrani u hrani za životinje. Također, zbog visokog sadržaja topljive suhe tvari, odnosno šećera, oba uzorka jabučne komine mogu se podvrgnuti fermentaciji u svrhu proizvodnje jakih alkoholna pića. Iz svega navedenog može se zaključiti kako su daljnja istraživanja organskih ostataka od jabuke nužna jer ona predstavlja vrijedan nusproizvod koji se prije svega može koristiti u brojne svrhe oporabe, a tek onda klasificirati kao otpad i takav zbrinjavati.

Literatura

AOAC (1995). Official Methods of Analysis. 16th Edition, Association of Official Analytical Chemists, Washington DC.

Bhushan S., Kalia K., Sharma M., Singh B., Ahuja P. S. (2008). Processing of Apple Pomace for Bioactive Molecules. Critical Reviews in Biotechnology. 28(4): 285-296.

Dan C., Şerban C., Sestras A. F., Militaru M., Morariu P., Sestras R. E. (2015). Consumer Perception Concerning Apple Fruit Quality, Depending on Cultivars and Hedonic Scale of Evaluation – a Case Study. Notulae Scientia Biologicae. 7(1): 140-149.

Drogoudi P. D., Michailidis Z., Pantelidis G. (2007). Peel and flesh antioxidant content and harvest quality characteristics of seven apple cultivars. Scientia Horticulturae. 155: 149-153.

Eisenstecken D., Panarese A., Robatscher P., Huck C. W., Zanella A., Oberhuber M. (2015). A Near Infrared Spectroscopy (NIRS) and Chemometric Approach to Improve Apple Fruit Quality Management: A Case Study on the Cultivars “Cripps Pink” and “Braeburn”. Molecules. 20(8): 13603-13619.

FAOSTAT (2021). Production quantities of Apples. http://www.fao.org/faostat/en/#data/QC. Pristupljeno 15. lipnja 2020.

Fernandes P.A.R., Ferreira S.S., Bastos R., Ferreira I., Cruz M.T., Pinto A., Coelho E., Passos C.P., Coimbra M.A., Cardoso S.M., Wessel D.F. (2019). Apple pomace extract as a sustainable food ingredient. Antioxidants. 8(6): 1-16.

Ghafir S. A. M., Gadalla S. O., Murajei B. N., El-Nady M. F. (2009). Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions. African Journal of Plant Science. 3(6): 133-138.

Henríquez C., Almonacid S., Chiffelle I., Valenzuela T., Araya M., Cabezas L., Simpson R., Speisky H. (2010). Determination of antioxidant capacity, total phenolic content and

mineral composition of different fruit tissue of five apple cultivars grown in Chile. Chilean Journal of Agricultural Research. 70(4): 523-536.
Kalinowska M., Gryko K., Wróblewska A. M., Jabłońska-Trypuć A., Karpowicz D. (2020). Phenolic content, chemical composition and anti-/pro-oxidant activity of Gold Milenium and Papierowka apple peel extracts. Scientific Reports. 10(14951): 1-15.
Katalinić V. (2006). Kemija mediteranskog voća i tehnologija prerade. Kemijski sastav. Skripta, 1. dio. 15-22. Kemijsko-tehnološki fakultet u Splitu. Sveučilište u Splitu.
Lyu F., Luiz S.F., Perdomo Azeredo D.R., Cruz A.G., Ajlouni S., Senaka Ranadheera C. (2020). Apple Pomace as a Functional and Healthy Ingredient in Food Products: A Review. Processes. 8(3): 1-15.
Mezey J., Serralegri D. (2017). Selected Qualitative and Quantitative Parameters Comparison of Apples from Bio- and Conventional Production. Acta Scientific Nutritional Health. 1(3): 23-29.
Michailidis M., Karagiannis E., Nasiopoulou E., Skodra C., Molassiotis A., Tanou G. (2021). Peach, Apple and Pear Fruit Quality: To Peel or Not to Peel? Horticulturae. 7(4): 85.
O'Shea N., Arendt E. K., Gallagher E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. Innovative Food Science and Emerging Technologies. 16: 1-10.
Puntarić E., Kušević – Vukšić M., Kufrin J., Marić T., Požgaj Đ. (2020). Izvješće o komunalnom otpadu za 2019. godinu. Ministarstvo gospodarstva i održivog razvoja, Zagreb. Raspoloživo: http://www.haop.hr/sites/default/files/uploads/inline-files/OTP_Izvje%C5%A1%C4%87e%20o%20komunalnom%20otpadu%20za%202019_5.pdf
Skinner R.C., Gigliotti J.C., Ku K., Tou J.C. (2018). A comprehensive analysis of the composition, health benefits, and safety of apple pomace. Nutrition Reviews. 76(12): 1-17.
Šic Žlabur J., Voća S., Dobričević N., Pliestić S., Galić A. i Novak B. (2013). Nutritional Composition of Different Varieties of Apple Purees Sweetened with Green and White Stevia Powder. Agriculturae Conspectus Scientificus. 78(1): 57-63.

Physico-chemical properties of apple organic residue

Abstract
Apple pomace is an organic residue from apple fruit after processing that is classified as biowaste and must be disposed of appropriately as such. In addition to existing disposal methods for apple pomace, there are many opportunities for additional use or recovery due to its significant nutritional composition. The objective of this study was to determine the physicochemical properties of apple pomace and the possibilities of its utilization. The apple pomace samples studied showed a high content of total dry matter (20%), soluble solids content (13.5%) and a low content of total acids (0.65%), which is why they can be considered a valuable by-product for further utilization from the food industry to biotechnology and only then to be classified as waste and disposed of.

Key words: recovery, bioowaste, dry matter, sugars, total acids

PREGLEDNI RAD

Skladištenje energetskih kultura za potrebe proizvodnje bioplina

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Sažetak
Proizvodnja temeljena na održivoj energiji obvezna je od stupanja na snagu Europskih direktiva kojima su zaključeni ciljevi smanjenja emisija štetnih plinova. Poljoprivredni potencijal korištenja biomase kao obnovljivog i održivog izvora energije postavljen je kao jedan od načina dobivanja energije. Radom će se dati pregled negativnog utjecaja korištenja dosadašnjih tehnologija proizvodnje bioplina iz jednogodišnjih poljoprivrednih kultura kao što je kukuruz. Održivost proizvodnje može se postići korištenjem višegodišnjih energetskih kultura. Cilj rada je dati pregled mogućnosti skladištenja energetskih kultura kako bi se ustanovilo mogu li dosadašnje tehnologije biti primjenjive na energetske kulture.

Ključne riječi: energetske kulture, skladištenje, bioplin, ruralni razvoj

Uvod

Porast zabrinutosti zbog utjecaja energije fosilnog podrijetla na okoliš, ubrzala je razvoj održive i zelene energije na globalnoj razini. Jedna od potencijalnih zamjena energije fosilnih goriva je biomasa iz poljoprivrede budući da predstavlja dugoročno obnovljiv i $C0_2$ neutralan izvor (Johnson i sur., 2007.). Moguća primjena biomase u proizvodnji energije je putem bioplinskih postrojenja (Špicnagel i sur., 2014.), a najčešće korišteni supstrat u proizvodnji bioplina, uz životinjski otpad, je kukuruz uskladišten u obliku silaže. Prema posljednjim EU smjernicama, takav način korištenja jednogodišnjih kultura nije održiv. Smatra se da prevelika zastupljenost jednogodišnjih kultura poput kukuruza ima negativan utjecaj na tlo te dolazi do povećanja emisija stakleničkih plinova. Uz navedene negativne posljedice korištenja kukuruzne silaže u proizvodnji bioplina, jedna od najvećih je izravna kompeticija s proizvodnjom hrane jer se kukuruz koristi u dobivanju raznih proizvoda u prehrambenoj industriji za potrebe ljudi, ali i doradi i preradi za potrebe hrane za životinje (Kiesel i Lewandowski, 2014.). Stoga je u proizvodnji bioplina potrebno ubrzano početi koristiti kulture koje neće imati navedene negativne utjecaje.

S namjerom smanjenja negativnog utjecaja korištenja jednogodišnjih konvencionalnih kultura u proizvodnji bioplina održivost bi se mogla postići primjenom višegodišnjih energetskih kultura. Višegodišnje energetske kulture poput *Miscanthus x giganteus*, *Panicum virgatum* i *Arundo donax L.* predstavljaju lignoceluloznu sirovinu visokog prinosa nadzemne biomase po jedinici površine (Bilandžija i sur., 2017.). Bioplinski potencijal kukuruzne silaže kreće se oko 6.000 m^3 ha^{-1}, dok višegodišnja energetska kultura *Miscanthus x giganteus* može dati i prinose bioplina do 6.150 m^3 ha^{-1}. No, da bi se ostvario takav potencijal potrebo je osigurati prinos biomase u rasponu od 19-26 t s. tv. ha^{-1} (Whittaker i sur., 2016.). *Panicum virgatum*, odnosno divlje proso također predstavlja potencijalnu višegodišnju energetsku kulturu za korištenje u procesu proizvodnje bioplina. Provedenim istraživanjima postignuti su prinosi i do 25 t s. tv. ha^{-1} (Collins i sur., 2008.). *Arundo donax L.* je invazivna višegodišnja energetska kultura koja je široko rasprostranjena u Hrvatskoj. Nadzemna stabljika dosegne visinu i do 9 m, a sklop biljke je vrlo gust zbog čega ova kultura može ostvariti prinos biomase i do 30 t s. tv. ha^{-1} (Riffaldi i sur., 2010.). Kako bi se ostvario

potpuni energetski potencijal ovih kultura nakon žetve potrebno ih je pravilno uskladištiti. Stoga, cilj rada je dati pregled mogućnosti skladištenja višegodišnjih energetskih kultura s obzirom na trenutne tehnologije skladištenja.

Skladištenje energetskih kultura

Tehnologija i način skladištenja prvenstveno će ovisiti o načinu žetve energetskih kultura. Za potrebe proizvodnje bioplina preporučuje se žetva u jesen jer se time omogućuje ostvarivanje većih prinosa biomase i bioplinskog potencijala (Lewandowski i Heinz, 2003.). Žetva višegodišnjih energetskih kultura za potrebe bioplina može se provesti korištenjem različitih tehnika košnje usjeva. Krmni kombajni mogu se koristiti za dobivanje silirane mase, odnosno silaže, dok se košnjom i prešanjem u bale dobiva sjenaža (Bilandžija i sur., 2017.).

Skladištenje podrazumijeva posljednju fazu koja prethodi njihovoj daljnjoj doradi i preradi energetskih kultura i proizvodnji bioplina, to jest predstavlja mjesto gdje se biomasa čuva u adekvatnim prostorima, na određeni period (Renko, 2011.; Krička i sur., 2021.). O samoj tehnologiji i načinu skladištenja ovisit će kvaliteta i kvantiteta sirovine što znači da je potrebno obratiti posebnu pozornost na procese skladištenja. Nepravilno skladištenje može utjecati na pojavu određenih gubitaka poput mase, vlage, suhe tvari, ali i bioplinskog potencijala (Emery, 2013.).

Postoji nekoliko mogućnosti skladištenja energetskih kultura s obzirom na njihovu formu i to u obliku bala u otvorenim ili zatvorenim tipovima skladišta, a u obliku silaže u horizontalnim i vertikalnim skladištima, te plastičnim crijevima (Mastilović i sur., 2011.; Assirelli i sur., 2018.; Krička i sur., 2021.).

Skladištenje energetskih kultura u obliku bala

Energetske kulture formirane u obliku bala predstavljaju najjednostavnije rješenje s aspekta njihova skladištenja. Omatanjem bala u plastične folije, postiže se smanjenje gubitaka u masi i sadržaju suhe tvari, ali i stvaranju povoljnih uvjeta za proces anaerobne fermentacije unutar bale čime se dobiva sjenaža. Sirovinom uskladištenom u obliku sjenaže ostvarit će se veći bioplinski potencijal u usporedbi s balama bez omotane folije iz razloga što procesom fermentacije dolazi do djelomične razgradnje biomase te smanjenja udjela teško razgradivih struktura poput lignina. Skladištenje bala u zatvorenom prostoru podrazumijeva sagrađene objekte za skladištenje, dok je skladištenje na otvorenom prostoru najrentabilniji i najčešće korišteni oblik skladištenja. No, potrebno je imati u vidu utjecaj vremenskih prilika, ali i odabir podloge skladišta. Nepovoljan način skladištenja na otvorenom može dovesti do negativnog utjecaja na sirovinu, što može rezultirati stvaranjem gubitaka u vidu mase i suhe tvari. Nastajanje gubitaka u konačnici će dovesti do smanjenog bioplinskog potencijala energetskih kultura. Iz tog razloga, za skladištenje na otvorenom prvenstveno je potrebno koristiti određene tipove podloge (palete, kamen) kako bi se izbjegao kontakt s tlom. Za prekrivanje bala dovoljno je koristiti cerade ili plastične folije kojima će se djelomično umanjiti navedeni gubitci (Emery, 2013.; Krička i sur., 2017.; Leto i sur. 2017.).

Skladištenje silaže energetskih kultura

Horizontalni silos najčešći je oblik silosa koji se koristi na trenutnim postrojenjima i gospodarstvima. Na velikim postrojenjima najčešće su smješteni u obliku baterija, odnosno nalaze se jedan kraj drugog, dok su na manjim gospodarstvima građeni kao samostalni objekti. Prostor za skladištenje može biti u obliku silo hrpe ili trenč silosa. Silo hrpa predstavlja oblik horizontalnog silosa bez građenih bočnih stranica, pri čemu se silirana sirovina istovaruje iz transportnih sredstava na jedno mjestu, pri čemu se stvara hrpa. Ipak, u proizvodnji silaže pretežno se koriste trenč silosi koji imaju izgrađene betonske bočne

stranice, čime se kapacitet silosa znatno povećava (Brkić i sur., 2000.). Tehnologija punjenja horizontalnih silosa provodi se traktorima i transportnim sredstvima (prikolicama), koji silažu energetskih kultura istovaruju u prostor horizontalnog silosa. Po završetku istovara, zaprimljenu sirovinu potrebno je sabijati traktorima koji ujedno jednoliko raspodjeljuju silažu duž silosa. Uloga sabijanja silaže može se promatrati u vidu smanjenja volumena sirovine, odnosno s ciljem povećanja kapaciteta silosa, ali i stvaranja anaerobnih uvjeta za povoljnu fermentaciju silaže (Grbeša, 2016.). Lisowski i sur. (2020.) zaključuju kako najveći utjecaj na zbijenost silaže ima sadržaj suhe tvari, veličina čestica silaže, broj prohoda, debljina sloja i pritisak. Stoga je, prilikom sabijanja, potrebno koristiti traktore sa što većim opterećenjem po jedinici površine, pri čemu silaža mora biti dobro usitnjena, a žetva obavljena kada je sadržaj suhe tvari nizak.

Iako znatno manje korišteni, vertikalni silosi predstavljaju jedan od mogućih načina skladištenja energetskih kultura u obliku silaže. Prednost gradnje takvih tipova silosa proizlazi iz njihovog oblika, silo ćelija, promjera 5-8 m i visine do 20 m, koji u prostoru ne zauzimaju puno jedinične površine na postrojenju. No, zbog kompleksnosti izgradnje, cijene, ali i tehnologije punjenja i pražnjenja ovog tipa silosa, mali broj gospodarstava odlučuje se na ovaj tip skladištenja. Punjenje silosa izvodi se putem pneumatskih transportera koji silažu transportiraju na vrh silosa, pri čemu silaža unutar silosa gravitacijskim putem pada na dno. Pražnjenje ćelije može se izvesti putem freza smještenih na dnu, čija je uloga zahvaćanje silaže i transportiranje do mjesta za uporabu (Savoie i sur., 2003.). Takav način izuzimanja smanjuje prodiranje i izravni kontakt zraka sa silažom što znači da će kvarenje biti manje izraženo u usporedbi s horizontalnim silosima. Zbijenost silaže unutar ovog tipa silosa postiže se postavljanjem sustava s rotirajućim betonskim ili metalnim valjcima nakon što je silosna ćelija napunjena. No, zbog toga dolazi do velike neujednačenosti zbijenosti silaže po vertikalnom presjeku ćelije što predstavlja problem pri procesu fermentacije (Adesogan i Newman, 2014.). Za oba načina skladištenja vrlo je bitno ostvarivanje povoljnih uvjeta za proces anaerobne fermentacije. Uspješan proces anaerobne fermentacije podrazumijeva dobivanje kvalitetne silirane sirovine s visokom energetskom vrijednosti, povoljnom za korištenje u bioplinskom postrojenju. Za sam proces fermentacije, najvažnije je postići stabilnost silaže smanjenjem pH vrijednosti sirovine. Smanjenje pH vrijednosti događa se shodno procesu fermentacije, odnosno uslijed djelovanja bakterija mliječne kiseline i ugljikohidrata topivih u vodi, u anaerobnim uvjetima, te proizvodnje organske kiseline. Prema Silva (2017.), čuvanje silaže u stabilnoj fazi gdje nema bioloških aktivnosti može trajati neograničeno, Pakarinen i sur. (2008.) navodi kako nije uočen gubitak bioplinskog potencijala energetskih trava nakon 11 mjeseci skladištenja.

Skladištenje u plastična crijeva

Silaža energetskih kultura u horizontalno postavljena plastična crijeva predstavlja način skladištenja koje nije vezano uz stalni gospodarski objekt i određenu površinu za skladištenje. Dužina crijeva može se odrediti proizvoljno, što znači da bi jedno napunjeno crijevo moglo odgovarati dnevnim potrebama bioplinskog postrojenja. Na taj način onemogućili bi se već spomenuti gubitci zbog utjecaja prodora zraka unutar silosa. Za punjenje plastičnih crijeva, silažu je potrebno transportirati do mjesta za punjenje, silo-preše, koja utiskuje silažu u plastična crijeva. Takav sustav skladištenja dovodi do smanjenja ulaganja u usporedbi s klasičnim horizontalnim silosima jer za potrebe izrade iste količine sirovine potrebno je uložiti 8 % manje energije što dovodi do smanjenja troškova za 7 %. Nedostatak ovog načina skladištenja može se gledati kroz velike potrebe za plastičnom folijom, koja služi za omatanje silaže u oblik crijeva, ali i problematičnom izuzimanju. Stoga, u posljednje vrijeme, provode se istraživanja na različitim tehnikama izuzimanja s

posebnim naglaskom na silažu energetskih kultura s ciljem promicanja korištenja skladištenja u formi crijeva (Zimmer, 2009.; Assirelli i sur., 2018.).

Zaključak

Klimatske promjene koje se događaju utječu na pojavu ekstremnih temperatura koje posljedično utječu na sušu, smanjenje prinosa te genetske promjene na pojedinim kulturama. Jedna od najznačajnijih kultura u svijetu koja se našla pod utjecajem klimatskih promjena je kukuruz koji se koristi za hranu i dobivanje energije. Vodeći se spomenutim, upotreba sirovina višegodišnjih energetskih kultura u obliku silaže ili sjenaže moguća je za dobivanje energije na bioplinskim postrojenjima. Pri tome je važno istaknuti kako nije potrebno razvijati nove tehnike i tehnologije skladištenja. Moguće je koristiti dosadašnje skladišne objekte, što znači da nisu potrebna nova ulaganja na već izgrađenim postrojenjima za proizvodnju bioplina. Svi navedeni oblici skladišta mogu se koristiti za potrebe skladištenja energetskih kultura, no potrebno je dodatno istražiti utjecaj skladištenja na bioplinski potencijal energetskih kultura.

Napomena

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Literatura

Adesogan A.T., Newman Y.T., (2014). Silage Harvesting, Storing, and Feeding. University of Florida.

Assirelli A., Santangelo E. (2018). An extractor for unloading the wet biomass stored in silo bag. Industrial Crops and Products. 123: 128-134.

Bilandžija N., Leto J., Fabijanić G., Sito S., Smiljanović I. (2017). Tehnike žetve poljoprivrednih energetskih kultura. Glasnik zaštite bilja. 40(4): 112-119.

Brkić D., Vujčić M., Šumanovac L., Jurišić, M. (2000). Strojevi i uređaji za spremanje silaže, Vinkovci. Poljoprivredni fakultet Osijek.

Collins H.P., Fransen S., Hang A., Boydston R.A., Kruger C. (2008). Biomass production and nutrient removal by switchgrass (Panicum virgatum) under irrigation. In: The American Society of Agronomy, the Crop Science Society of America, and the Soil Science Society of America *International annual meetings abstract*, Houston.

Emery I.R. (2013). Direct and indirect greenhouse emmissions from biomass strorage: Implications for life cycle assessment of biofuels. Purde University.

Grbeša D. (2016). Hranidbena svojstva kukuruza. Sveučilište u Zagrebu, Agronomski fakultet.

Johnson J.M.F., Franzluebbers A.J., Weyers S.L., Reicosky D.C. (2007). Agricultural opportunities to mitigate greenhouse gas emissions. Environmental Pollution. 150: 107–24.

Kiesel A., Lewandowski I. (2014). Miscanthus as biogas substrate. ETA-Florence Renewable Energies.

Krička T., Leto J., Bilandžija N., Grubor, M., Jurišić V., Matin A., Voća N., Dović D., Horvat I. (2017). Priručnik: Tehnologija uzgoja, dorade i skladištenja energetske kulture Sida hermaphrodita (L.) Rusby. Sveučilište u Zagrebu, Agronomski fakultet.

Krička T., Matin A., Grubor M. (2021). Utjecaj skladištenja na higroskopnost sječke Side hermaphrodite. Agricultural Technics. Agronomski fakultet, Sveučilište u Zagrebu.

Leto J., Bilandžija N., Voća N., Grgić Z., Jurišić V. (2017). Uzgoj i korištenje miskantusa (*Miscanthus* sp.). Sveučilište u Zagrebu, Agronomski fakultet.

Lewandowski I., Heinz A. (2003). Delayed harvest of Miscanthus - influences on biomass quantity and quality and environmental impacts of energy production. European Journal of Agronomy. 19(1): 45-63.

Lisowski A., Wójcik J., Klonowski J., Sypuła M., Chlebowski J., Kostyra K., Nowakowski T., Strużyk A., Świętochowski A., Dąbrowska M., Mieszkalski L., Piątek M. (2020). Compaction of chopped material in a mini silo. Biomass and Bioenergy. 139.

Mastilović J., Janić Hajnal E., Torbica A., Pojić M., Živančev D., Kevrešan Ž., Novaković A., Radusin T. (2011). Suvremeni pristup upravljanju skladištem za zrnaste kulture. Univerzitet u Novom Sadu, Institut za prehrambene tehnologije.

Pakarinen O., Lehtomaki A., Rissanen S., Rintala J. (2008). Storing energy crops for methane production: Effects of solids content and biological additive. Bioeresource Technology. 99: 7074-7082.

Renko S., (2011). Poslovna logistika. Skripta, Ekonomski fakultet, Sveučilište u Zagrebu

Riffaldi R., Saviozzi A., Cardelli R., Bulleri F., and Angelini L. (2010). Comparison of soil organic matter characteristics under the energy corp giant reed cropping sequence and natural grass. Communications in Soil Science and Plant Analysis. 41: 173-180.

Savoie P., Jofriet J.C. (2003). Silage storage. Silage Science and Technology, American Society of Agronomy, Madison. 1: 405-468.

Silva T.C., Silva D.L., Santos E.M., Oliveira J.S., Perazzo, A.F. (2017). Importance of the Fermentation to Produce High-Quality Silage. Department of Animal Science, Federal University of Goias, Brazil

Špicnagel A.M., Puškarić T., Raymaekers F., Driessche J.V., Radočaj M.LJ., Verspecht A. (2014). Potencijal bioplinskih postrojenja u hrvatskom poljoprivrednom sektoru.

Whittaker C., Hunt J., Misselbrook T., Shield I. (2016). How well does Miscanthus ensile for use in an anaerobic digestion plant?. Biomass and Bioenergy. 88: 24-34.

Zimmer R., Pichler S., Košutić S., Jelošek D. (2009). Uzgoj, košnja i uskladištenje silažnog kukuruza u Ag-Bag fleksibilno crijevo. *37. međunarodni simpozij iz područja mehanizacije poljoprivrede*, 195-200. Zagreb. Agronomski fakultet Zagreb.

Storage of energy crops for biogas production

Abstract

Production based on the sustainable energy is promoted and guided by the Europe legislative, with the ultimate goal of reducing GHG emissions. The agricultural potential of providing biomass as a renewable and sustainable energy source has been set as one of the ways to produce energy. This study provides an overview of the negative impact of the use of existing technologies for the production of biogas from annual crops such as corn. Sustainability of the production could be achieved by using perennial energy crops. The aim of this paper is to provide an overview of the possibilities of storing energy crops in order to determine whether the current storage technologies can be applied when storing energy crops.

Key words: energy crops, storage, biogas, rural development

IZVORNI ZNANSTVENI RAD

Razlike u lignoceluloznom sastavu energetskih kultura u dva roka žetve

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Sažetak
Lignocelulozni sastav energetskih kultura od velike je važnosti za određivanje energetskog potencijala biomase, kao i za koju vrstu biogoriva ju je bolje iskoristiti. Iz tog razloga cilj ovog rada bio je istražiti lignocelulozni sastav tri energetske kulture: miskantusa, divljeg prosa i virdžinijskog sljeza u dva roka žetve. Nakon provedenih analiza utvrđeno je da rok žetve značajno utječe na sadržaj vlakana u biomasi. Udio lignina se povećao odgodom žetve do proljeća, dok se ne može sa sigurnošću utvrditi utjecaj roka žetve na udio celuloze i hemiceluloze.

Ključne riječi: lignocelulozna biomasa, energetske kulture, rok žetve

Uvod

Posljednjih desetljeća okoliš je pretrpio negativne posljedice masovnog korištenja fosilne energije (Brusca i sur., 2018.). Kako bi se pokušao zaustaviti takav negativan trend, proizvodnja energije iz obnovljvih izvora predstavlja jedno od mogućih alternativnih rješenja. Potencijal biomase, kao obećavajuće sirovine u proizvodnji celuloznog etanola, godinama se istražuje zbog svojih prednosti ispred bioetanola dobivenog iz kultura namijenjenih za proizvodnju hrane (Cerazy-Waliszewska i sur., 2019.). Biomasa se može definirati kao organski dio proizvoda, otpada ili ostatka proizveden u poljoprivredi (Perea-Moreno i sur., 2019.). Lignocelulozni materijal se sastoji od tri polimera: lignina, celuloze i hemiceluloze, koji su međusobno povezani ovisno o vrsti biljnog materijala i ključan su čimbenik u određivanju optimalne energije biomase (Bajpai, 2016.). Ukoliko biljka sadrži više lignina, pogodnija je za izravno izgaranje odnosno proizvodnju električne i toplinske energije (Krička i sur., 2017.) dok povećan sadržaj celuloze i hemiceluloze pogoduje produkciji tekućih biogoriva (Jönsson i sur., 2013.). Lignin čini najmanji udio u biomasi i glavna je glavna komponenta stanične stijenke. Starenjem primarna stanična stijenka prelazi u sekundarnu što rezultira povećanim udjelom lignina čime biljka dobiva na čvrstoći (Stolarski i sur., 2013.). Celuloza i hemiceluloza sadrže više kisika u odnosu na lignin što rezultira nižom ogrjevnom vrijednosti te je zbog toga biomasa s većim udjelom lignina povoljnija za izravno izgaranje (Lewandowski i sur., 2003.). Odgodom roka žetve do proljeća, dolazi do prirodnog sušenja na polju čime se smanjuje sadržaj vlage biomase, a povećava udio suhe tvari što rezultira povećanjem udjela lignina. Najviše perspektive za proizvodnju energije pokazuju višegodišnji energetski usjevi koji svojim karakteristikama u vidu visokog prinosa, trajnosti i niskih zahtjeva odnosno mogućnost uzgoja na marginalnim tlima, odgovaraju zahtjevima za obnovljivost i rješavaju sukob između proizvodnje hrane i proizvodnje goriva (Bilandžija i sur., 2018.). Miskantus (*Miscanthus x giganteus*) je višegodišnja drvenasta rizomatska trava sa životnim vijekom 18-20 godina (Wang i sur., 2020.). Jako je otporan na visoke i niske temperature, suše i povećanu vlažnost. Virdžinijski sljez (*Sida hermaphrodita* L.) višegodišnja je vrsta iz porodice sljezova (Cumplido-Marin i sur., 2020.). U usporedbi s miskantusom daje nešto manji prinos, ali ima sličnu ogrjevnu vrijednost do 20 MJ kg^{-1} (Kurucz i sur., 2018.). Koristi se za proizvodnju biogoriva, a zbog visokog sadržaja celuloze važna je i u industriji papira i drvoprerađivačkoj industriji

(Borkowska i Styk, 2006.). Divlje proso (*Panicum virgatum* L.) višegodišnja je trava visine do 3 metra, s dobro razvijenim korijenom (Elbersen i sur., 2013.). Prilagodljiv je na različite agroekološke uvjete.

Cilj rada je karakterizacija lignoceluloznog sastava energetskih kultura: miskantusa, virdžinijskog sljeza i divljeg prosa u dva roka žetve (jesenski i proljetni).

Materijal i metode

U ovom istraživanju analiziran je lignocelulozni sastav biomase tri energetske kulture: miskantus *(Miscanthus x giganteus*), virdžinijski sljez (*Sida hermaphrodita* L.) i divlje proso (*Panicum virgatum* L.), uzgajane na pokusnim poljima Sveučilišta u Zagrebu Agronomskog fakulteta (Centar za travnjaštvo-Medvednica, Maksimir i Šašinovec). Miskantus je posađen početkom svibnja 2011. godine reznicama rizoma. Divlje proso posijano je u proljeće 2016. godine, dok je virdžinijski sljez posađen u svibnju 2017. godine, sadnjom presadnica. Uzorci požete biomase uzeti su u studenom 2019. godine i ožujku 2020. godine, prilikom jesenske odnosno proljetne žetve. Svi laboratorijski postupci provedeni su u tri ponavljanja, a podaci su iskazani preko srednje vrijednosti suhe tvari. Biomasa je sušena u sušioniku 48 h na 60 °C te potom usitnjena u laboratorijskom mlinu (IKA, Njemačka) nakon čega je standardnim metodama određen udio: celuloze, lignina i hemiceluloze (ISO 5351-1:1981). Na dobivenim rezultatima provedena je analiza varijance ANOVA, dok su razlike između srednjih vrijednosti testirane t-testom (5 %) (SAS Institute, 2018).

Rezultati i rasprava

Kako bi se utvrdilo utječe li rok žetve na lignocelulozni sastav energetskih kultura za proizvodnju bioetanola potrebno je utvrditi sadržaj vlakana u jesenskoj i proljenoj biomasi. U proljetnom roku žetve miskantus je imao 13,8 % veći sadržaj lignina u odnosu na jesenski rok žetve ($p \leq 0.05$) (tablica 1).

Tablica 1. Sadržaj lignina energetskih kultura

%	Lignin		
	Miskantus	Virdžinijski sljez	Divlje proso
Jesen	$12{,}89^{b} \pm 0{,}73$	$14{,}88^{b} \pm 0{,}74$	$10{,}57^{b} \pm 0{,}51$
Proljeće	$14{,}67^{a} \pm 1{,}17$	$17{,}78^{a} \pm 0{,}97$	$12{,}60^{a} \pm 0{,}28$

Prikazane su srednje vrijednosti ± standardna devijacija. Različita slova unutar kolone pridružena prosječnim vrijednostima označavaju statistički značajnu razliku između sorti ($p \leq 0{,}05$)

Dobiveni rezultati odgovaraju rezultatima koji su dobili Brosse i sur. (2012.) gdje je miskantus sadržavao oko 12 % lignina jesen, a u proljeće oko 14 %. Virdžinijski sljez je u proljeće sadržavao 19,5 % više lignina, a divlje proso 19,2 % više lignina nego u jesen ($p \leq 0.05$). Rezultati koji su dobili Bilandžija i sur., (2018.) na virdžinijskom sljezu nešto su nižih vrijednosti, odnosno lignin je u jesen iznosio 19,88 %, a u proljeće 25,45 %. Bez obzira na niže vrijednosti i razlike u rezultatima u sadržaju lignina, istraživanja pokazuju da dolazi do značajnog povećanja udjela lignina odgodom roka žetve. Prema Jablanoeski i sur. (2017.) sadržaj lignina povećava se zbog smanjenog sadržaja vode do kojeg dolazi uslijed prirodnog sušenja na polju. Isti rezultat dobiven je i u ovom istraživanju gdje se sadržaj lignina kod sve tri promatrane kulture povećao.

Autori Cerazy-Waliszewska i sur. (2019.) navode kako se u proljetnoj biomasi miskantusa sadržaj lignina, hemiceluloze, a posebno celuloze povećao. Tablica 2 prikazuje rezultate slične literaturnim navodima koji su dobivene u ovom istraživanju, ali samo kod miskantusa i divljeg prosa.

Tablica 2. Sadržaj celuloze i hemiceluloze energetskih kultura

%	Celuloza		
	Miskantus	Virdžinijski sljez	Divlje proso
Jesen	$46,65^{b} \pm 1,58$	$56,95^{a} \pm 3,42$	$42,23^{b} \pm 1,45$
Proljeće	$54,00^{a} \pm 1,13$	$55,89^{a} \pm 0,96$	$49,69^{a} \pm 0,86$
	Hemiceluloza		
Jesen	$25,06^{a} \pm 1,77$	$15,03^{b} \pm 1,35$	$33,10^{a} \pm 1,01$
Proljeće	$24,66^{a} \pm 1,3$	$20,34^{a} \pm 2,02$	$31,38^{b} \pm 0,8$

Prikazane su srednje vrijednosti ± standardna devijacija. Različita slova unutar kolone pridružena prosječnim vrijednostima označavaju statistički značajnu razliku između sorti ($p \leq 0,05$)

Naime, udio celuloze kod miskantusa u proljeće je bio 15,8% viši nego u jesen ($p \leq 0.05$). Odgodom roka žetve zabilježen je porast udjela celuloze divljeg prosa u proljeće u odnosu na jesen za 17,7 % ($p \leq 0.05$). Studija koju su radili Bergs i sur., (2020.) također prikazuje povećanje udjela celuloze miskantusa s 42 % u jesen na 54 % u proljeće. Liu i sur. (2013.) dobili su povećanje udjela celuloze divljeg prosa u studenom (oko 41 %) u odnosu na proljeće (oko 47 %). Zanetti i sur. (2019.) utvrdili su da se udio hemiceluloze miskantusa smanjio odgodom roka žetve uslijed povećanja sadržaja celuloze i taloženja lignina. Takav rezultat dobiven je i u ovom istraživanju, gdje se celuloza povećala u proljetnoj biomasi miskantusa. S druge strane, udio celuloze virdžinijskog sljeza nije se značajno mijenjao s rokom žetve, te je iznosio prosječno 56,4 2%, dok se udio hemiceluloze značajno povećao za 35,3 % u proljetnom roku ($p \leq 0.05$). Bilandžija i sur., (2018.) utvrdili su da nije bilo razlike u sadržaju hemiceluloze virdžinijskog sljeza između jesenskog i proljetnog roka žetve. Dobivene razlike u ovom istraživanju vezane uz smanjenje ili povećanje celuloze odnosno hemiceluloze pokazuju da osim roka žetve, klimatski uvjeti i vrsta tla također utječu na lignocelulozni sastav biomase (Haberzettl i sur., 2021.).
Allison i sur. (2012.) i Scordia i sur. (2014.) zaključuju da celuloza ima najveći udio u biomasi bez obzira na rok žetve, a do smanjenja sadržaja hemiceluloze moguće dolazi zbog smanjenog sadržaja pepela odgodom roka žetve do proljeća uslijed prirodnog sušenja biomase.

Zaključak

Lignocelulozni sastav miskantusa, virdžinijskog sljeza i divljeg prosa značajno se razlikuje obzirom na rok žetve. Odgodom roka žetve do proljeća udio lignina značajno se povećao kod svih kultura. Obzirom na lignocelulozni sastav, virdžinijski sljez i miskantus pokazuju potencijal u proizvodnji tekućih biogoriva, dok za izravno izgaranje veći udio lignina ima virdžinijski sljez. Prilikom odabira energetske kulture za poizvodnju bilo koje vrst biogoriva u obzir svakako treba uzeti i prinose po jedinici površine kod biomase.

Napomena

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Literatura

Allison G.G., Morris C., Lister S.J., Barraclough T., Yates N., Shield I., Donnison I.S. (2012). Effect of nitrogen fertiliser application on cell wall composition in switchgrass and reed canary grass. Biomass and Bioenergy. 40: 19-26.

Bajpai P. (2016). Pretreatment of Lignocellulosic Biomass for Biofuel Production. Chapter 2 Structure of Lignocellulosic Biomass SpringerBriefs in Molecular Science. 7-12.

Bergs M., Do X.T., Rumpf J., Kusch P., Monakhova Y., Konow C., Volkering G., Pudeef R., Schulze M. (2020). Comparing chemical composition and lignin structure of Miscanthus × giganteus and Miscanthus nagara harvested in autumn and spring and separated into stems and leaves. RSC Advances. 10: 10740.

Bilandžija N., Krička T., Matin A., Leto J., Grubor M. (2018). Effect of Harvest Season on the Fuel Properties of Sida hermaphrodita (L.) Rusby Biomass as Solid Biofuel. Energies. 11: 3398

Borkowska H., Styk B. (2006). Virginia fanpetals (Sida hermaphrodita Rusby) - Cultivation and Use. Monograph, WAR, Lublin, 69.

Brosse N., Dufour A., Meng X., Ragauskas A. (2012). Miscanthus: A fast-growing crop for biofuels and chemicals production, Biofuels Bioproducts and Biorefining. 6(5): 580-598.

Brusca S., Cosentino S.L., Famoso F., Lanzafame R., Mauro S., Messina M., Scandura P.F. (2018). Second generation bioethanol production from Arundo donax biomass: an optimization method. Energy Procedia. 148: 728–735.

Cerazy-Waliszewska J., Jeżowski S., Łysakowski P., Waliszewska B., Zborowska M., Sobańska K., Ślusarkiewicz-Jarzina A., Białas W., Pniewski T. (2019). Potential of bioethanol production from biomass of various Miscanthus genotypes cultivated in three-year plantations in west-central Poland. Industrial Crops and Products. 141: 111790.

Cumplido-Marin L., Graves A.R., Burgess P.J., Morhart C., Paris P., Jablonowski N.D., Facciotto G., Bury M., Martens R., Nahm M. (2020). Two Novel Energy Crops: *Sida hermaphrodita* L. Rusby and *Silphium perfoliatum* L.-State of Knowledge. Agronomy. 10(7): 928.

Egner H., Riehm H., and Domingo W. R. (1960). Investigations on the chemical soil analysis as a basis for assessing the soil nutrient status II. Chemical extraction methods for phosphorus and potassium determination. Kungliga Lantbrukshügskolans Annaler. 26: 199-215.

Elbersen W., Poppens R., Bakker R. (2013). Switchgrass (Panicum virgatum L.). A perennial biomass grass for efficient production of feedstock for the biobased economy. NL Agency Ministry of Economic Affairs, Agriculture and Innovation. Focus on energy and climate change. 8-28.

Haberzettl J., Hilgert P., Von Cossel M.A. (2021). Critical Review on Lignocellulosic Biomass Yield Modeling and the Bioenergy Potential from Marginal Land. Agronomy. 11: 2397.

Jablonowski N.D., Kollmann T., Nabel M., Damm T., Klose H., Müller M., Bläsing M., Seebold S., Krafft S., Kuperjans I. et al. (2017). Valorization of Sida (Sida hermaphrodita) biomass for multiple energy purposes. GCB Bioenergy: Bioproducts for a Sustainable Bioeconomy. 9: 202–214.

Jönsson L.J., Alriksson B., Nilvebrant N.O. (2013). Bioconversion of lignocellulose: inhibitors and detoxification. Biotechnology for Biofuels and Bioproducts. 6: 16.

Krička T., Matin A., Bilandžija N., Jurišić V., Antonović A., Voća N., Grubor M. (2017). Biomass valorisation of *Arundo donax* L., *Miscanthus* x *giganteus* and *Sida hermaphrodita* for biofuel production. International Agrophysics. 575-581.

Kurucz E., Fári M.G., Antal G., Gabnai Z., Popp J., Bai A. (2018). Opportunities for the production and economics of Virginia fanpetals (Sida hermaphrodita). Renewable and Sustainable Energy Reviews. 90: 824–834.

Lewandowski I., Clifton-Brown J.C., Andersson B., Basch G., Christian D.G., Jorgensen U., Jones M.B., Riche A.B., Schwarz K.U., Tayebi K. (2003) Enviroment and harvest time affect the combustion qualities of Miskantus genotypes. Agronomy Journal. 95: 1274–1280.

Liu X.-J.A., Fike J.H., Galbraith J.M., Fike W.B., Parrish D.J., Evanylo G.K., Strahm B.D. (2013). Effects of harvest frequency and biosolids application on switchgrass yield, feedstock quality, and theoretical ethanol yield. GCB Bioenergy: Bioproducts for a Sustainable Bioeconomy. 7(1): 112–121.

Perea-Moreno M.A., Samerón-Manzano E., Perea-Moreno A.J. (2019). Biomass as Renewable Energy: Worldwide Research Trends. Sustainability. 11: 863.

SAS Institute 2018. SAS/STAT Software: Changes and enhancements through Rel. 6.12. Sas Inst., Cary, NC, USA.

Scordia D., Testa G., Cosentino S.L. (2014). Perennial grasses as lignocellulosic feedstock for second-generation bioethanol production in Mediterranean environment. Italian Journal of Agronomy. 9(581): 84–92.

Smuga-Kogut M., Piskier T., Walendzik B., Szymanowska-Powałowska D. (2019). Assessment of wasteland derived biomass for bioethanol production. Electronic Journal of Biotechnology. 41: 1-8.

Stolarski M.J., Szczukowski S., Tworkowski J., Krzyz˙aniak M., Gulczyn´ ski P., Mleczek M. (2013). Comparison of quality and production cost of briquettes made from agricultural and forest origin biomass. Renewable Energy. 57.

Zanetti F., Scordia D., Calcagno S., Acciai M., Grasso A., Cosentino S.L., Monti A. (2019). Trade-off between harvest date and lignocellulosic crop choice for advanced biofuel production in the Mediterranean area. Industrial Crops and Products. 138: 111439.

Wang C., Kong Y., Hu R., Zhou G. (2020). Miscanthus: A fast growing crop for environmental remediation and biofuel production. Research review. GCB Bioenergy: Bioproducts for a Sustainable Bioeconomy. 1-12.

Differences in lignocellulosic composition of energy crops in two harvest season

Abstract

The lignocellulosic composition of energy crops is of great importance in determining the energy potential of biomass as well as which type of biofuel is more suitable. Therefore, the objective of this study was to investigate the lignocellulosic composition of three energy crops: Miscanthus, Switchgrass and Virginia mallow in two harvest periods. The analysis showed that harvest timing significantly affected the fiber content in the biomass. Lignin content increased with the delay of harvest until spring, while the influence of harvest period on cellulose and hemicellulose content cannot be determined with certainty.

Key words: lignocellulosic biomass, energy crops, harvest season

IZVORNI ZNANSTVENI RAD

Utjecaj brzine sjetve na raspodjelu sjemena kukuruza (*Zea mays* L.) po površini i dubini

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Sažetak
U radu je prikazan utjecaj pet različitih brzina sjetve na kvalitetu sjetve kukuruza sijačicom PSK-4. U cilju utvrđivanja rezultata rada sijačice PSK-4 napravljen je profilogram tla, uzorkovanje i prosijavanje tla, mjerenje biljnih ostataka u sjetvenom sloju. U istraživanju je detaljno opisana metoda sjetve. Nakon provedenih mjerenja rezultati su obrađeni statistički u Excel-u te grafički prikazani. U istraživanju je dokazano da brzina sjetve najviše utječe na dubinu sjetve, odnosno većom brzinom dolazi do pliće sjetve.

Ključne riječi: kukuruz, sjetva, brzina i dubina sjetve, OLT PSK-4 sijačica

Uvod

Kukuruz, Zea mays *L*. je jednogodišnja kulturna biljka, s vrlo velikim uzgojnim arealom. Kukuruz je trenutno treća svjetska kultura po zasijanim površinama, s oko 130 milijuna ha (nakon riže i pšenice), s prosječnim prinosom zrna od 3,7 t ha^{-1} (AGBASE; Čakija, 2016.; Gagro 1997.; Bari i sur., 2014.). Najveći proizvođači kukuruza su SAD (oko 35 milijuna ha), Kina (38 milijuna ha), Brazil (14 milijuna ha), Meksiko (7 milijuna ha) (FAOSTAT, 2016.; Frketić, 2018.). U Njemačkoj kukuruz je druga najveća kultura poslije pšenice (Statistisches Bundesamt, 2014.), dok je u Kini osnovni usjev te se koristi kao hrana za životinje, industrijske kemikalije i bioenergiju (Wei i sur., 2017.; Cui i sur., 2018.). Prema podatcima Državnog zavoda za statistiku, kukuruz je 2016. godine u RH zasijan na 252 072 ha gdje ostvaruje ukupni prinos od 2 154 470 t (www.dzs.hr). Većina proizvodnje, oko 90 %, ostvaruje se na području istočne Hrvatske; Slavonija, Baranja i zapadni Srijem gdje se ostvaruju i najviši prinosi (Martinčić i Kozumplik, 1996.). Ogroman svjetski gospodarski značaj kukuruza očituje se njegovom velikom iskoristivošću, jer se koristi kao hrana ili za industrijsku preradu, što mu daje i dodatni ekonomski značaj (Šimić, 2008.; Musila, 2010.; Jović, 2013.; Qian i sur. 2019.). Prinos zrna kukuruza uvjetovan je različitim agroekološkim uvjetima kao i agrotehničkim mjerama poput vlažnosti i temperature zraka, plodnosti tla, izabranim hibridom, kvalitetnom gnojidbom i odgovarajućim sklopom. Pravilnim agrotehničkim mjerama mogu se stvoriti povoljni uvjeti za kvalitetan rast i razvoj kukuruza (Kovačević i Rastija, 2009.). Sjetva je jedan od najvažnijih koraka u proizvodnji kukuruza. Kvalitetna sjetva osigurava povoljne uvjete za rast i razvoj biljaka (Kurkutović, 2014.; Savjetodavna služba, 2016.; Nemet, 2017.). Kako bi se postigao što viši i kvalitetniji prinos zrna bitno je u dobro pripremljeno tlo za sjetvu, uložiti sjeme na zadanu dubinu i zadani razmak u redu i između redova (Kramer, 2017.). Sjetvu kukuruza najbolje je obaviti u optimalnom agrotehničkom roku. U sjeverozapadnijim krajevima to je od polovice do kraja travnja, a za istočni dio Hrvatske od 10. travnja do 25. travnja. Sjetvu treba započeti kada su temperature sjetvenog sloja veće od 10 ^{0}C (Butorac, 1999.). Ranijom sjetvom osiguravamo ranije klijanje i nicanje, bolje korištenje zimske vlage, ranije metličanje, svilanje, cvatnju i oplodnju pa se izbjegavaju velike vrućine i suh zrak u najosjetljivijim fazama razvoja

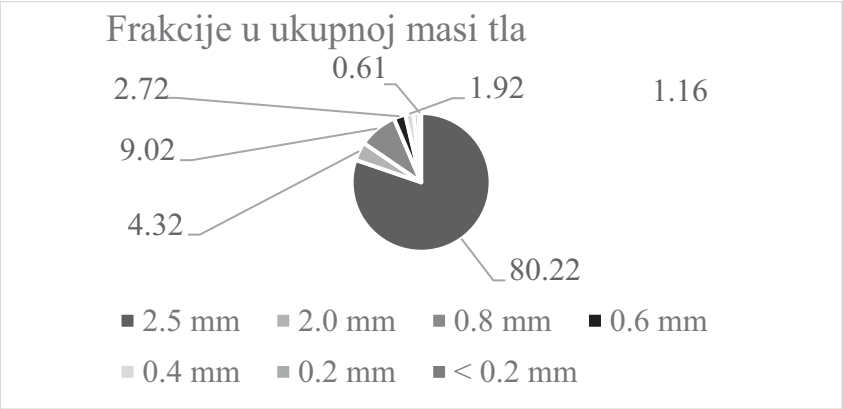
kukuruza. Kukuruz se sije sijačicama (mehaničkim ili pneumatskim) na međuredni razmak od 70 cm (Zimmer i sur., 1997.; Zimmer i sur., 2009.; Kveštek 2015.; Dušak, 2016.). Paulić, (2015.) navodi da se raniji hibridi siju na gušće sklopove, jer imaju nižu i tanju stabljiku, manju lisnu površinu te slabiji korijenov sustav, pa zauzimaju manji vegetacijski prostor. Kasniji hibridi se siju u manjim sklopovima jer imaju već biljnu masu i duži vegetacijski period. U većini proizvodnih područja u našim krajevima gustoća usjeva kreće se od 35 000-40 000 pa 70 000-80 000 biljaka ha^{-1}. Kako bi se postigla što kvalitetnija i preciznija sjetva uz nisku razinu oštećenja zrna dolazi do razvoja pneumatskih sijačica. Andričević (2017.) navodi da se promjenom brzine sjetve direktno utječe na raspored i dubinu sjemena kukuruza.

Materijal i metode

Na poljoprivrednoj parceli u vlasništvu Poljoprivrednog instituta Osijek obavljeno je istraživanje utjecaja brzine sjetve kukuruza na raspodjelu sjemena po površini i dubini. Poljoprivredna površina je prema izvodu iz Arkoda veličine 5,16 hektara. Sjetva kukuruza obavljena je agregatom Fendt Vario 415 i sijačicom OLT PSK-4. Prilikom istraživanja utjecaja brzina sjetve na razmak sjetve u redu sijačica je promatrana kroz pet brzina izvođenja 4, 6, 8, 10 i 12 km h^{-1} u tri ponavljanja. Kako bi se spriječila moguća pogreška prilikom izvođenja sjetve, postavljen je plan pokusa prema kojem se rukovatelj stroja treba pridržavati. Potreban je plan rada sa smjernicama kako ne bi došlo do pogrešaka pri izvođenju sjetve. Rukovatelj za svaku brzinu sjetve ima 20 m za usklađivanje agregata i brzine prema planu pokusa. Kako bi se olakšala uočljivost, sva područja su označena trasirkama. Nakon što je izvršena sjetva, u polja se postavljaju oznake sa legendom na čeličnoj žici. Oznake služe kako bi u daljnjem istraživanju i nakon nicanja kukuruza bilo lakše pronaći dio polja na kojem je obavljena sjetva pri određenoj brzini. U svaki red, na jednom označenom polju od 25 m gdje je sijačica određenom zadanom brzinom prošla te obavila sjetvu, postavlja se zastavica s podacima o brzini, redu te broju ponavljanja. Nakon što je obavljeno zadnje mjerenje sve zastavice se uklanjaju s proizvodne površine zbog skore kultivacije kukuruza. Na promatranoj površini prije obavljanja sjetve kukuruza napravljen je profilogram tla u tri ponavljanja nasumično odabranih mjesta. Profilogram tla je dobiven mjerenjem visinske vrijednosti čestica tla. Na površini gdje se obavljalo istraživanje korištenjem standardne metodike uzet je uzorak tla za utvrđivanje granulometrijskog sastava tla (Grafikon 1.) prema postupku Suhi postupak (Kvasnička i sur., 2007.). Postupak uzorkovanja obavljen je u tri ponavljanja nasumično odabranih mjesta. Prosijavanje je obavljeno u laboratoriju Fakulteta agrobiotehničkih znanosti Osijek. Na površini gdje se obavlja istraživanje također su prikupljeni uzorci biljnih i građevinskih ostataka (cigla, blok, itd.) na tri nasumično odabrana mjesta pojedine veličine površine 1 m^2. Najviše je pronađeno građevinskih ostataka odnosno 946,6 kg ha^{-1} jer je na mjestu izvođenja pokusa nedavno srušen građevinski objekt dok je biljnih ostataka 143,30 kg ha^{-1}. Nakon nicanja pristupilo se posljednjem segmentu istraživanja. Na mjestima gdje su postavljene čelične žice sa oznakama i legendom, u svakom redu se iskopava pokraj 5 biljaka te se centimetrom mjeri udaljenost zrna do površine tla. Osim mjerenja dubine sjetve, također je izmjeren međuredni razmak i razmak sjetve u redu.

Rezultati i rasprava

Na površini gdje se obavlja istraživanje nakon uzorkovanja tla i prosijavanja dobivene su frakcije, odnosno prikazana je raspodjela čestica tla po veličini, izražena u postotku mase. (Grafikon 1.).



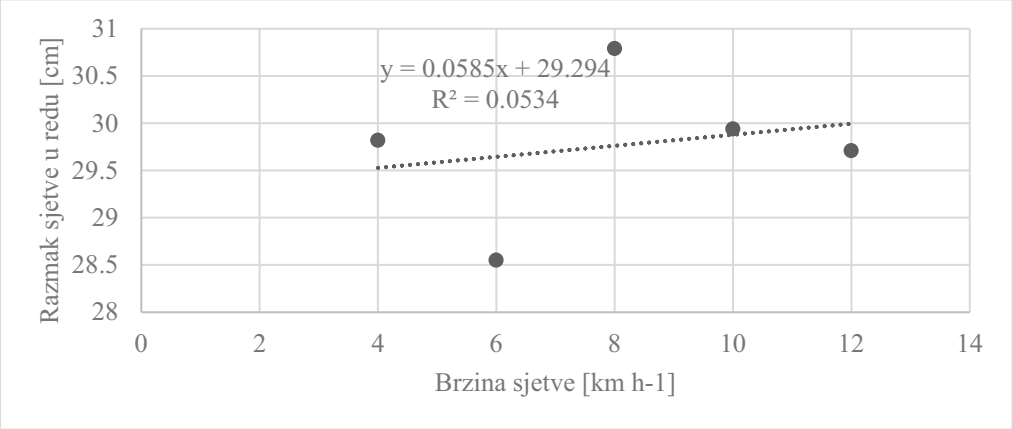
Grafikon 1. Prikaz frakcija u ukupnoj masi tla izraženo u %

Razmak sjetve u redu, međuredni razmak i dubina sjetve su uprosječeni (Tablica 1.).

Tablica 1. Prikaz vrijednosti prosjeka razmaka sjetve, međurednog razmaka i dubine sjetve

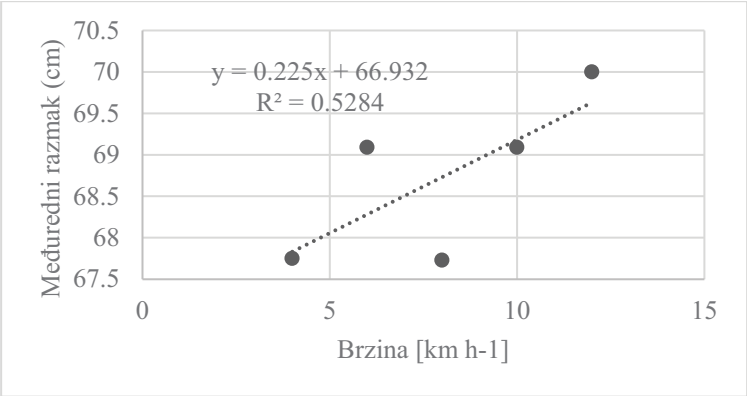
Brzina (km h^{-1})	Prosjek razmaka sjetve u redu (cm)	Prosjek međurednog razmaka (cm)	Prosjek dubina sjetve (cm)
4	29,82	67,75	4,83
6	28,55	69,09	5,49
8	30,79	67,73	4,56
10	29,94	69,09	5,12
12	29,71	70,00	3,69

U programu Microsoft Excel (2013.) izračunat je koeficijent korelacije po Pearson-u, koji iznosi r = 0,23. Iz koeficijenta korelacije (r) po Roemer-Orphalovoj raspodjeli jačina korelacije je slaba. Reprezentativnost modela iznosi R^2 = 0,0534, veza je objašnjena linearnim regresijskom modelom y = 0,0585x + 29,294 (Grafikon 2.).



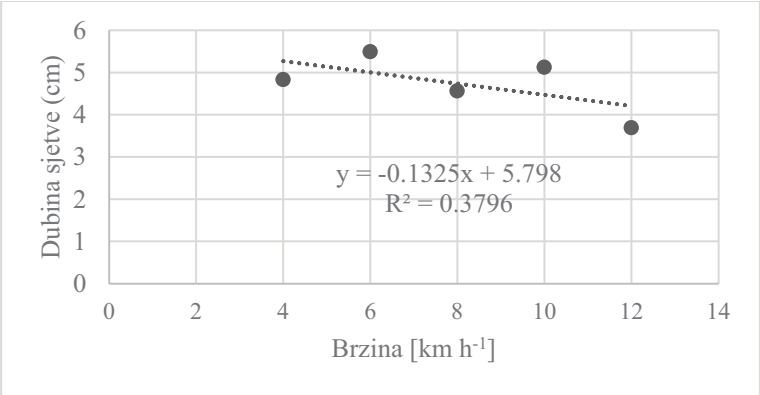
Grafikon 2. Prikaz korelacije brzine na razmak sjetve u redu

U istraživanju utjecaja brzina sjetve na međuredni razmak sjetve (Tablica 1.) utvrđeno je da koeficijent korelacije po Pearson-u je pozitivan te iznosi r = 0,72. Iz koeficijenta korelacije (r) po Roemer-Orphalovoj raspodjeli jačina korelacije je srednja. (Grafikon 3.). Reprezentativnost modela iznosi R^2 = 0,5284, dok je veza objašnjena linearnim regresijskom modelom y = 0,2255x + 66,932



Grafikon 3. Prikaz korelacije brzine na međuredni razmak

Prilikom istraživanja utjecaja brzina sjetve na dubinu sjetve (Tablica 1.) utvrđeno kako je koeficijent korelacije po Pearson-u negativan te iznosi r = -0,61. Iz koeficijenta korelacije (r) po Roemer-Orphalovoj raspodjeli jačina korelacije je srednje negativna. Reprezentativnost modela iznosi R^2 = 0,3796, veza je objašnjena linearnim regresijskom modelom y = -0,1325x + 5,798 (Grafikon 4.).



Grafikon 4. Prikaz korelacije brzine na dubinu sjetve

Zaključak

Na poljoprivrednoj parceli u vlasništvu Poljoprivrednog instituta Osijek obavljena je sjetva kukuruza koristeći PSK-4 sijačicu Osječke tvrtke OLT. Sjetva je obavljena sa pet različitih brzina u tri ponavljanja. Prije postavljanja pokusa na površini je obavljeno uzorkovanje tla i prosijavanje te je najviše dobiveno frakcija čestica tla od 2.5 mm što se poklapa sa istraživanjem Kremer, 2017. Prilikom istraživanja utjecaja brzina sjetve na razmak sjetve u redu koeficijent korelacije po Pearson-u iznosi r = 0,23. Po Roemer-Orphalovoj raspodjeli jačina korelacije je slaba, dok reprezentativnost modela iznosi R^2 = 0,0534. Prilikom istraživanja utjecaja brzina sjetve na međuredni razmak sjetve koeficijent korelacije po Pearson-u je pozitivan te iznosi r = 0,72. te je korelacija srednja, dok reprezentativnost modela iznosi R^2 = 0,5284. Kod istraživanja utjecaja brzina sjetve na dubinu sjetve koeficijent korelacije po Pearson-u je negativan te iznosi r = -0,61 te je jačina korelacije srednje negativna, dok reprezentativnost modela iznosi R^2 = 0,3796. U istraživanju je dokazano da brzina sjetve najviše utječe na dubinu sjetve, odnosno većom brzinom dolazi do pliće sjetve. Ovim istraživanjem nije se došlo do logičkog rezultata i očekivanja utjecaja radnih brzina i razmaka unutar reda. U nekim budućim istraživanjima trebalo bi se tu nelogičnost detaljno istražiti i pojasniti.

Literatura

Andričević F. (2017). Utjecaj brzine sjetve sijačicom „OLT PSK-4“ na raspodjelu sjemena po površini i dubini, Poljoprivredni fakultet u Osijeku.

Bari, M. A. A., Carena M. J. (2015). Can expired proprietary maize (Zea mays L.) industry lines be useful for short-season breeding programs? II. grain quality and nutritional traits. Euphytica. 202(1): 157-171.

Butorac A. (1999). Opća agronomija, „Školska knjiga“ Zagreb.

Cui J., Yan P., Wang X., Yang J., Li Z., Yang X., Chen Y. (2018). Integrated assessment of economic and environmental consequences of shifting cropping system from wheat-maize to monocropped maize in the North China Plain. Journal of Cleaner Production. 193: 524-532.

Čakija D. (2016). Proizvodnja silažnog kukuruza na „OPG-u Čakija“ za potrebe bio-plinskog postrojenja bio-plinara organica, Gregurovec. Visoko gospodarsko učilište u Križevcima.

Dušak M. (2016). Korištenje i održavanje traktora na OPG Bukal, Završni rad, Visoko gospodarsko učilište u Križevcima.

DZS https://www.dzs.hr (12.02.2019.)

FAOSTAT, FAO Statistic Division (2016) http://www.fao.org/faostat/en/#data/QC (20.03.2018.)

Frketić M. (2018). Kukuruz, Završni rad, Veleučilište u Karlovcu, Karlovac.

Gagro M. (1997). Ratarstvo obiteljskog gospodarstva - Žitarice i zrnate mahunarke. Zagreb. Hrvatsko agronomsko društvo.

Jović J. (2013): Utjecaj fosfatizacije i godine uzgoja na prinos zrna kukuruza, Diplomski rad, Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.

Kovačević V., Rastija M. (2013). Žitarice, Interna skripta, Poljoprivredni fakultet u Osijeku.

Kremer M. (2017). Utjecaj brzine na kvalitetu sjetve kukuruza sijačicom „Gaspardo magica 6MTR“, Diplomski rad, Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.

Kurkutović L. (2014). Razrada i grafički prikaz pneumatske sijačice kukuruza, Završni rad, Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.

Kovačević V., Rastija M. (2009). Osnove proizvodnje žitarica–interna skripta, Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.

Kvasnička P., Domitrović D. (2007). Mehanika tla, Sveučilište u Zagrebu, Rudarsko–geološko–naftni fakultet.

Martinčić J., Kozumplik V. (1996). Plant breeding, Poljoprivredni fakultet Osijek, Agronomski fakultet Zagreb.

Musila R. N., Makumbi D., Njoroge K. (2010). Combining ability of early-maturing quality protein maize inbred lines adapted to Eastern Africa. Field Crops Research. 119(2-3): 231-237.

Nemet F (2017). Proizvodnja kukuruza na „OPG-u Kate Nemet“ od 2012. do 2016. godine. Sveučilište J. J. Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku.

OBZ (2018): AGBASE – Kukuruz.
http://www.obz.hr/vanjski/CD_AGBASE2/PDF/Kukuruz.pdf (13.02.2018.)

Savjetodavna služba (2016). Priprema sijačica za okopavine za proljetnu sjetvu.
http://www.savjetodavna.hr/adminmax/File/savjeti/Priprema_sijacica_proljetna_sjetva.pdf (13.02.2018.).

Statistisches Bundesamt (2014) Land und Forstwirtschaft, Fischerei: Bodennutzung derBetriebe, Agrarstrukturerhebung, Fachserie 3, Reihe.

Šimić B. (2008). Kukuruz skripta pdf.

Statistički ljetopis Republike Hrvatske (2013): Državni zavod za statistiku Republike Hrvatske, Zagreb.
Zimmer R., Banaj Đ., Brkić D., Košutić S. (1997). Mehanizacija u ratarstvu, Udžbenik Poljoprivredni fakultet u Osijeku.
Zimmer R., Košutić S., Zimmer D. (2009). Poljoprivredna tehnika u ratarstvu, Udžbenik Poljoprivredni fakultet u Osijeku.
Qian Y., Ren Q., Zhang J., Chen L. (2019). Transcriptomic analysis of the maize (Zea mays L.) inbred line B73 response to heat stress at the seedling stage. Gene. 692: 68-78.
Wei T., Glomsrød S., Zhang T. (2017). Extreme weather, food security and the capacity to adapt–the case of crops in China, Food Security. 9(3): 523-535.

Influence of sowing speed on the distribution of corn seed (*Zea mays* L.) on surface and depth

Abstract
The paper presents the influence of five different sowing rates on the quality of corn sowing with PSK-4 seeder. In order to determine the results of the PSK-4 sowing work, soil profiling, soil sampling and sifting was carried out, measurements of plant residues in the sowing layer. The study describes the sowing method in detail. After the measured measurements the results were processed statistically in Excel and presented graphically. The research has shown that sowing speeds have the greatest impact on the depth of sowing, with faster seeding speed comes shallow sowing.

Key words: corn, sowing, sowing speed and depth, OLT PSK-4 sowing machine

PREGLEDNI RAD

Utjecaj konstrukcijskih razlika sijačica na kvalitetu sjetve kukuruza

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Sažetak
U radu je prezentirano istraživanje o utjecaju pet brzina sjetve na kvalitetu sjetve kukuruza s dvije *Horsch* sijačice koje funkcioniraju na principu podtlaka i nadtlaka. Nakon provedenih mjerenja rezultati su obrađeni statistički u Microsoft Excel-u te prikazani putem grafikona. U istraživanju je utvrđeno da brzina sjetve najviše utječe na razmak sjetve unutar reda (r=0,70) koji je i dalje bio u zadanim okvirima, dok dubina sjetve tek pri visokoj brzini neznatno odstupa od zadane (r=0,20).

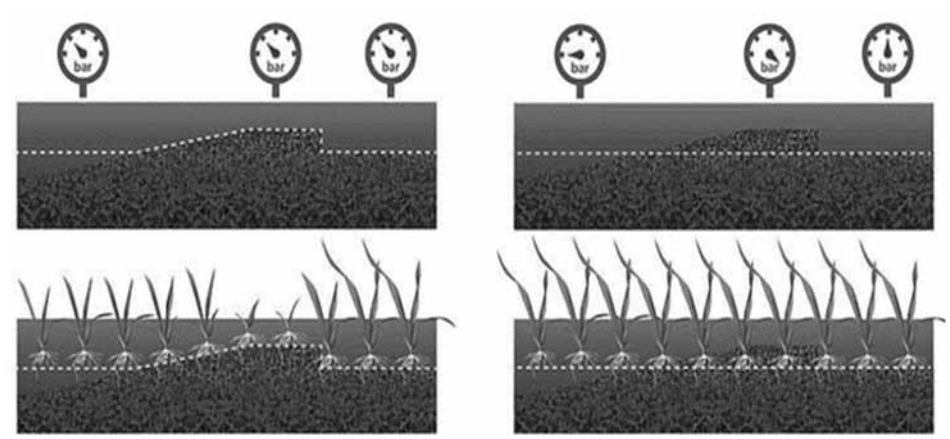
Ključne riječi: kukuruz, brzina sjetve, dubina sjetve, *Horsch* sijačice, podtlak, nadtlak

Uvod

Prema podatcima DZS (2021.), u Hrvatskoj je 2020. kukuruz uzgajan na 282.000 ha, prosječni prinos je bio 9 t ha^{-1}, a ukupna proizvodnja iznosila je 2.524.000 t. Prema Zrakić i sur. (2017.) površine pod kukuruzom u svijetu su na razini 182 milijuna ha uz prosječni prinos od 5,6 t/ha. Ukupna svjetska proizvodnja kukuruza iznosila je oko tisuću milijuna tona, a najveći svjetski proizvođači kukuruza su SAD, Kina i Brazil s ukupno dvije trećine svjetske proizvodnje. U prosjeku od 2013. godine do 2018. godine prema podacima FAOSTATA kukuruz se uzgajao na oko 191 milijun hektara, a ukupna proizvodnja je iznosila oko 1 milijarde i 90 milijuna tona kukuruza godišnje uz prosječni prinos od 5,69 tona po hektaru (FAOSTAT). Prinos zrna kukuruza uvjetovan je različitim agroekološkim uvjetima kao i agrotehničkim mjerama, kojima se mogu mogu omogućiti povoljni uvjeti za kvalitetan rast i razvoj kukuruza (Kovačević i Rastija, 2009.). U Republici Hrvatskoj u hranidbi stoke kukuruz se najčešće koristi kao silaža cijele biljke i po tome ima karakter voluminoznog krmiva (Zimmer i sur., 2009.). Sjetva je jedan od najvažnijih agrotehničkih zahvata u proizvodnji kukuruza. Za kvalitetan i visok prinos zrna iznimno je značajno uložiti sjeme u dobro pripremljeno tlo (Li i sur., 2017.). Sawan (2018.) navodi kako pravilni datumi sjetve imaju važnu ulogu u povećanju kvantitativnog i kvalitetnog prinosa i minimiziranju negativnih učinaka biotičkih i abiotičkih stresova. Brojni autori (Zimmer i sur., 1997.; Zimmer i sur., 2009.; Kveštek 2015.; Dušak, 2016.) navode kako se sjetva kukuruza obavlja mehaničkim i pneumatskim sijačicama na međuredni razmak od 70 cm, dok je u današnje vrijeme sve zastupljenija sjetva upotrebom pneumatskih sijačica. Andričević (2017.) u svojim istraživanjima navodi da se promjenom brzine sjetve izravno utječe na raspored i dubinu sjemena kukuruza. Precizne sijačice su strojevi od velike važnosti kod moderne tehnološke poljoprivrede, gdje je potrebna najveća preciznost uz najmanju potrošnju energije i troškova (Xing i sur., 2017.). Turan i sur. (2015.) navode kako prednost sadnje kukuruza s preciznom sijačicom uključuje uštedu sjemena, manje radnih sati, postiže ujednačeniji razmak u redu i dubinu sadnje.

Materijal i metode

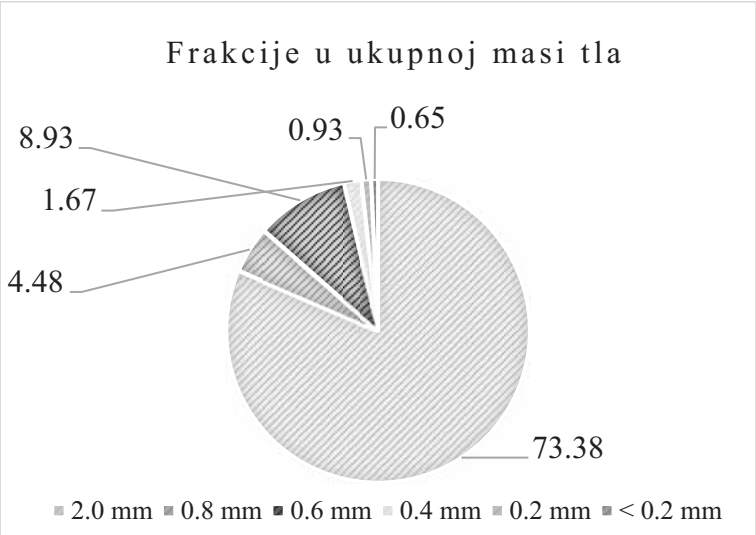
Na ispitnoj proizvodnoj površini u okolici Koške u suradnji s tvrtkom Jerković d.o.o. istraživane su pneumatske sijačice proizvođača *Horsch*, čija se tehnologija precizne sjetve posebno ističe ekstremno malim jedinstvenim sjetvenim mehanizmom i kontrolom kvalitete sjetve. Ispitivanje je obavljeno na dva tipa sijačica i to sijačica na principu podtlaka RV i sa nadtlakom RX. Tehničke karakteristike ove sijačice su: radna brzina 8-12 km h^{-1}, spremnik za mineralna sredstva obujma 2 800 litara, spremnici za sjeme obujma 70 litara na svakoj sjetvenoj sekciji, mogućnost varijacije broja redova (6, 8 i 12 redova), podešavanje razmaka između redova od 45-80 cm, pritisak po ulagaču sjemena od 125-300 kg hidraulički podesiv. Sijačica *Horsch* koristi patentirani sistem izuzimanja sjemena koji je jedinstven po kombinaciji izdvajanja, pogona i kontrole u jednom kućištu. Sjetveni mehanizam je temeljen na potpuno novom disku s prorezima koji se otvaraju prema vanjskom dijelu diska. Ovo, zajedno sa novim skidačem sjemena, omogućuje odlične rezultate u sjetvi s visokim rasponom učestalosti isijavanja sjemena (0-30 Hz). Učestalost isijavanja sjemena od 30 Hz postiže se brzinom sjetve od 12 km h^{-1}, pri normi sjetve od 90 000 sjemenki kukuruza po hektaru. Ova izuzetna preciznost zavisi o brzini rotacije diska, a posebno se kontrolira putem senzora. Program na *Horsch* terminalu je podešen za lako uočavanje praznih mjesta ili isijavanja dvije sjemenke na jedno mjesto, kao i varijacije za svaku sjetvenu sekciju posebno, što omogućuje prilagođavanje svim uvjetima sjetve kako bi se postigao maksimalan učinak i kvaliteta sjetve. Za potrebe istraživanja i uspoređivanja praćen je rad dviju sijačica i to na principu podtlaka i nadtlaka. Kako bi se osigurali isti uvjeti za provođenje istraživanja, ispitna proizvodna površina bila je istih pedo-mehaničkih značajki te je pripremljena prije istraživanja (kasno jesensko oranje, prohod sjetvospremačem u proljeće i nakon gnojidbe još jedan prohod sjetvospremačem). Sjetva je obavljena sjemenom proizvođača *Dekalb* DKC5830, odnosno hibridom izuzetno visokog potencijala rodnosti i kvalitetne silaže te toleranog na stresne uvjete uzgoja. Obje istraživane sijačice su podešene na gustoću sjetve od 75.000 sjemena ha^{-1} s međurednim razmakom od 70 cm. Prije početka sjetve obavljeno je mjerenje zbijenosti tla upotrebom konusnog 60° penetrometra proizvođača *Eijkelkamp* za mjerenje do dubine od 100 cm. Mjerenje zbijenosti tla obavljeno je na sedam različitih dubina (5, 10, 15, 20, 25, 30 i 35 cm) u tri ponavljanja za svaku pojedinu dubinu. Na ispitnoj površini, na nasumično odabranim mjestima, izvršeno je uzorkovanje tla za utvrđivanje granulometrijskog sastava tla (Grafikon 1.) prema suhom postupku (Kvasnička i sur., 2007.). Ova analiza je obavljena u laboratoriju Fakulteta agrobiotehničkih znanosti u Osijeku. Prilikom istraživanja utjecaja brzina sjetve na razmak unutar reda sjetva je izvršena u pet brzina: 6, 8, 10, 11 i 13 km h^{-1} u tri ponavljanja. Sijačice su agregirane s traktorom proizvođača *Claas* Arion 630 snage motora 110 kW. Za kvalitetno izvođenje precizne sjetve istraživane sijačice bile su navođene sustavom GPS sa *RTK* (eng. *Real Time Kinematic*) signalom proizvođača *Trimble* i postavljenim baznim stanicama tvrtke Jerković d.o.o. za korekciju signala i konačne točnosti od 2 cm. Osim toga, obje istraživane sijačice bile su priključene na *E-manager* te je korišten suvremeni software *AutoSpeed* za automatsku regulaciju brzine ovisno o parametrima sjetve. Za jednaku dubinu polaganje sjemena bez obzira na profil sjetvene površine korišten je *AutoForce* sustav za automatsko opterećenje sjetvenih sekcija kojim se postiže jednaka dubina polaganja sjemena u heterogenim tlima. Kontrolom pritiska cilindra na paralelogramu sijačice regulira se jednaka sila pritisaka za sve sjetvene sekcije. Sustavom AutoForce pritisak radnih tijela se automatski prilagođava od 1 470 do 3 432 N i time svaka sjetvena sekcija ostvaruje istu kvalitetu sjetve (Slika 1.)



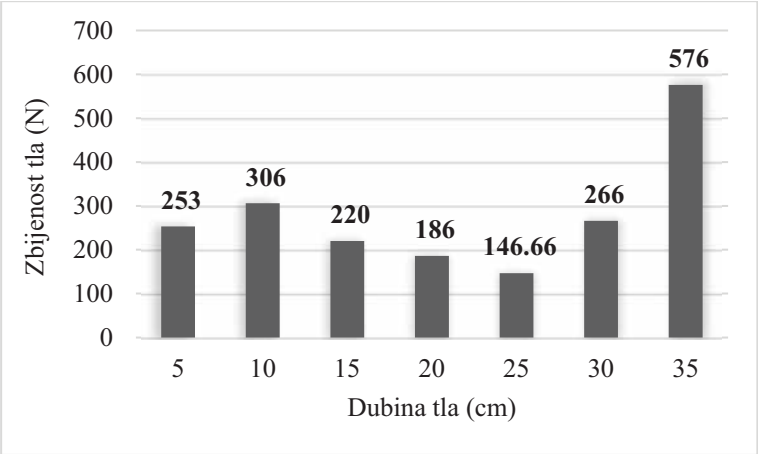
Slika 1. Primjena *AutoForce* sustava u sjetvi (desno).

Rezultati i rasprava

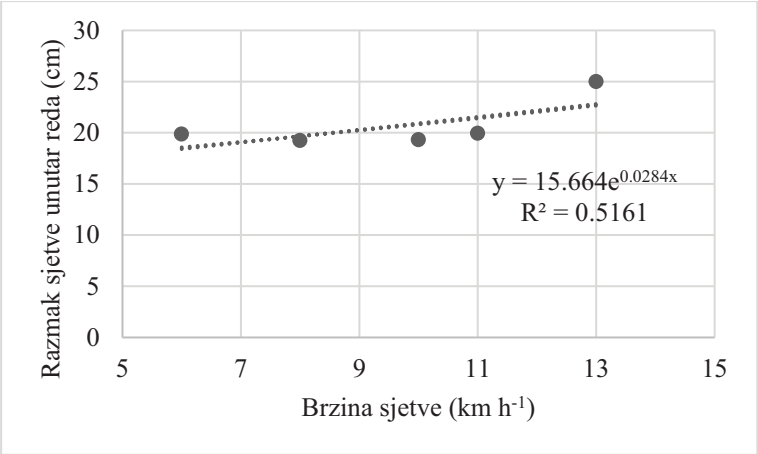
Na ispitnim površinama prije ovog istraživanja obavljeno je uzorkovanje tla, a raspodjela čestica tla izražena je u postotku mase (Grafikon 1). Podaci dobiveni mjerenjem zbijenosti tla upotrebom penetrometra na sedam različitih dubina (5, 10, 15, 20, 25, 30 i 35 cm.) u tri ponavljanja za pojedinu dubinu prikazani su u grafikonu 2. Pri istraživanju utjecaja brzine na kvalitetu sjetve korišten je Microsoft Excel (2013.) te je izračunat koeficijent korelacije po Pearson-u, koji za utjecaj brzine na razmak sjetve unutar reda iznosi r = 0,70*. Koeficijent korelacije (r) po Roemer-Orphalovoj raspodjeli upućuje kako je jačina korelacije jaka. Reprezentativnost modela iznosi R^2 = 0,4862, a oblik veze je objašnjen eksponencijalnim modelom $y = 15{,}664e^{0{,}0284x}$ (Grafikon 3.). Pri istraživanju utjecaja brzine na kvalitetu međurednog razmaka izračunat je koeficijent korelacije po Pearson-u (r = -0,26). Koeficijent korelacije (r) upućuje kako je korelacija slaba. Reprezentativnost modela iznosi R^2 = 0,0721, a veza je opisana linearnim modelom y = -0,0562x + 70,269 (Grafikon 4).



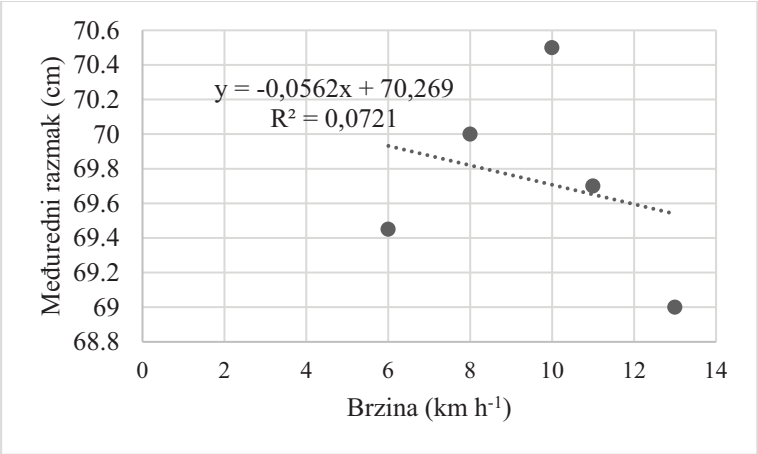
Grafikon 1. Prikaz frakcija u ukupnoj masi tla (%)



Grafikon 2. Prikaz zbijenost tla po različitim dubinama



Grafikon 3. Prikaz korelacije brzine i razmaka sjetve unutar reda

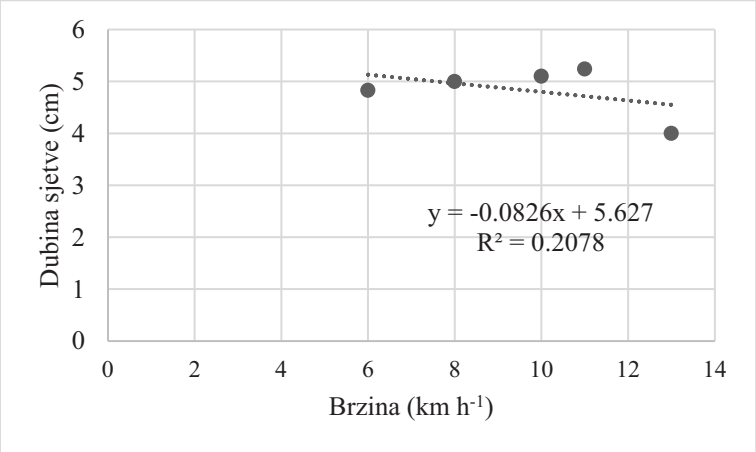


Grafikon 4. Prikaz korelacije brzine i međurednog razmaka

Razmak sjetve unutar reda, međuredni razmak i dubina sjetve uprosječeni su te prikazani u tablici 1. Pri istraživanju utjecaja brzine na kvalitetu polaganja sjemena izračunat je koeficijent korelacije po Pearson-u, koji iznosi r= -0,45. Promatrajući koeficijent korelacije (r) po Roemer-Orphalovoj raspodjeli vidi se kako je jačina korelacije srednja. Reprezentativnost modela iznosi R^2 = 0,2078, veza je objašnjena linearnim modelom y = -0,0826x + 5,627 (Grafikon 5).

Tablica 1. Prikaz vrijednosti prosjeka razmaka sjetve, međurednog razmaka i dubine sjetve

Brzina (km h^{-1})	Prosjek razmaka sjetve unutar reda (cm)	Prosjek međurednog razmaka (cm)	Prosjek dubina sjetve (cm)
6	19,86	69,45	4,83
8	19,24	70,00	5,00
10	19,32	70,50	5,10
11	19,95	69,70	5,24
13	25,00	69,90	4,00



Grafikon 5. Prikaz korelacije brzine na dubinu polaganja

Zaključak

Provedenim istraživanjem utjecaja brzine na površinsku i dubinsku kvalitetu raspodjele sjemena utvrđeno je kako su *Horsch* pneumatske sijačice iznimno precizne u radu čak i pri većim brzinama (≥13 km h^{-1}). Statistički promatrano jaka korelacija evidentna je između utjecaja brzine i razmaka sjetve u redu (r=0,70*), dok je ista korelacija slaba kod utjecaja brzine na međuredni razmak, što je shvatljivo obzirom na konstrukciju i pripremljenost ispitne proizvodne površine. Utjecaj na dubinu polaganja uočava se tek pri brzini rada sijačice iznad 13 km h^{-1} i to korelacijom srednje jakosti, što znači da je sjetva po površini i dubini izvedena kvalitetno. Sijačica s korištenjem nadtlaka pokazala je u izvjesnoj mjeri bolje rezultate u pogledu kvalitete sjetve, kao važnog preduvjeta za više i stabilnije prinose u odnosu na sijačicu sa principom rada na podtlak. Korištenjem suvremenih sustava navođenja pomoću GPS *RTK* signala sa baznim stanicama, software-ima za asistenciju u radu (*AutoSpeed*, *AutoForce*) omogućena je kvalitetna raspodjela sjemena bez izraženijih gubitaka.

Literatura

Andričević F. (2018). Utjecaj brzine sjetve sijačicom" PSK-4" na raspodjelu sjemena po površini i dubini, Završni rad, Sveučilište J. J. Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Osijek.

Dušak M. (2016). Korištenje i održavanje traktora na OPG Bukal, Završni rad, Visoko gospodarsko učilište u Križevcima.

FAOSTAT www.fao.org/faostat/en/ (pristupljeno: 27.05.2022.)

Kvasnička P., Domitrović D. (2007). Mehanika tla, Sveučilište u Zagrebu, Rudarsko–geološko–naftni fakultet.

Li Y., Bingxin Y., Yiming Y., Xiantao H., Quanwei L., Zhijie L., Dongxing Z. (2016). Global overview of research progress and development of precision maize planters. International Journal of Agricultural and Biological Engineering. 9(1): 9-26.

OBZ (2018): AGBASE – Kukuruz.
http://www.obz.hr/vanjski/CD_AGBASE2/PDF/Kukuruz.pdf (pristupljeno: 24.01.2022.)

Kovačević V., Rastija M. (2013). Žitarice, Interna skripta, Poljoprivredni fakultet u Osijeku.

Kveštek M. (2015). Proizvodnja silažnog kukuruza na obiteljskom poljoprivrednom gospodarstvu Burek u 2014, Završni rad, Visoko gospodarsko učilište u Križevcima, Križevci.

Sawan Z.M. (2018). Climatic variables: Evaporation, sunshine, relative humidity, soil and air temperature and its adverse effects on cotton production. Information Processing in Agriculture. 5 (1): 134-148.

Turan J., Višacki V., Sedlar A., Pantelić S., Findura P., Máchal P., Mareček J. (2015). Seeder with different seeding apparatus in maize sowing. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. 63(1): 137-141.

Zimmer R., Pichler S., Košutić S., Jelošek D. (2009). Uzgoj, košnja i uskladištenje silažnog kukuruza u Ag-Bag fleksibilno crijevo. *37. međunarodni simpozij iz područja mehanizacije poljoprivrede*, Silvio Košutić. 195-200. Zagreb: Agronomski fakultet Zagreb.

Zimmer R., Košutić S., Zimmer D. (2009). Poljoprivredna tehnika u ratarstvu. Poljoprivredni fakultet u Osijeku, 193-199.

Zimmer R., Banaj Đ., Brkić D., Košutić S. (1997). Mehanizacija u ratarstvu, Poljoprivredni fakultet u Osijeku, Osijek.

Zrakić M., Hadelan L., Prišenk J., Levak V., Grgić I. (2017). Tendencije proizvodnje kukuruza u svijetu, Hrvatskoj i Sloveniji. Glasnik zaštite bilja. 40(6): 78-85.

Xing H, Wang Z M, Luo X W, Cao X M, Liu C B, Zang Y (2017). General structure design and field experiment of pneumatic rice direct seeder, International Journal of Agricultural and Biological Engineering. 10(6): 31 –42.

Influence of precision seed drill construction on maize sowing quality

The paper presents a study on the influence of five sowing speeds on the quality of sowing corn with two Horsch seed drills that operate on the principle of vacuum and overpressure. After the measurements, the results were processed statistically in Microsoft Excel and presented via graphs. The research found that the sowing speed had the greatest effect on the sowing distance within the row (r =0.70), which was still within the given limits, while the sowing depth deviates slightly from the given sowing only at high speed (r =0.20).

Keywords: corn, sowing speed, sowing depth, Horsch drills, vacuum, overpressure

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Klima i poljoprivreda

10

Climate and Agriculture

ORIGINAL SCIENTIFIC PAPER

Soil fluxes of carbon dioxide in winter wheat (*Triticum aestivum* L.) agroecosystem

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Abstract
The increasing release of greenhouse gasses from soil to the atmosphere is an important contributor to global climate change. A research study was conducted to compare data of carbon dioxide emission from soil during winter wheat vegetation in 2012 and 2016. Also, the relationships between soil CO_2 emissions, agro-ecological factors, and N fertilization were determined. The soil CO_2 emissions were higher in 2012 compared to 2016 due to the higher temperature during summer period. The lower precipitation in 2012 did not reduce the soil respiration. Statistical analyses determined that the same N fertilization treatments significantly differed between studied years in I, II, IV, VI treatment. The observed differences among treatments in soil CO_2 emissions show that the addition of N fertilizers increased soil respiration.

Key words: CO_2 flux, soil temperature, soil moisture, vegetation

Introduction

Including all of the problems that the humanity is facing on a daily basis, climate change caused by the increase of greenhouse gas emissions, represents one of the major problems today (Mohammed et al., 2020). In many agricultural regions, including Croatia, climate trends have been fairly rapid (Galić et al., 2020). The three largest individual contributors to global warming are CO_2, CH_4, and N_2O. The agricultural sector, including the agricultural activities also represents a source of greenhouse gasses. Depending on how they are managed, soils have the capacity to generate or store greenhouse gases. Thus, many scientists have contributed in study of the factors which influence CO_2 production and emissions rates from the soil. Emissions are released because of respiration of soil microorganisms and plant roots, microbial decay and burning of plant residue (Raich and Tufekcioglu, 2000; Smith et al., 2008). Furthermore, soil properties including soil temperature, soil moisture, and soil organic matter also affect the CO_2 emissions (Smith et al., 2008; Bilandžija et al., 2014). Recently, the greenhouse gas emissions from different types of agricultural activities including irrigation, tillage, soil amendments and fertilization have been estimated in many studies (Bilandžija et al., 2016; Galić et al., 2019).
This study aimed to measure, analyze, and compare data of carbon dioxide emission from soil obtained in 2012 and 2016 during winter wheat vegetation. Also, the goal was to analyze the relationships between soil CO_2 emissions, agro-ecological factors, and fertilization.

Materials and methods

Site description with experimental design and cover crop

The study area with 10 different fertilization treatments is located in Popovača in the Western Pannonian sub-region of Croatia (N 45° 33' 21.42", E 16° 31' 44.62"). The soil type is classified as district Stagnosols (IUSS Working group, WRB 2015). Soil texture of arable

topsoil was defined as loam (Šestak et al., 2019). Soil physical and chemical properties are described in Mesić et al. (2011). Each trial treatment has a dimension of 30 × 130 m^2 including blank space. The distance between treatments was 2 m by each side, as well as between 4 replicates. 10 fertilization treatments were: I) control treatment; II) 0 kg N ha^{-1}+P+K; III) 100 kg N ha^{-1}+P+K; IV) 150 kg N ha^{-1}+P+K; V) 200 kg N ha^{-1}+P+K; VI) 250 kg N ha^{-1}+P+K; VII) 250 kg N ha^{-1}+P+K+ 20 t ha^{-1} of solid farmyard mixed manure; VIII) 250 kg N ha^{-1}+P+K+ 40 t ha^{-1} of solid farmyard mixed manure; IX) 300 kg N ha^{-1}+P+K and X) black fallow. The fertilization with phosphorus (P) and potassium (K) was uniform for all treatments (120 kg ha^{-1} for P and 180 kg ha^{-1} for K).
The cover crop at the experimental plot in investigated 2012 and 2016 was winter wheat (*Triticum aestivum* L. – Srpanjka).

Table 1. Dates of winter wheat sowing and harvesting

Triticum aestivum L.	**2012**	**2016**
Seeded	05.11.2011.	12.11.2015.
Harvested	11.07.2012.	09.07.2016.

Measurements of soil CO_2 concentration and agro-ecological factors
Field measurements of soil CO_2 concentrations were conducted once per month during the vegetation season except in some winter months when measurement was not possible considering unfavorable weather conditions (e.g. to wet, frozen soil, snow cover). Seven measurements of soil CO_2 concentrations were conducted during the investigated 2012, and ten measurements during the investigated 2016, in three repetitions on each of 10 treatments. Measurement of soil CO_2 concentrations were done by in situ closed static chamber method with portable infrared detector of carbon dioxide (Galić et al., 2020), where the soil carbon dioxide flux was calculated according to Bilandžija et al. (2014). Air temperature (°C), relative air humidity (%) and air pressure (hPa) were measured with the Testo 610, 2011 and Testo 511, 2011. Soil temperature, soil moisture and electroconductivity were measured with instrument IMKO HD2 – probe Trime, Pico64, 2011 at 10 cm depth near each chamber on each measurement date.
Data analysis
SAS software (version 9.1.3.) was used for statistical analyse of the data. Variability between years on each treatment was evaluated with analysis of variance (ANOVA) and tested with Fisher's least significant difference procedure. In all statistical tests the significance level was 5 %.

Results and discussion
Meteorological conditions
Climate of study area is continental, with an average annual air temperature of 10.6 °C and average annual precipitation amount of 865 mm, in the reference period 1961. – 1990. (Galić, 2019). According to the Lang´s rain factor, the reference period was characterized by a humid climate (Lf=82). In investigated years, average annual temperature and average annual precipitation amount was as follows: 2012 (12.4°C, 811 mm, Lf=65.3—semiarid climate), 2016 (12.1 °C, 1024 mm, Lf=84.5—semihumid climate) (Gračanin, 1950). Average air temperature of the growing season 2012 was 1.8 °C higher compared to reference period as well as 2016 (1.5 °C). Difference in precipitation amounts is also determined between studied 2012/2016 and 1961–1990 average. In the terms of the annual amount of total precipitation, investigated 2012 was very similar to the reference period (7 % less precipitation occurred in the 2012 compared with the reference period). In 2016 occurred 18

% more precipitation compared to the reference period. In 2012 and 2016 the high variability and extremes in the amount of precipitation between the months was noted.

Contribution of agro-ecological factors and vegetation to soil CO_2 flux

Average monthly agro-ecological factors and daily CO_2 flux in 2012 and 2016 were in the range, respectively: soil temperatures 14.2–39.0 °C and 17.6–37.7 °C; soil moisture 11.1 %–34.6 % and 19.7 %–34.1 %; average daily soil CO_2 flux 0.7–85.3 kg ha^{-1} day^{-1} and 1.9–54.3 kg ha^{-1} day^{-1} (Figure 1 (a) and (b)). Average monthly CO_2 flux ranged from 6.5–53.4 kg ha^{-1} day^{-1} for 2012 and 2.9–37.9 kg ha^{-1} day^{-1} for 2016. Numerous studies have investigated the influence of soil temperature and soil moisture on the GHG emissions (Galić et al., 2019; Ray et al., 2020). In Zhang et al. (2014) research, soil temperature (0-5 cm depth) and soil moisture (0-20 cm) combined together explained 55-70 % of soil CO_2 emissions, with root interference, and 62-78 % without root interference. In this research, in year 2012, there was no correlation between average CO_2 fluxes and soil temperatures at 10 cm depth (r=0.01), but average CO_2 fluxes were moderately positively correlated with soil moisture content at 10 cm depth (r=0.46) (Reis et al., 2014). In 2016 average CO_2 fluxes were strongly positively correlated with soil temperatures at 10 cm depth (r=0.56) and soil moisture content at 10 cm depth (r=0.51) (Galić et al., 2019). In Velasco et al. (2013) states that vegetation with corresponding belowground activity, through photosynthesis, removes CO_2 from the atmosphere during the day and releases a fraction of it through respiration. On Figure 1 (a) and (b) we can notice higher CO_2 flux in months where vegetation was present compared with the months without the plant cover. Treatments without plant cover noted the lowest CO_2 flux (Figure 2). The soil CO_2 emissions measured in this study were higher than in Bilandžija et al. (2016) on the same soil type and culture.

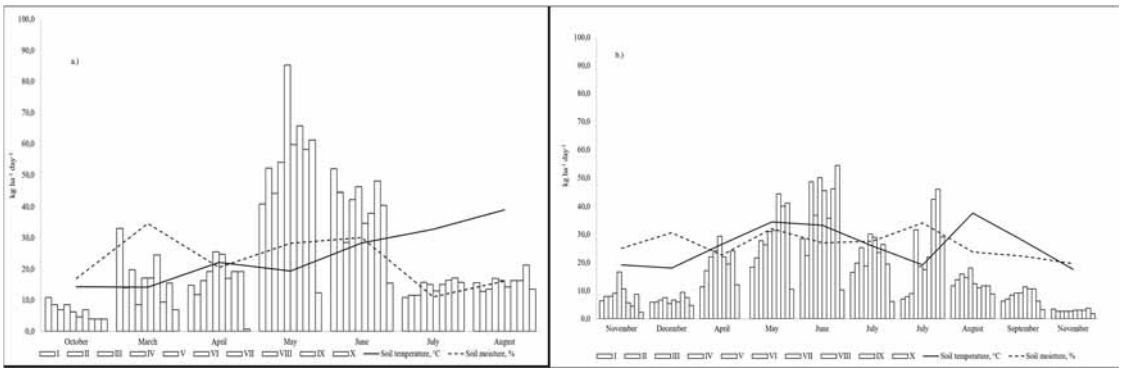

Figure 1 (a) and (b). Average daily values of soil respiration (C-CO_2 kg ha^{-1} day^{-1}) including soil temperature (°C) and soil moisture (%) in 2012 (a) and 2016 (b)

Fertilization and soil CO_2 flux relationship

According to Zhang et al. (2014), changes and releases of soil C and N to the atmosphere are particularly sensitive to the management practices such as fertilization. Figure 2 shows the average annual soil CO_2 flux considering fertilization treatments in 2012 and 2016. In 2012, CO_2 flux ranged from 9.8 C-CO_2 kg ha^{-1} day^{-1} on treatment X (black fallow) to 30.3 C-CO_2 kg ha^{-1} day^{-1} on the treatment V (200 kg N ha^{-1}+P+K). In 2016, CO_2 flux ranged from 7.7 C-CO_2 kg ha^{-1} day^{-1} on treatment X (black fallow) to 21.9 C-CO_2 kg ha^{-1} day^{-1} on the treatment VIII (250 kg N ha^{-1}+P+K+40 t ha^{-1} of solid farmyard mixed manure). This indicates that the black fallow reduces soil CO_2 flux, while fertilization with higher N quantities increases CO_2 flux. Statistical analyses determined that the same fertilization treatments significantly differed between studied years in I, II, IV, VI treatment. Soil CO_2 flux was higher on each treatment during the investigated 2012 compared to the 2016 growing season.

The effects of mineral and organic fertilization on CO_2 emission were studied by Mignon et al. (2011). Obtained results indicated a higher soil respiration rate in organically fertilized soil compared with mineral fertilized soil and control treatments where respiration was lower. After the disposals of organic wastes, increases in soil CO_2 fluxes in agricultural soils have been frequently observed by Ryals and Silver (2013). On the other hand, several studies showed that the replacement of the chemical fertilizer with the organic manure significantly decreased the emission of GHGs (Ryals and Silver, 2013; Liu et al., 2014), indicating the possibility that the organic farming can potentially reverse the agriculture ecosystem from a carbon source to a carbon sink. In this study case, the addition of organic manure had no significant impacts on the soil CO_2 flux in addition to the mineral fertilizers. Thus, the appropriate applications of farmland manure could potentially mitigate the soil CO_2 emissions.

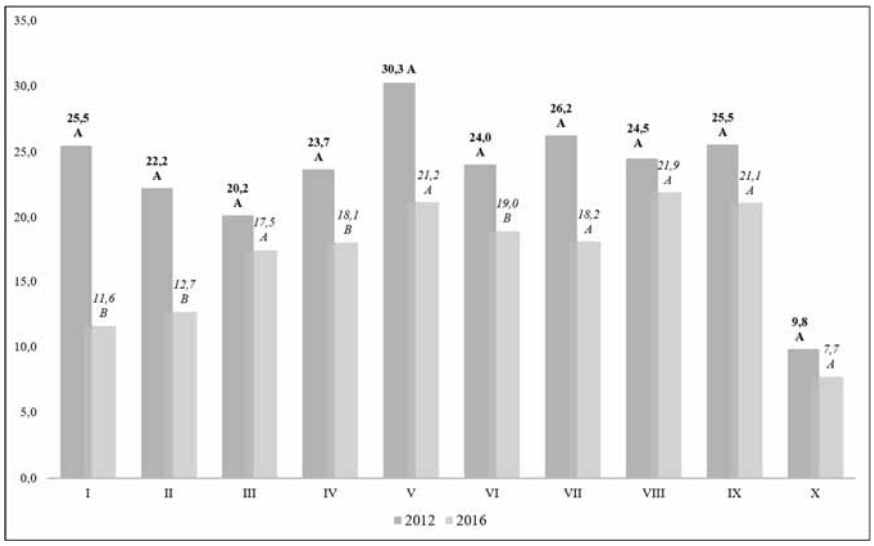

Figure 2. Average annual CO_2 flux (kg ha^{-1} day^{-1}) in 2012 and 2016 considering fertilization treatments *(Mean values marked with the same letter are not significantly different (SAS 9.1 $p < 0,05$)*

Conclusion

Intensification without suitable management and the use of chemical fertilizers has negative implications for the ecosystem and environment. Thus, the study was conducted to measure, analyze, and compare data of carbon dioxide emission from soil obtained in 2012 and 2016 during winter wheat vegetation, and to determine the relationships between soil CO_2 emissions, agro-ecological factors, and fertilization. The soil CO_2 emissions were higher in 2012 compared to 2016 due to the higher temperature during summer period in which the soil respiration is at its peak. The lower precipitation in 2012 did not reduce the soil respiration. The amount of the precipitation was sufficient to keep soil moisture at the level where soil microorganisms and roots can breathe. The soil CO_2 emissions are related to the agro-ecological factors like air temperature and precipitation as those factors are affecting the soil properties tied to soil respiration like soil moisture and temperature. The observed differences among treatments in soil CO_2 emissions show that the addition of N fertilizers increased soil respiration. The addition of solid farmyard mixed manure did not significantly impact the soil CO_2 flux as the manure was applied along with N fertilizer, which might have masked the impacts of the manure. Further research is needed in understanding the impacts of fertilizers on soil respiration in order to develop good agricultural practices that will reduce GHG emissions.

References

Bilandžija D., Zgorelec Ž., Kisić I. (2014). The Influence of Agroclimatic Factors on Soil CO_2 Emissions. Collegium Antropologicum. 38: 77 – 83.

Bilandžija D., Zgorelec Ž., Kisić I. (2016). Influence of Tillage Practices and Crop Type on Soil CO_2 Emissions. Sustainability. 8 (1): 90.

Galić M., Zgorelec Ž., Bilandžija D. (2019). Soil carbon dioxide emissions in winter wheat vegetation influenced by agro-ecological factors and fertilization. 12th International scientific/proffesional conference Agriculture in nature and environment protection. Osijek, 181 – 187.

Galić M., Mesić M., Zgorelec Ž. (2020). Influence of Organic and Mineral Fertilization on Soil Greenhouse Gas Emissions. A Review. ACS - Agriculturae conspectus scientificus. 85 (1): 1-8.

Gračanin, M. (1950): Mjesecni kisni faktori i njihovo znacenje u pedoloskim istrazivanjima. Poljoprivredna znanstvena smotra, Sv. Zagreb, 51 – 66.

IUSS Working group WRB (2015). World Reference Base for Soil Resources. International soil classification system for naming soils and creating legends for soil maps. World soil resources reports no. 106. Rome: FAO. Available at: http://www.fao.org/3/a-i3794e.pdf

Liu H., Li J., Li X., Zheng Y., Feng S., Jiang G. (2014). Mitigating greenhouse gas emissions through replacement of chemical fertilizer with organic manure in a temperate farmland. Science Bulletin 60: 598 – 606.

Mesić, M., Zgorelec, Ž., Šestak, I., Jurišić, A. (2011). Nitrogen fertilization acceptable for environment (scientific report for 2010). Zagreb: University of Zagreb, Faculty of Agriculture.

Mignon S., Maxim A., Opruia C. (2011). Soil Respiration in Mineral and Organic Fertilized Soils During Springtime in a Potato Field. ProEnvironment. 4: 316 – 319.

Mohammed S., Alsafadi K., Takács I., Harsányi E. (2020). Contemporary changes of greenhouse gases emission from the agricultural sector in the EU-27. Geology, Ecology and Landscapes. 4 (4): 282–287.

Raich J.W., Tufekcioglu A. (2000). Vegetation and soil respiration: Correlations and controls. Biogeochemistry. 48: 71 – 90.

Ray R. L., Griffin R. W., Fares A., Elhassan A., Awal R., Woldesenbet S., Risch E. (2020). Soil CO_2 emission in response to organic amendments, temperature, and rainfall. Scientific Reports. 10: 5849.

Reis I. (2014). Mjerenje emisije ugljikovog dioksida iz tla u vegetaciji ozime pšenice. Diplomski rad. Sveučilište u zagrebu Agronomski fakultet, Zagreb.

Ryals R., Silver W.L. (2013). Effects of organic matter amendments on net primary productivity and greenhouse gas emissions in annual grasslands. Ecological Applications. 23 (1): 46 – 59.

Šestak I., Mihaljevski Boltek L., Mesić M., Zgorelec Ž., Perčin A. (2019). Hyperspectral sensing of soil pH, total carbon and total nitrogen content based on linear and non-linear calibration methods. Journal of Central European Agriculture. 20 (1): 504 – 523.

Smith P., Martino D., Cai Z., Gwary D., Janzen H., Kumar P. et al. (2008). Greenhouse gas mitigation in agriculture. Philosophical Transactions of the Royal Society Biological Sciences. 363 (1492): 789–813.

Velasco E., Roth M., Tan S. H., Quak M., Nabarro S. D. A., Norford L. (2013). The role of vegetation in the CO_2 flux from a tropical urban neighbourhood. Atmospheric Chemistry and Physics. 13: 10185 – 10202.

Zhang X., Wu L., Sun N., Ding X., Li J., Wang B., Li D. (2014). Soil CO_2 and N_2O Emissions in Maize Growing Season Under Different Fertilizer Regimes in an Upland Red Soil Region of South China. Journal of Integrative Agriculture. 13 (3): 604 – 614.

Emisije ugljičnog dioksida iz tla u agroekosustavu ozime pšenice (*Triticum aestivum* L.)

Sažetak
Sve veće oslobađanje stakleničkih plinova iz tla u atmosferu važan je doprinos globalnim klimatskim promjenama. Istraživanje je provedeno u svrhu usporedbe podataka o emisiji ugljičnog dioksida iz tla tijekom vegetacije ozime pšenice 2012. i 2016. godine. Također, utvrđeni su odnosi između emisije CO_2 u tlu, agroekoloških čimbenika i N gnojidbe. Emisije CO_2 iz tla bile su veće u 2012. u odnosu na 2016. godinu zbog više temperature tijekom ljetnog razdoblja. Niže oborine u 2012. godini nisu umanjile disanje tla. Statističkim analizama utvrđeno je da se tretmani I, II, IV i VI međusobno značajno razlikuju između ispitivanih godina. Uočene razlike između tretmana u emisiji CO_2 iz tla pokazuju da je dodavanje N gnojiva povećalo disanje tla.

Ključne riječi: CO_2 fluks, temperature tla, vlaga tla, vegetacija

IZVORNI ZNANSTVENI RAD

Alelopatski potencijal korova iz različitih konzervacijskih sustava obrade tla u uvjetima klimatskih promjena

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Sažetak
Cilj rada bio je utvrditi alelopatski potencijal korovnih vrsta iz različitih konzervacijskih sustava obrade tla. Alelopatski potencijal vodenih ekstrakata, pripremljenih od nadzemne mase običnog koštana (*Echinocloa crus-galli* (L.) P.Beauv.) i zelenog muhara (*Setaria viridis* (L.) P.Beauv.) prikupljenih na tretmanima s konvencionalnom (ST), dubokom konzervacijskom (CTD) i plitkom konzervacijskom (CTS) obradom tla, testiran je na klijavost i rast klijanaca salate. Alelopatski potencijal ovisi o korovnoj vrsti, sustavu obrade tla i mjerenom parametru. U prosjeku su najveći inhibitorni potencijal na salatu imali vodeni ekstrakti pripremljeni od jedinki prikupljenih s tretmana duboke konzervacijske obrade tla.

Ključne riječi: alelopatija, klijavost, konzervacijska obrada tla, korovi, inhibicija

Uvod

Alelopatija predstavlja biološki fenomen koji je definiran kao izravni ili neizravni, pozitivni ili negativni utjecaj jedne biljke na klijavost i rast druge putem kemijskih supstanci (alelokemikalija) koje se izlučuju u okoliš (Rice, 1984.). U poljoprivrednim sustavima alelopatija i alelopatske interakcije imaju značajan utjecaj na promjenu sastava korovne flore te rast i prinos usjeva (Singh i sur., 2001.). Alelopatski potencijal i količina alelokemikalija u biljkama ovise o brojnim okolišnim utjecajima, kao što su mikroklimatska lokacija, svjetlost, dostupnost hraniva, temperatura i drugi abiotski i biotski čimbenici (Safdar i sur., 2014., Motamedi i sur., 2016., Khanh i sur., 2018.).

Konzervacijska poljoprivreda temelji se na minimalnom narušavanju tla obradom, permanentnoj pokrovnosti i pravilnom plodoredu, te ima važnu ulogu u prilagodbi i sprječavanju negativnog utjecaja klimatskih promjena na produktivnost, stabilnost i održivost biljne proizvodnje (Nichols i sur., 2015., Jug i sur., 2021.). Različiti sustavi obrade tla utječu na promjene u korovnoj populaciji poput strukture korovne zajednice i dinamike rasta, intezitet zakorovljenosti, dominaciju jednogodišnjih ili višegodišnjih korovnih vrsta, vertikalnu distribuciju sjemena u tlu (Knežević i sur., 2012., Jug i sur., 2017.), međutim malo je podataka o alelopatskom odgovoru korova u odnosu na obradu tla.

Cilj rada bio je utvrditi utjecaj različitih konzervacijskih sustava obrade tla na alelopatski potencijal korovnih vrsta.

Materijal i metode

Kao donor vrste za pripremu vodenih ekstrakata izabrane su korovne vrste obični koštan (*Echinocloa crus-galli* (L.) P.Beauv.) i zeleni muhar (*Setaria viridis* (L.) P.Beauv.). Nadzemna masa korovnih vrsta prikupljena je u fenološkoj fazi cvatnje (Hess i sur., 1997.). Jedinke običnog koštana prikupljene su na lokaciji Čačinci, a jedinke zelenog muhara na lokaciji Križevci. Na obje lokacije jedinke korovnih vrsta uzete su iz tretmana s različitom obradom tla i to: ST- konvencionalna obrada (oranje), CTD – duboka konzervacijska obrada (rahljenje s minimalno 30 % žetvenih ostataka) i CTS – plitka konzervacijska obrada (obrada do 10 cm dubine uz minimalno 50 % žetvenih ostataka).

Prikupljena masa korovnih vrsta osušena je u sušioniku na 70 °C tijekom 72 sata. Suha biljna masa je izmljevena u prah električnim mlinom te čuvana u papirnatim vrećicama na suhome mjestu do izvođenja pokusa.

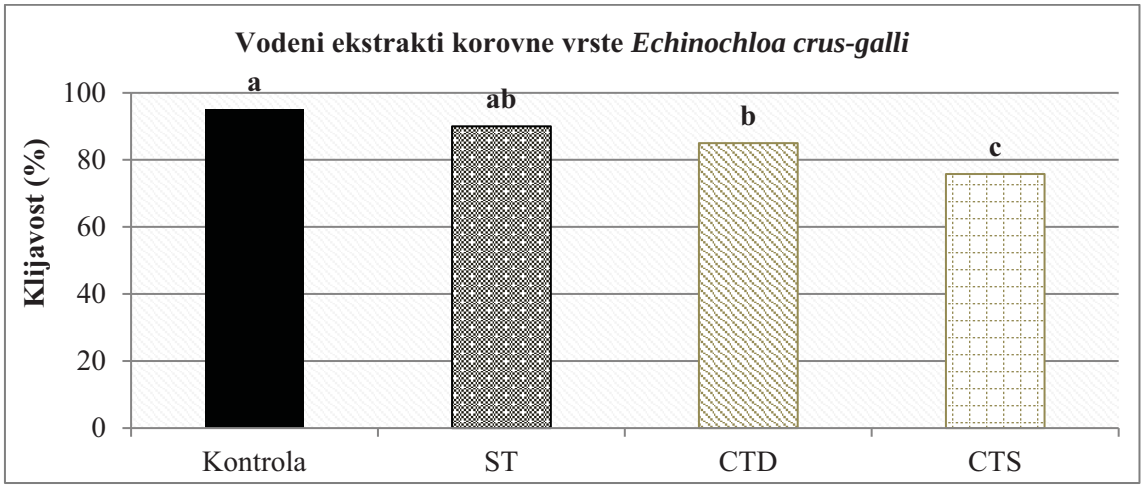
Vodeni ekstrakti pripremljeni su prema metodi Norsworthy (2003.) miješanjem 5 grama suhe biljne mase sa 100 ml destilirane vode. Nakon 24 sata mješavine su procijeđene kroz muslinsko platno te filtrirane kroz filter papir čime su dobiveni ekstrakti koncentracije 5 %. U pokusu je kao test vrsta korišteno sjeme salate (*Lactuca sativa* L.) (cv. Majska kraljica).

Pokus je proveden u laboratorijskim uvjetima u Petrijevim zdjelicama prema potpuno slučajnom planu u četiri ponavljanja. Svaki tretman sastojao se od naklijavanja 30 sjemenki salate na filter papiru navlaženom s 3 ml ekstrakta, odnosno destilirane vode u kontrolnom tretmanu. Sjeme salate naklijavano je 5 dana na temperaturi od 22 (± 2) °C.

Alelopatski potencijal ekstrakata korova procijenjen je mjerenjem sljedećih parametara: klijavost sjemena (%), duljina korijena i izdanka (cm), svježa i suha masa klijanaca (mg) salate. Prikupljeni podatci analizirani su statistički analizom varijance (ANOVA), a razlike između srednjih vrijednosti tretmana testirane su LSD testom na razini 0,05.

Rezultati i rasprava

Statistički značajni alelopatski potencijal na klijavost sjemena salate utvrđen je pri primjeni vodenih ekstrakata običnog koštana iz različitih sustava obrade tla. Vodeni ekstrakti običnog koštana prikupljenog na tretmanima duboke i plitke konzervacijske obrade značajno su smanjili klijavost sjemena salate za 10,5 % i 20,2 % u odnosu na kontrolu (grafikon 1.).



Grafikon 1. Alelopatski potencijal vodenih ekstrakata običnog koštana (*Echinochloa crus-galli* (L.) P.Beauv.) iz različitih konzervacijskih sustava obrade tla na klijavost sjemena salate

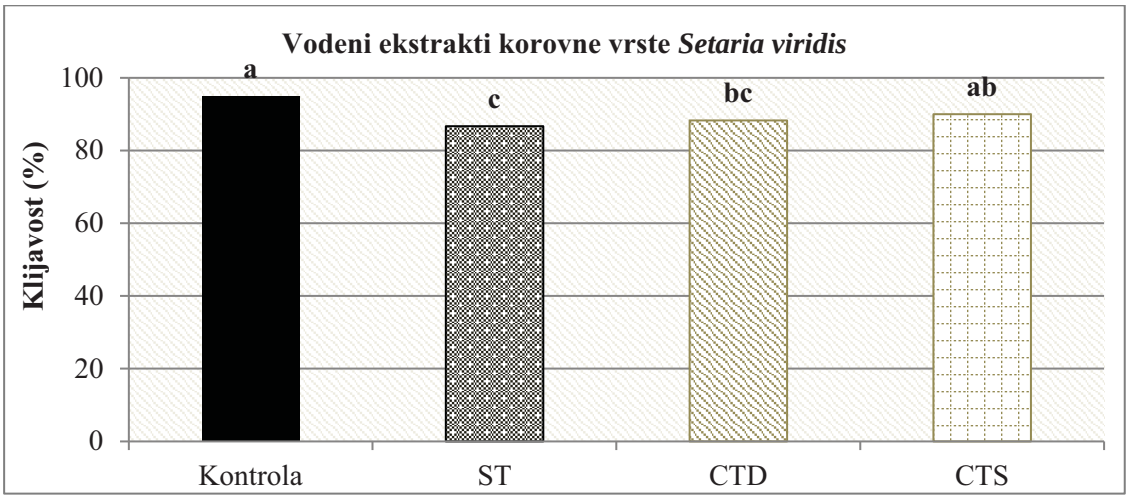
Vodeni ekstrakti običnog koštana statistički su značajno inhibirali duljinu korijena i izdanka te svježu masu klijanaca salate (tablica 1.). Najveća redukcija duljine korijena zabilježena je u tretmanu s vodenim ekstraktom običnog koštana iz tretmana duboke konzervacijske obrade i to statistički značajno u odnosu na kontrolu i druge tretmane obrade tla. Slično, vodeni ekstrakti običnog koštana iz duboke i plitke konzervacijske obrade tla smanjili su duljinu izdanka klijanaca za 52,9 % i 39,5 % u odnosu na kontrolu i 37,2 % i 19,4 % u odnosu na vodeni ekstrakt običnog koštana iz konvencionalne obrade tla. Svježa masa klijanaca bila je najniža u tretmanima s vodenim ekstraktima običnog koštana iz konvencionalne i duboke konzervacijske obrade tla.

Tablica 1. Alelopatski potencijal vodenih ekstrakata običnog koštana (*Echinochloa crus-galli* (L.) P.Beauv.) iz različitih konzervacijskih sustava obrade tla na rast klijanaca salate

tretman	duljina korijena (cm)	duljina izdanka (cm)	svježa masa (mg)
kontrola	1,86 a	1,72 a	7,06 a
ST	0,63 b	1,29 b	5,04 b
CTD	0,32 c	0,81 c	4,90 b
CTS	0,67 b	1,04 bc	7,15 a

abc - razlike između vrijednosti s istim slovom za svaki mjereni parametar nisu statistički značajne ($P < 0,05$)

Vodeni ekstrakti zelenog muhara prikupljenog na tretmanima s konvencionalnom i dubokom konzervacijskom obradom tla značajno su smanjili klijavost sjemena salate za 8,7 % odnosno 7,1 % (grafikon 2.).



Grafikon 2. Alelopatski potencijal vodenih ekstrakata zelenog muhara (*Setaria viridis* (L.) P.Beauv.) iz različitih konzervacijskih sustava obrade tla na klijavost sjemena salate

Duljina korijena i izdanka salate bila je najniža u tretmanima s vodenim ekstraktima zelenog muhara iz duboke konzervacijske obrade tla te smanjena u odnosu na kontrolni tretman za 77,9 % i 59,9 % (tablica 2.). Duljina korijena i izdanka značajno je smanjena i pri primijeni vodenih ekstrakata zelenog muhara iz plitke konzervacijske obrade tla, dok su vodeni ekstrakti zelenog muhara iz konvencionalne obrade tla imali najslabiji alelopatski potencijal. Svježa masa klijanaca statistički je značajno smanjena u odnosu na kontrolu samo u tretmanu s vodenim ekstraktom zelenog muhara iz duboke konzervacijske obrade tla i to za 29,6 %. Vodeni ekstrakti korovnih vrsta pokazali su značajan alelopatski potencijal na klijavost i rast salate. U prosjeku, neovisno o tretmanu obrade tla, vodeni ekstrakti običnog koštana smanjili su klijavost, duljinu korijena i izdanka te svježu masu klijanaca salate za 12 %, 70,9 %, 38,9 % odnosno 19 %. Nešto slabiji utjecaj imali su vodeni ekstrakti zelenog muhara koji su navedene parametre reducirali za 7,1 %, 69,3 %, 32,6 % i 14 %. Prema Xuan i sur. (2006.) korijenovi eksudati običnoga koštana između ostalog sadrže fenole, dugolančane masne kiseline, laktone, benzojevu i ftalnu kiselinu, te su značajno inhibirali klijavost sjemena i rast klijanaca salate i riže. Smanjenje klijavosti i rasta klijanaca salate u tretmanu s vodenim ekstraktima od nadzemne mase zelenog muhara navode Valcheva i sur. (2017.), posebice pri povećanju koncentracije vodenog ekstrakta.

Tablica 2. Alelopatski potencijal vodenih ekstrakata zelenog muhara (*Setaria viridis* (L.) P.Beauv.) iz različitih konzervacijskih sustava obrade tla na klijavost rast klijanaca salate

tretman	duljina korijena (cm)	duljina izdanka (cm)	svježa masa (mg)
kontrola	1,86 a	1,72 a	7,06 a
ST	0,69 b	1,63 a	5,85 ab
CTD	0,41 c	0,69 c	4,97 b
CTS	0,61 bc	1,16 b	7,33 a

abc - razlike između vrijednosti s istim slovom za svaki mjereni parametar nisu statistički značajne ($P < 0,05$)

Sustav obrade tla značajno je utjecao na alelopatski potencijal obje korovne vrste. Najveći inhibitorni potencijal na salatu imali su vodeni ekstrakti pripremljeni od jedinki prikupljenih s tretmana duboke konzervacijske obrade tla, pa je tako prosječno smanjenje svih mjerenih parametara u tretmanu s vodenim ekstraktom običnog koštana iznosilo 44,2 %, a s vodenim ekstraktom zelenog muhara 43,6 %. Vodeni ekstrakti običnoga koštana iz tretmana konvencionalne i plitke konzervacijske obrade tla u prosjeku su smanjili mjerene parametre za 31,2 % i 30,6 %, a vodeni ekstrakti zelenog muhara za 23,5 % i 25,3 %. Alelopatski potencijal i koncentracija alelokemikalija u pojedinoj biljnoj vrsti ovisi o brojnim čimbenicima. Safdar i sur. (2014.) istraživali su alelopatski potencijal korovne vrste *Parthenium hysterophorus* L. s različitih lokacija te utvrdili da je vodeni ekstrakt pripremljen od jedinki koje su rasle blizu proizvodnih površina statistički značajno jače inhibirao klijavost i rast klijanaca kukuruza u odnosu na vodene ekstrakte od jedinki koje su rasle blizu kanala i jezera. Slično, Motamedi i sur. (2016.) bilježe jači alelopatski potencijal biljnih ostataka različitih genotipova šafranike uzgajane u uvjetima stresa izazvanog nedostatkom vode. Klijavost i rast salate i rotkvice značajno je smanjena u tretmanima s vodenim

ekstraktima klijanaca riže uzgajane pri temperaturama višim od 30 °C navode Khanh i sur. (2018.). Autori su također utvrdili ukupno povećanje fenola u ekstraktima klijanaca riže uzgajane na višim temperaturama u odnosu na niže temperature. U istraživanju Bae i sur. (2019.) povišeni CO_2 uzrokovao je povećanje koncentracije alelokemikalija u listovima ambrozije (*Ambrosia artemisiifolia* L.).

Zaključak

Vodeni ekstrakti korovnih vrsta obični koštan i zeleni muhar imali su statistički značajni alelopatski utjecaj na klijavost sjemena i rast klijanaca salate. Sustav obrade tla značajno je utjecao na alelopatski potencijal, pa su u prosjeku vodeni ekstrakti obje korovne vrste prikupljene na tretmanu duboke konzervacijske obrade tla iskazali jači inhibitorni potencijal.

Napomena

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Literatura

Bae J., Byun C., Ahn Y.G., Choi J.H., Lee D., Kang H. (2019). Effect of elevated atmospheric carbon dioxide on the allelopathic potential of common ragweed. Journal of Ecology and Environment, 43, 21.

Hess M., Barralis G., Bleiholder H., Buhr H., Eggers T., Hack H., Stauss R. (1997). Use of the extended BBCH scale – general for the description of the growth, stages of mono- and dicotyledonous species. Weed Research, 37: 433-441.

Jug J., Brozović B., Đurđević B., Wilczewski E., Vukadinović V., Stipešević B., Jug D. (2021). Response of crops to conservation tillage and nitrogen fertilization under different agroecological conditions. Agronomy, 11(11): 2156.

Jug D., Jug I., Vukadinović V., Đurđević B., Stipešević B., Brozović B. (2017). *Konzervacijska obrada tla kao mjera ublažavanja klimatskih promjena*. Hrvatsko društvo za proučavanje obrade tla (HDPOT): Hrvatska.

Khanh D.T., Anh L.H., Nghia L.T., Trung K.H., Hien P.B., Trung D.M., Xuan T.D. (2018). Allelopathic responses of rice seedlings under some different stresses. Plants, 7(2): 40.

Knežević M., Baličević R., Ravlić M., Ravlić J. (2012). Impact of tillage systems and herbicides on weeds and soybean yield. Herbologia, 13(2): 29-39.

Motamedi M., Karimmojeni H., Sini F.G. (2016). Evaluation of allelopathic potential of safflower genotypes (*Carthamus tinctorius* L.). Journal of Plant Protection Research, 56(4): 364-371.

Nichols V., Verhulst N., Cox R., Govaerts B. (2015). Weed dynamics and conservation agriculture principles: A review. Field Crops Research,

Norsworthy, J.K. (2003). Allelopathic potential of wild radish (*Raphanus raphanistrum*). Weed Technology, 17: 307-313.

Rice E.L. (1984). *Allelopathy*. 2nd Edition, Academic Press, New York.

Safdar M.E., Tanveer A., Khaliq A., Naeem M.S. (2014). Allelopathic action of parthenium and its rhizospheric soil on maize as influenced by growing conditions. Planta Daninha, 32(2): 243-253.

Singh H.P., Batish D.R., Kohli R.K. (2001). Allelopathy in agroecosystems: an overview. Journal of Crop Production, 14(4): 1-42.

Valcheva E., Popov V., Marinov-Serafimov P., Golubinova I., Nikolov B., Velcheva I., Petrova S. (2017). Allelopathic effect of some weed species on germination and initial development of *Lactuca sativa*. *Book of Proceedings VIII International Scientific Agriculture Symposium,* Kovačević, D., 170-175. Jahorina, Bosnia and Herzegovina.

Xuan T.D., Chung I.M., Khan T.D., Tawata S. (2006). Identification of phytotoxic substances from early growth of barnyard grass (*Echinochloa crusgalli*) root exudates. Journal of Chemical Ecology, 32: 895-906.

Allelopathic potential of weeds from different conservation tillage systems under climate change conditions

Abstract

The aim of this study was to determine the allelopathic potential of weed species from different conservation tillage systems. Allelopathic potential of water extracts prepared from the aboveground mass of barnyardgrass (Echinocloa crus-galli (L.) P.Beauv.) and green foxtail (Setaria viridis (L.) P.Beauv.) collected on treatments with conventional (ST), deep conservation (CTD) and shallow conservation (CTS) tillage was tested on germination and growth of lettuce seedlings. Allelopathic potential depended on weed species, tillage system and measured parameter. On average, water extracts prepared from plants collected from deep conservation tillage treatment had the greatest inhibitory potential on lettuce.

Key words: allelopathy, germination, conservation tillage, weeds, inhibition

IZVORNI ZNANSTVENI RAD

Utjecaj konzervacijske obrade na poljski vodni kapacitet i gustoću pakiranja čestica tla u uvjetima klimatskih promjena

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Sažetak

Istraživanja u Čačincima i Križevcima tijekom 2021. godine imala su za cilj utvrditi utjecaj tipa tla (na lokalitetu Čačinci - Stagnosol i na lokalitetu Križevci - Gleysol), sustava obrade (ST – standardna obrada, CTD – konzervacijska obrada duboka i CTS – konzervacijska obrada plitka) i dubine tla (0 – 20 i 20 – 40 cm), kao i njihovih interakcija na poljski vodni kapacitet (PVK) i gustoću pakiranja (Gp). Sadržaj gline u Stagnosolu kretao se od 31,82 % (na dubini 0 – 20 cm) do 35,23 % (na dubini 20 – 40 cm), a u Gleysolu od 8,84 % (na dubini 0 – 20 cm) do 10,37 % (na dubini 20 – 40 cm). Tekstura tla u Stagnosolu je praškasto glinasta ilovača (PrGI), a praškasta (Pr) u Gleysolu. Na vrijednosti PVK je značajno utjecao tip tla, obrada i dubina, te su utvrđene značajne interakcije tipa tla s obradom i tipa tla s dubinom. Najveći PVK izmjeren je na Gleysolu na CTS tretmanu (46,14 %) i na dubini 20 – 40 cm (46,25 %), dok je najmanji PVK izmjeren na Stagnosolu na CTD tretmanu (30,34 %) i na dubini 20 - 40 cm (33,28 %). Gustoća pakiranja je bila pod značajnim utjecajem tipa tla. Utvrđene su značajne interakcije između tipa tla s obradom i tipa tla s dubinom. Najmanja gustoća pakiranje izmjerena je na Gleysolu na CTD tretmanu (1,42 g cm^{-3}) i na dubini 20 - 40 cm (1,44 g cm^{-3}). Najveća gustoća pakiranje izmjerena je na Stagnosolu na CTD tretmanu (2,02 g cm^{-3}) i na dubini od 20 - 40 cm (1,93 g cm^{-3}).

Ključne riječi: konzervacijska obrada, zbijanje, poljski vodni kapacitet, gustoća pakiranja

Uvod

Obrada tla, kao mehanička intervencija u tlo kako bi se proizvela dovoljna količina hrane za sve brže rastući broj stanovnika, značajno utječe na svojstva tla. Prema Busari i sur. (2015.) obrada tla utječe na smanjivanje rezervi vode u tlu, temperaturu tla, a posljedično i na infiltraciju i evaporaciju te u značajnoj mjeri na okoliš. Jug i sur. (2018.) ističu kako je jedan od najranjivijih aspekata sve izraženijih klimatskih promjena upravo proizvodnja hrane, odnosno poljoprivreda. Intenzivna obrada (Lal i sur., 2007.) uzrokuje eroziju izlažući tlo jakom intenzitetu oborina i utjecaju vjetra. Iz tog razloga su razvijeni konzervacijski sustavi obrade kako bi usporili, a u konačnici i zaustavili degradacijske procese fizikalnih, kemijskih i bioloških svojstava tla. Zbijanje tla je degradacijski proces (Khan i sur., 2012.) koji direktno utječe na fizikalna, a indirektno i na ostala svojstva tla. U konvencionalnoj poljoprivredi najčešći uzrok je pojačani promet na oranicama (Ahmad i sur., 2009) i veliko vanjsko opterećenje mehanizacije ne samo na površini tla nego i dublje u solumu. Time se smanjuje volumen pora (Weisskopf i sur., 2009.) što znači i narušene vodno zračne odnose u tlu. U vlažnim razdobljima, prema Vukadinović i sur., (2013.), nastaje suficitno vlaženje oraničnih i podoraničnih slojeva zbog otežane perkolacije vode. Posljedica je niži pH tla i smanjenja pristupačnost hraniva biljkama. Cilj istraživanja je utvrditi u kojoj mjeri tip tla, sustav obrade tla i dubina tla utječu na poljski vodni kapacitet i gustoću pakiranja kao jednog od indikatora zbijenosti tla.

Materijal i metode

U prvoj godini istraživanja (2021. god.) na projektu Hrvatske zaklade za znanost „Procjena konzervacijske obrade tla kao napredne metode uzgoja usjeva i prevencije degradacije tla - ACTIVEsoil“ organizirana su istraživanja na dvije lokacije: Čačinci (OPG Knežević) i Križevci (pokusno polje Visokog gospodarskog učilišta). Pokusno polje Čačinci predstavlja referentna grupa Stagnosol, a Križevce Gleysol (IUSS Working Group WRB, 2015.). Pokus je postavljen po split-split-plot pokusnoj shemi gdje je lokalitet, definiran tipom tla i agroekološkim pokazateljima, glavni faktor, kalcizacija – podfaktor, te gnojidba – podpodfaktor. Rezultati ovog rada samo su manji dio provedenih istraživanja te obuhvaćaju rezultate na istraživanim tipovima tala pri 3 različita sustava obrade tla (ST - standardna, konvencionalna uz obvezno duboko oranje i sve dodatne agrotehničke operacije u proizvodnji kukuruza, CTD - konzervacijska duboka, rahljenje do dubine 30 cm i CTS - konzervacijska plitka obrada uz površinsko rahljenje do 10 cm) i na dvije dubine (0 - 20 cm i 20 – 40 cm).

U proljeće 2021. godine zasijan je merkantilni kukuruz na oba lokaliteta. Uzorkovanja tla i biljke odvijala su se u fazi ranog porasta kukuruza (3 - 5 listova), svilanja i pune zriobe. Nenarušeni uzorci tla uzeti su u cilindre po Kopeckom volumena 100 cm^3 na svim sustavima obrade tla. Uzorkovalo se u sloju 0 - 20 cm i 20 - 40 cm prema HRN ISO 11272:2004 (Pernar i sur., 2013.). U uzorcima je određen sadržaj vlage i volumna gustoća. Za potrebe izrade ovog rada odabrane su vrijednosti poljskog vodnog kapaciteta (PVK) i gustoće pakiranja (Gp) u fazi pune zriobe kukuruza.

Gustoća pakiranja izračunata je pomoću izraza: $Gp = \rho_v + (0{,}009 \times \text{glina, \%})$. Dobiveni podaci statistički su obrađeni pomoću programa Statistika 12.5 (StatSoft, Inc., 2014.).

Rezultati i rasprava

Lokaliteti pokusnih polja predstavljeni su s dva različita tipa tla u kojima je utvrđena razlika u sadržaju gline. Tekstura u Stagnosolu je praškasto glinasta ilovača sa sadržajem gline u površinskih 20 cm tla od 31,82 do 34,05 %, a u sloju 20 – 40 cm od 33,54 % do 35,23 %. (Tablica 1). U Križevcima Gleysol je praškaste teksture s 8,84 - 9,97 % gline u sloju do 20 cm dubine. U dubljem sloju (20 – 40 cm) prosječni sadržaj gline 10,10 %, a kreće se u granicama od 9,87 -10,37 %.

Tablica 1. Sadržaj gline i teksturna klasa u Stagnosolu (Čačinci) i Gleysolu (Križevci)

	Stagnosol			Gleysol		
	0-20 cm	20-40 cm		0-20 cm	20-40 cm	
	glina, %		tekstura	glina, %		tekstura
ST	31,82	33,54	PrGI	9,97	10,37	Pr
CTD	34,05	35,23	PrGI	9,73	9,87	Pr
CTS	33,81	34,22	PrGI	8,84	10,06	Pr
prosjek	33,23	34,33		9,51	10,10	
min	31,82	33,54		8,84	9,87	
max	34,05	35,23		9,97	10,37	
CV	3,68	2,48		6,26	2,50	

Tumač kratica: ST – standardna obrada, CTD – konzervacijska obrada duboka, CTS – konzervacijska obrada plitka, min – najmanja vrijednost, max – najveća vrijednost, CV – koeficijent varijabilnosti, PrGI – praškasto glinasta ilovača, Pr – prah

Sadržaj gline ima izravan utjecaj na zbijanje poljoprivrednih površina. U tlima s više gline odnos makro i mikropora je nepovoljan. Prevladavaju mikropore u kojima se uglavnom zadržava voda, pa su česti anaerobni uvjeti u vlažnim periodima tijekom godine. Zbijanje je intenzivnije izraženo upravo zbog malog udjela makropora.

Tablica 2. Prosječne vrijednosti PVK (%) i Gp (g cm^{-3}) na različitim tipovima tala (Stagnosol, Gleysol), pri različitim sustavima obrade (ST, CTD, CTS) i na dvije dubine (0 - 20 cm i 20 - 40 cm)

	Stagnosol			Gleysol			
	0 -20 cm	20-40 cm	prosjek	0-20 cm	20-40 cm	prosjek	prosjek
			PVK (%)				
ST	37,18	35,70	*36,44*	41,41	44,31	*42,86*	***39,65***
CTD	29,96	30,73	*30,34*	43,73	47,04	*45,38*	***37,86***
CTS	33,94	33,42	*33,68*	44,87	47,41	*46,14*	***39,91***
prosjek	**33,69**	**33,28**	***33,48***	**43,33**	**46,25**	***44,79***	***39,14***
			Gp (g cm^{-3})				
ST	1,82	1,94	1,88	1,53	1,46	1,50	***1,69***
CTD	2,01	2,03	2,02	1,42	1,42	1,42	***1,72***
CTS	1,91	1,96	1,93	1,47	1,43	1,45	***1,69***
prosjek	**1,91**	**1,98**	***1,94***	**1,47**	**1,44**	***1,46***	***1,70***

Tumač kratica: ST – standardna obrada, CTD – konzervacijska obrada duboka, CTS – konzervacijska obrada plitka, PVK – poljski vodni kapacitet, %, Gp – gustoća pakiranja, g cm^{-3}

Poljski vodni kapacitet u prosjeku je iznosio 39,14 % i bio je pod značajnim utjecajem tipa tla i sustava obrade tla (Tablica 2). Utvrđene su statistički značajne interakcije između tipa tla i sustava obrade, kao i između tipa tla i dubine (Tablica 3). Porast vlažnosti s dubinom soluma u fazi pune zriobe kukuruza zabilježen je u Gleysolu koji je imao za 11,31 % veći PVK u odnosu na Stagnosol.

Pagliai i sur. (2004.) su istražujući utjecaj minimalne i konvencionalne obrade na fizikalna svojstva ustanovili povećanje volumena skladišnih (0,5 – 50 mm) i izduženih transportnih pora promjera 50 do 500 mm u sustavu minimalne obrade. Najveći PVK izmjeren je na Gleysolu na CTS tretmanu (46,14 %) i na dubini 20 – 40 cm (46,25 %), dok je najmanji PVK izmjeren (Tablica 2) na Stagnosolu na CTD tretmanu (30,34 %) i na dubini 20 - 40 cm (33,28 %).

Tablica 3. Utjecaj pojedinih istraživanih čimbenika i njihovih interakcija na poljski vodni kapacitet (PVK) za razinu značajnosti $P < 0,05$

	A	B	C	AxB	AxC	BxC	AxBxC
$LSD_{0,05}$	0,958	1,277	-	1,964	2,022	-	-
F test	593,561	6,845	ns	26,986	6,446	ns	ns

Tumač kratica: A – tip tla, B – obrada tla , C – dubina tla, AxB – interakcija tipa tla i obrade, AxC – interakcija tipa tla i dubine, BxC – interakcija obrade tla i dubine, AxBxC – interakcija tipa tla, obrade i dubine

Zbijenost tla može se izraziti kroz vrijednost volumne gustoće tla, ali Jug i sur. (2019.) navode da je Renger 1970. godine predložio gustoću pakiranja, naročito za procjenu u podoraničnim slojevima. U tlima koja sadrže više gline, čestice tla su gušće pakirane, ukupna poroznost je niža, prevladavaju mikropore pa je izražen i nedostatak zraka.

Na Gp, koja je u prosjeku iznosila 1,70 g cm^{-3}, statistički je značajno utjecao samo tip tla (Tablica 4). Utvrđene su statistički značajne interakcije između tipa tla i obrade, kao i tipa tla i dubine. Najveća gustoća pakiranje (Tablica 2) izmjerena je na Stagnosolu na CTD tretmanu (2,02 g cm^{-3}) i na dubini od 20 - 40 cm (1,93 g cm^{-3}). Najmanja gustoća pakiranja izmjerena je na Gleysolu na CTD tretmanu (1,42 g cm^{-3}) i na dubini od 20 do 40 cm (1,44 g cm^{-3}).

Povećana zbijenost tla usporava rast korijena, a ako se intenzitet zbijanja nastavi tijekom duljeg vremenskog perioda opada prinos i kvaliteta konačnog proizvoda. Prema Kaufmannu i sur. (2010.) optimalni uvjeti za rast biljaka su uz vrijednosti Gp < 1,70 g cm^{-3}. U Križevcima, na Gleysolu zbijenost tla je bila niža (Gp je u prosjeku iznosio 1,46 g cm^{-3}), dok je u Čačincima, na Stagnosolu Gp iznosila 1,94 g cm^{-3} što je rezultat jače zbijenosti tla.

Tablica 4. Utjecaj pojedinih istraživanih čimbenika i njihovih interakcija na gustoću pakiranja (Gp) za razinu značajnosti P < 0,05

	A	B	C	AxB	AxC	BxC	AxBxC
$LSD_{0,05}$	0,034	-	-	0,090	0,039	-	-
F test	852,236	ns	ns	7,725	15,249	ns	ns

Tumač kratica: A – tip tla, B – obrada tla , C – dubina tla, AxB – interakcija tipa tla i obrade, AxC – interakcija tipa tla i dubine, BxC – interakcija obrade tla i dubine, AxBxC – interakcija tipa tla, obrade i dubine

Zaključak

Rezultati istraživanih pedofizikalnih svojstava na različitim tipovima tala, pri različitim sustavima obrade i na dvije dubine ukazuju na prednost primjene konzervacijskih sustava obrade tla. Najveći PVK i najmanja Gp zabilježeni su u Gleysolu lagane praškaste teksture, na CTD tretmanu na dubini 20-40 cm.

Napomena

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Literatura

Busari, M.A., Kukal, S.S., Kaur, A., Bhatt, R., Dulazi, A.A. (2015). Conservation tillage impacts on soil, crop and the environment. International Soil and Water Conservation research. 3: 119-129.

IUSS Working Group WRB (2015). World Reference Base for Soil Resources 2014, update 2015 International soil classification system for naming soils and creating legends for soil maps. World Soil Resources Reports No. 106. FAO, Rome.

Jug, D., Đurđević, B., Birkás, M., Brozović, B., Lipiec, J., Vukadinović, V., Jug, I. (2019). Effect of conservation tillage on crop productivity and nitrogen use efficiency. Soil & Tillage Research. 194: 1-15.

Jug, D., Jug, I., Brozović, B., Vukadinović, V., Stipešević, B., Đurđević, B. (2018). The role of conservation agriculture in mitigation and adaptation to climate change. Poljoprivreda. 24(1): 35-44.

Kaufmann, M., Tobias, S., Schulin, R., 2010. Comparison of critical limits for crop plant growth based on different indicators for the state of soil compaction. J. Plant Nutr. Soil Sci. 173(4), 573-583.

Khan, S.R., Abbasi, M.K., Hussan, A. (2012). Effect of Induced Soil Compaction on Changes in Soil Properties and Wheat Productivity under Sandy Loam and Sandy Clay Loam Soils: A Greenhouse Experiment. Communications in Soil Science and Plant Analysis. 43: 2550-2563.

Lal, R., Reicosky, D.C., Hanson, J.D. (2007). Evolution of the plow over 10,000 years and the rationale for no-till farming. Soil & Tillage Research. 93: 1-12.

Pagliai, M., Vignozzi, N., Pellegrini, S. (2004). Soil structure and the effect of management practices. Soil & Tillage Research. 79: 131-143.

Pernar, N., Bakšić, D., Perković, I. (2013). Terenska i laboratorijska istraživanja tla, priručnik za uzorkovanje i analizu. Udžbenici Sveučilišta u Zagrebu, Šumarski fakultet. Zagreb.

StatSoft, Inc. (2014). Statistica (data analysis software system), version 12. www.statsoft.com

Vukadinović, V., Jug, D., Jug, I., Đurđević, B., Cvjetković, S., Stipešević, B., Brozović, B. (2014). Impact of soil tillage at compaction physical parameters. Objavljeno u: Proceedings 7th International Soil Conference: Soil management in sustainable farming systems, Badalikova, B. (ed.),151-154. Křtiny near Brno, Czech Republic.

Vukadinović, V., Jug, D., Đurđević, B., Jug, I., Vukadinović, V., Stipešević, B., Lović, I., Kraljičak, Ž. (2013). Agricultural compaction of some soil types in eastern Croatia. Objavljeno u: Proceedings & Abstracts 2nd International Scientific Conference, Soil and Plant Management: Adaptation and Mitigation of Climate Changes, Jug, I., Vukadinović, V., Đurđević, B. (ed.), 38-45. Osijek, RH.

Influence of conservation soil tillage on field water capacity and packing density under climate change

Abstract

The aim of the experiment (conducted on two localities in Cacinci and Krizevci) was to determine the impact of soil type (Cacinci – Stagnosol and Krizevci – Gleysol), soil tillage (ST – standard tillage, CTD – conservation tillage deep and CTS – conservation tillage shallow) and depth (0 -20 and 20 – 40), as well as their interactions, on field water capacity (FWC) and packing density (PD). Clay content in Stagnosol was from 31.82% (0 – 20 cm) to 35.23% (20 – 40 cm) and in Gleysol from 8.84% (0 – 20 cm) to 10.37% (20 – 40 cm). The soil texture in Stagnosol was silty clay loam and silty in Gleysol. FWC values were significantly influenced by soil type, tillage and depth, and significant interactions of soil type with tillage and soil type with depth were determined. The highest FWC values were measured on Gleysol on CTS (46.14%) and on soil depth 20 – 40 cm (46.25%), while the lowest FWC was measured on Stagnosol on CTD (30.34%) on soil depth 20 - 40 cm (33.28%). Packing density was significantly influenced by soil type. Significant interactions between soil type with tillage and soil type with depth, were determined. The lowest packing density was measured on Glaysol on CTD (1.42 g cm-3) and on depth 20 - 40 cm (1.44 g cm-3). The highest packing density was measured on Stagnosol on CTD (2.02 g cm-3) and on depth 20 - 40 cm (1.93 g cm-3).

Key words: conservation tillage, soil compaction, field water capacity, packing density