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DIGITALNI AKADEMSKI ARHIVI I REPOZITORIJI

Influence of Dietary Incorporation of Vegetable Oils and Microalgae on Laying Hens Egg Yolk Fatty Acids Profile and Health Lipid Indices

Utjecaj uvođenja biljnih ulja i mikroalgi u hranu kokoši nesilica na profil masnih kiselina u žumanjku jajeta i zdravstvene lipidne indekse

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THE INFLUENCE OF DIETARY INCORPORATION OF VEGETABLE OILS AND MICROALGAE ON THE LAYING HENS' EGG-YOLK FATTY ACID PROFILE AND THE HEALTH-LIPID INDICES

Kralik, G., Kralik, Z., Košević, M., Kralik, I., Gvozdanović, K.

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SUMMARY

Table eggs are a good source of nutrients. The nutritional properties of eggs are influenced by the composition of laying hens' feed mixtures. An important component in the feed mixtures are the oils, which, in addition to their energy value, are also important for the absorption of liposoluble vitamins. Oils differ in the content of fatty acids, which play a significant role in metabolism and health maintenance. The lipid indices, such as the atherogenic (AI), thrombogenic (TI), and hypo/hypercholesterolemic fatty-acid ratio index (HH), are used when evaluating the lipid-quality indicators in eggs. This study aimed to produce the eggs enriched with the n-3 PUFA and to determine which feeding treatment yields the eggs with the most favorable lipid quality. The three groups of layers (treatments A, B, and C) were used in the experiment, which were fed with the mixtures of a modified composition considering the oils and microalgae used. The mixtures were balanced at the level of 17% crude protein and 12.10 MJ/kg ME. The produced eggs were used for an analysis of fatty-acid content (SFA, MUFA, n-6 PUFA, and n-3 PUFA), on the basis of which the lipid indices were calculated. The research results of eggs from the A, B, and C feeding treatments showed that the AIs were 0.485, 0.476, and 0.453; the TIs were 0.802, 0.681, and 0.653, and the HH indices amounted to 2.976, 2.983, and 3.068. The activities of the $\Delta 5$ - and $\Delta 6$ desaturases in the synthesis of the n-6 PUFA and the n-3 PUFA in eggs differed between the feeding treatments. The influence of a composition of feed mixtures in the laying hens' diet on the quality of lipid indices was determined, and the eggs from the treatment C had the most favorable health-lipid indices.

Keywords: eggs, fatty acids, AI, TI, HHI

INTRODUCTION

Eggs are a good source of essential nutrients. They are a good source of micronutrients such as the vitamins (A, D, E, K, and B complex) and macro- and microelements (P, Ca, Zn, K, Na, Mg, and Se). Eggs also contain cholesterol (250-400 mg/50g eggs), which, according to the latest clinical studies, does not significantly affect the blood-cholesterol levels in humans (Lopez Sobaler et al., 2017; Kim and Campbell, 2018). An excessive intake of the n-6 polyunsaturated fatty acids (n-6 PUFA), relative to the n-3 polyunsaturated fatty acids (n-3 PUFA), is associated with a risk of cardiovascular disease, type 2 diabetes, inflammatory processes, and cancer (Ulbrich and Southgate, 1991; Simopoulos, 2002). For this reason, a composition of the laying hens' mixtures is modified by using suitable feedstuffs. The studies have shown that a nutritional value of eggs depends on a composition and characteristics of the feed.

The use of feed with a high content of polyunsaturated fatty acids has an effect on their deposition in the egg lipids (Kralik et al., 2018; Kralik et al., 2021; Irwan et al., 2022; Radanović et al., 2023). The eggs enriched with the functional ingredients such as the n-3 PUFA influence one or more functions in a human body, in addition to their basic nutritional effect (Karoliy 2007; Vranešić Bender, 2012; Lešić et al., 2017; Stupin et al., 2020). The aim of this study was to investigate the quality of egg lipids from a health point of view when using the vegetable oils (soybeans, rapeseed, and linseed) and microalgae (*Schizochytrium limacinum*) in the laying hens' feeding.

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MATERIAL AND METHODS

The research involved 135 laying hens of the Tetra SL hybrid, which were divided into 3 experimental groups (A, B, and C). The laying hens' groups were housed in the same facility in the enriched cages. At the beginning of the experiment, the laying hens were the 47-week-old individuals. To increase the content of the n-3 PUFA in eggs, the feed mixtures were designed. The feed-mixture compositions are shown in Table 1 and

Table 2. The diets were adjusted to a level of 16.5% of crude protein and 12.1% of MJ/kg metabolizable energy. The hens of all groups consumed 112-115 g of feed mixture per day. laying hens were fed with different feed mixtures for four weeks. The class L eggs were sampled from each group on the last day of the feeding trial. The fatty-acid profiles in the yolks were analyzed on the collected samples. All laying hens' groups were housed in the same poultry house with a controlled microclimate and 16 hours of daylight.

Table 1. Specific of feeding treatments

Tablica 1. Specifičnosti hranidbenih tretmana

Treatment / Tretman	Soybean oil, % / Sojino ulje	Microalgae*, % / Mikroalge	Rapeseed oil, % / Repičino ulje	Linseed oil, % / Laneno ulje
A	5.0	-	-	-
B	2.0	0.5	1.2	1.3
C	1.5	1.0	1.2	1.3

* *Schizochytrium limacinum*

The microalgae preparation *ALL-GRich*, manufactured by the Alltech Company, USA, contained the SFA in the amount of 65.58%, MUFA in the amount of 4.31%, n-6 PUFA in the amount of 3.89%, and the n-3 PUFA in

the amount of 23.23%. The DHA was dominant with the amount of 21.23%. Soybean, rapeseed, and linseed oils contained 5.69%, 7.48%, and 47.91% of the n-3 PUFA, respectively (Kralik et al., 2020).

Table 2. Composition and chemical analysis of the basic feed mixture

Tablica 2. Sastav i kemijska analiza osnovne krmne smjese

Ingredients / Sastojci	%	Chemical analysis* / Kemijska analiza	%
Corn / Kukuruz	48.47	Moisture content / Sadržaj vlage	9.30
Soybean cake / Sojina pogača	22.33	Crude protein / Sirove bjelančevine	16.63
Toasted soybeans / Tostirana soja	3.00	Crude fat / Sirove masti	7.30
Sunflower cake / Suncokretova pogača	5.00	Crude fiber / Sirova vlakna	4.00
Alfa-Alfa / Alfa-Alfa	1.67	Ash / Pepeo	16.54
Calcium granules / Granule kalcija	10.63	ME MJ/kg	12.10
Monocalcium phosphate / Monokalcij fosfat	1.33		
Yeast / Kvasac	0.50	*Referential methods / Referentne metode	
Salt / Sol	0.33	*moisture HRN ISO 6496 / vlaga	
Acidifier / Zakiseljivač	0.33	*ash HRN EN ISO 5984 / pepeo	
Minerals nanofeed / Minerali nanokrmiva	0.33	*crude protein HRN ISO 5983-2 / sirove bjelančevine	
Methionine / Metionin	0.15	*fat HRN ISO 6459 / mast	
Premix / Premiks	1.20	*crude fiber HRN EN ISO 6865, modified CRO standard / sirova vlakna HRN EN ISO 6865, modificirani CRO standard	
Oil ¹ / Ulje ¹	5.00		
Total / Ukupno	100.00		

¹ The specifics of the A, B, and C treatments are presented in Table 1.

¹ Specifičnosti tretmana A, B i C prikazane su u Tablici 1.

A fatty-acid profile was analyzed on the two feed samples and 24 egg samples (8 per treatment). The fatty acids were determined according to the methods described by Chapo et al. (1986) and Kralik et al. (2020). Gas chromatography was performed on a Bruker 430-GC apparatus (Billerica, MA, USA), equipped with a FAMEWAX (RESTEK, Bellefonte, PA, USA) type capillary column (30 m × 0.32 mm internal diameter, 0.25 μm film) and a flame-ionization detector. The characteristic operating conditions were as follows: injector temperature

amounted to 220°C, detector temperature to 230°C, and helium flow to 25 mL min⁻¹. The oven temperature was graded: from 50 to 225°C: 6.0°C min⁻¹ and 21 min at 225°C. To identify the individual fatty acids in the chromatogram, a standard fatty-acid mixture Supelco 37 Component FAME Mix (Supelco Inc., Bellefonte, PA, USA) was used. The portions of a saturated fatty acid (SFA) and of a monounsaturated fatty acid (MUFA), as well as of the n-6 PUFA and the n-3 PUFA, were shown as a g 100 g⁻¹ of total fatty acids in the microalga, oil, and feed mixtures and in mg 100 g⁻¹

in eggs. The atherogenic (AI), thrombogenic (TI), and hypo/hypercholesterolemic indices (HHIs) according to Omri et al. (2019) and Panaite et al. (2020) were used to assess the egg yolks' lipid quality, as follows:

$$AI = (C12:0 + 4 \times C14:0 + C16:0 + C18:0) / (\Sigma MUFA + \Sigma n-6 PUFA + \Sigma n-3 PUFA)$$

$$TI = (C14:0 + C16:0 + C18:0) / 0.5 \times \Sigma MUFA + 0.5 \times \Sigma n-6 PUFA + 3 \times \Sigma n-3 PUFA + (\Sigma n-3 PUFA / \Sigma n-6 PUFA)$$

$$HHI = (C18:1 n-9 + C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3) / (C14:0 + C16:0)$$

The activity of the enzymes Δ -5 desaturase and Δ -6 desaturase in the metabolic processes of n-6 PUFA and the n-3 PUFA was calculated as follows (Untea et al., 2020):

$$n-6 \Delta 5 + \Delta 6 = [(C20:2 n-6 + C20:4 n-6) / (C18:2 n-6 + C20:2 n-6 + C20:4 n-6)] \times 100$$

$$n-3 \Delta 5 + \Delta 6 = [(C20:5 n-3 + C22:5 n-3 + C22:6 n-3) / (C18:3 n-3 + C20:5 n-3 + C22:5 n-3 + C22:6 n-3)] \times 100$$

The data collected as a part of the egg analysis were statistically processed using the *Statistica* software, version 13.5.0.17 (1984–2018 TIBCO® Statistica™ Inc.), and summarized in the tables. A significance of the differences between the treatments was tested while applying an analysis of variants (ANOVA). A significance of the differences between the MEAN values was determined using Fisher's LSD test.

RESULTS AND DISCUSSION

The obtained results are presented in Tables 3, 4, and 5 and in Figure 1. Table 3 shows the fatty-acid content in the laying hens' feed mixture depending on the feeding treatment (A, B, and C). The mixtures contained the Σ SFA in the amount of 21.23%, 17.94%, and 17.28% and the Σ MUFA in the amount of 35.83%, 29.66%, and 29.33%. For the Σ n-6 PUFA, only the linoleic acid (C18:2 n-6) was present, in the amount of 44.17%, 38.89%, and 37.90%, respectively. The Σ n-3 PUFA in the feeding treatments A, B, and C amounted to 4.73%, 13.07%, and 14.58%, respectively, while the Σ n-6 PUFA was 44.17%, 38.89%, and 37.49%, respectively. A ratio of the Σ n-6 PUFA/ Σ n-3 PUFA depending on a feeding treatment was 9.34 for A, 2.97 for B, and 2.57 for C. A combination of linseed and rapeseed oil in the laying hens' mixtures (treatments B and C) increased the ALA content by 2.91 and 3.16 times, respectively, when compared to the treatment A mixture. The Σ n-3 PUFA also increased 2.76 and 2.08 times in the mixtures of the treatments B and C, respectively, when compared to the mixture of the treatment A. Due to an increased concentration of the ALA in the diet, the content of this fatty acid in egg yolk increased (Table 3 and Table 4). In addition, a conversion of ALA into a long-chain EPA and DHA takes place in the poultry liver (Ehr et al., 2017). The LA:ALA ratio is important in the conversion of ALA to EPA and DHA, as these fatty acids compete for the same enzymes in the processes of elongation and saturation. Goldberg et al. (2013) found that their reduced ratio increases the synthesis of long-chain PUFAs in the liver.

Table 3. Content of fatty acids in feed mixture (%)

Tablica 3. Sadržaj masnih kiselina u krmnoj smjesi (%)

Fatty acids / Masne kiseline	Treatments / Tretmani		
	A	B	C
Myristic (C14:0) / Miristinska	0.17	0.29	0.35
Pentadecanoic (C15:0) / Pentadekanska	0.05	0.12	0.12
Palmitic (C16:0) / Palmitinska	14.69	12.10	11.96
Heptadecanoic (C17:0) / Heptadekanska	0.13	0.12	0.10
Stearic (C18:0) / Stearinska	5.52	4.51	4.01
Arachidic (C20:0) / Arahidonska	0.46	0.47	0.44
Behenic (C22:0) / Behenska	0.21	0.33	0.30
Σ SFA	21.23	17.94	17.28
Palmitoleic (C16:1) / Palmitoleinska	0.23	0.14	0.14
Oleic (C18:1) / Oleinska	28.27	29.06	28.74
Eicosenoic (C20:1) / Eikozaenska	0.17	0.46	0.45
Erucic (C22:1) / Eruka	1.11	-	-
Σ MUFA	29.78	29.66	29.33
Linoleic (C18:2 n-6) / Linolna	44.17	38.89	37.49
Σ n-6 PUFA	44.17	38.89	37.49
α linolenic (C18:3 n-3) / α -linolenska	4.24	12.35	13.41
Eicosapentaenoic (C20:5 n-3) / Eikosapentaenoična	0.49	0.13	0.10
Docosahexaenoic (C22:6 n-3) / Dokosapentaenoična	-	0.59	1.07
Σ n-3 PUFA	4.73	13.07	14.58
Σ n-6 PUFA / Σ n-3 PUFA	9.34	2.97	2.57

SFA = saturated fatty acid, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

SFA = zasićene masne kiseline, MUFA = mononezasićene masne kiseline, PUFA = polinezasićene masne kiseline

Table 4 shows the profiles of fatty acids (%) in the egg yolks of the treatments A, B, and C. Statistically significant differences ($p < 0.05$) were found in the composition of Σ SFA, Σ MUFA, Σ n-6 PUFA, and Σ n-3 PUFA between the eggs of the treatments A, B, and C. A statistical analysis showed that the content of Σ n-6 PUFA decreased from 26.02% to 21.34% and to 20.25% (the treatments A, B, and C; $p < 0.05$). Linoleic acid (C18:2 n-6) was significantly reduced from 23.81% to 19.88%, i.e. 18.55%, as was the arachidonic acid (C20:4n-6), from 2.01% to 1.20% and to 1.40% ($p < 0.05$). At the same time, the content of Σ n-3 PUFA increased from 2.14%

to 4.95%, i.e. 4.48%, in the eggs of the treatments A, B, and C. The analysis showed that the mixtures of the treatments B and C in the laying hens' diet increased the content of the n-3 PUFA and decreased the content of the n-6 PUFA in the yolk lipids. The influence of a mixture composition on a fatty-acid profile in eggs was reported by Goldberg et al. (2013), Dalle Zotte et al. (2015), Omri et al. (2015, 2019), and Kralik et al. (2023). The authors stated that the proportions of different feedstuffs in the mixtures influence the fatty-acid profile and consequently also their deposition in the eggs.

Table 4. Content of fatty acids in egg yolks (%)

Tablica 4. Sadržaj masnih kiselina u žumanjku jajeta (%)

Fatty acids / Masne kiseline	Treatments / Tretmani		
	A	B	C
Miristic (C14:0) / Miristinska	0.23	0.21	0.23
Pentadecanoic (C15:0) / Pentadekanska	0.06	0.05	0.08
Palmitic (C16:0) / Palmitinska	21.77	21.60	21.49
Heptadecanoic (C17:0) / Heptadekanska	0.25	0.23	0.24
Stearic (C18:0) / Stearinska	9.99	9.84	8.92
Behenic (C22:0) / Behenska	0.25	-	-
Σ SFA	32.55 ^a	31.93 ^b	30.96 ^b
Palmitoleic (C16:1) / Palmitoleinska	1.27 ^b	1.41 ^a	1.45 ^a
Heptadecanoic (C17:1) / Heptadekanska	0.12 ^b	0.13 ^b	0.34 ^a
Oleic (C18:1) / Oleinska	37.33 ^c	39.68 ^b	41.87 ^a
Eicosaenoic (C20:1) / Eikozaenska	0.20	0.19	0.24
Σ MUFA	38.92 ^c	41.41 ^b	44.00 ^a
Linoleic (C18:2 n-6) / Linolna	23.71 ^a	19.88 ^b	18.55 ^b
Gamma-linolenic (C18:3 n-6) / Gama-linolenska	0.12	0.10	0.07
Eicosadienoic (C20:2 n-6) / Eikosadienoična	0.18	0.16	0.13
Arachidonic (C20:4 n-6) / Arahidonska	2.01 ^a	1.20 ^b	1.40 ^b
Σ n-6 PUFA	26.02 ^a	21.34 ^b	20.15 ^b
α linolenic (C18:3 n-3) / α -linolenska	1.05 ^c	2.08 ^b	2.58 ^a
Eicosapentaenoic (C20:5 n-3) / Eikosapentaenoična	0.08 ^b	0.15 ^a	0.15 ^a
Docosahexaenoic (C22:6 n-3) / Dokosapentaenoična	1.31 ^b	2.08 ^a	2.13 ^a
Σ n-3 PUFA	2.44 ^c	4.95 ^a	4.86 ^b

SFA = saturated fatty acid, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

Means in the same rows followed by different lower-case subscript letters differ significantly ($p < 0.05$)

SFA = zasićene masne kiseline, MUFA = mononezasićene masne kiseline, PUFA = polinezasićene masne kiseline

Srednje vrijednosti u istim redovima iza kojih slijede različita mala slova statistički se razlikuju ($p < 0,05$)

Table 5 shows the quality of lipids in eggs based on the ratio of Σ SFA, Σ PUFA, Σ n-6 PUFA, and Σ n-3 PUFA in the yolk. The activity indices of $\Delta 5$ and $\Delta 6$ desaturases in the metabolism of the n-6 PUFA and the n-3 PUFA are also presented. The results showed that the

forementioned indicators depend on the feeding treatments in such a way that the higher values were found in the eggs of the treatment A if compared to eggs of the treatments B and C.

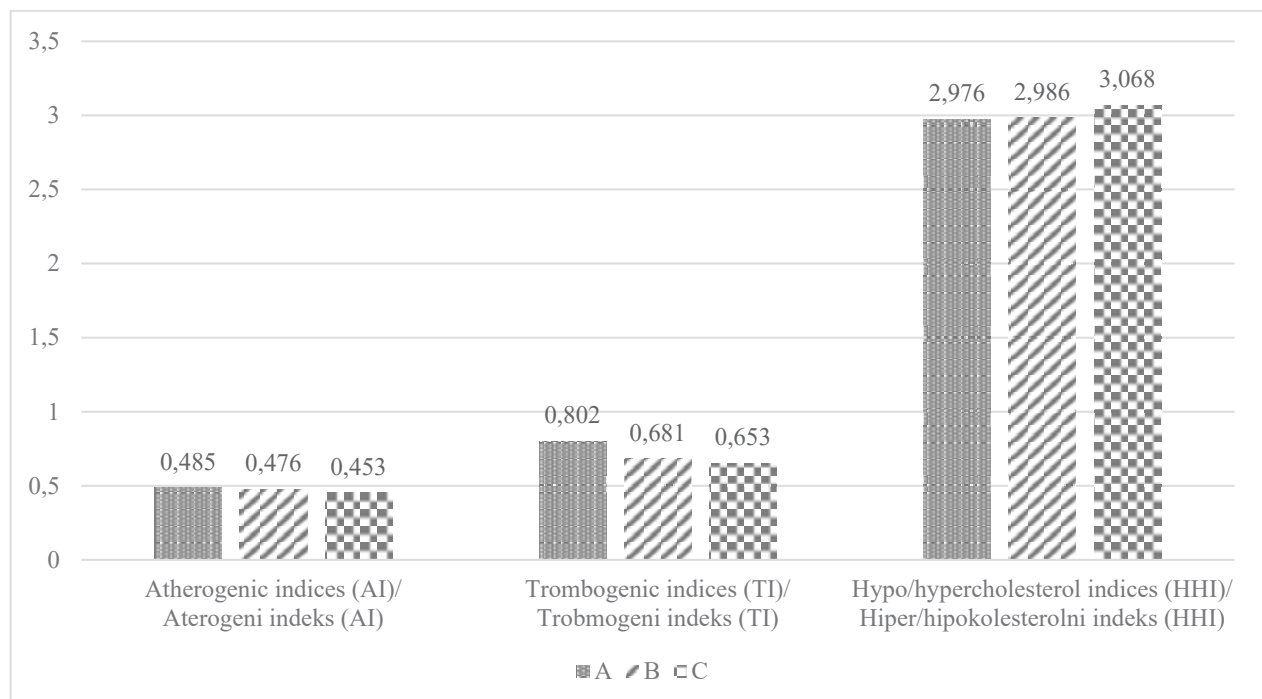
Table 5. Lipid quality of egg yolks

Tablica 5. Kakvoća lipida u žumanjku jajeta

Indicator / Indikator	Treatments / Tretmani		
	A	B	C
$\Sigma n-6$ PUFA / $\Sigma n-3$ PUFA ratio / $\Sigma n-6$ PUFA / $\Sigma n-3$ PUFA omjer	10.66	4.31	4.15
Σ SFA / Σ PUFA ratio / Σ SFA / Σ PUFA omjer	0.48	0.47	0.45
$\Sigma n-6$ PUFA / Σ MUFA ratio / $\Sigma n-6$ PUFA / Σ MUFA omjer	0.69	0.52	0.46
n-6 $\Lambda 5$ + $\Lambda 6$ activity index / n-6 $\Lambda 5$ + $\Lambda 6$ indeks aktivnosti	8.45	6.40	7.62
n-3 $\Lambda 5$ + $\Lambda 6$ activity index / n-3 $\Lambda 5$ + $\Lambda 6$ indeks aktivnosti	56.97	51.17	49.69

Figure 1 shows the values of the atherogenic, thrombogenic, and hypo/hypercholesterolemic indices. Depending on the feeding treatment A, B, and C, the AI values of the eggs were 0.485, 0.476, and 0.453, the TI values were 0.802, 0.681, and 0.653 and the HHI values were 2.976, 2.986, and 3.068. The atherogenic indices show the ratio of a sum of saturated and unsaturated fatty acids. Saturated fatty acids are considered proatherogenic, as they promote the deposition of lipids in the cells of the circulatory system. However, the unsaturated fatty acids have an antiatherogenic effect, as they inhibit the aggregation of plaques and prevent the occurrence of coronary disease (Omri et al., 2019). The

AI and the TI indicators consider the effects of certain fatty acids on the increase of pathogenic phenomena such as the atheroma and thrombi that affect the human health. The eggs from the treatments B and C had the lower values of AI and TI indicators than the eggs from the treatment A. The eggs from the treatments B and C also had the lower levels of the HHI indicators when compared to the eggs from the treatment A. The results of our study are consistent with the statements of Attia et al. (2015), Omri et al. (2019), Untea et al. (2020), and Panaita et al. (2020) on the trends of an increase and decrease in the indicated lipid indices.

**Figure 1. Lipid health quality indicators of eggs**

Grafikon 1. Indikatori lipidne kakvoće jaja

CONCLUSION

The results showed that the feeding treatments used in the research affect the fatty-acid profile of eggs. The highest content of the n-3 PUFA was determined in the eggs of the treatment B, while the narrowest ratio of the $\sum n-6$ PUFA/ $\sum n-3$ PUFA was in the eggs of treatment C. Given that the values of health-lipid indices were calculated from a fatty-acid profile using different mathematical expressions, a difference between the treatments was observed for these values. Although the eggs of the treatment B had the highest content of the n-3 PUFA, the most favorable fatty-acid profile was determined in the eggs of the treatment C, which also had the most favorable values of the health-lipid indices (AI, TI, and HHI).

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UTJECAJ UVOĐENJA BILJNIH ULJA I MIKROALGI U HRANU KOKOŠI NESILICA NA PROFIL MASNIH KISELINA U ŽUMANJKU JAJETA I ZDRAVSTVENE LIPIDNE INDEKSE

SAŽETAK

Konzumna jaja dobar su izvor hranjivih tvari. Na hranjiva svojstva jaja utječe sastav krmne smjese za kokoši nesilice. Važna komponenta u krmnim smjesama su ulja, koja su osim energetske vrijednosti važna i za apsorpciju vitamina topljivih u mastima. Ulja se razlikuju po sadržaju masnih kiselina, koje imaju značajnu ulogu u metabolizmu i očuvanju zdravlja. Lipidni indeksi, kao što su aterogeni (AI), trombogeni (TI) i indeks omjera hiperkolesterolemičnih masnih kiselina (HH), koriste se pri procjeni pokazatelja kvalitete lipida u jajima. Ovo istraživanje imalo je za cilj proizvesti jaja obogaćena n-3 PUFA-om, te utvrditi iz kojega hranidbenog tretmana jaja imaju najpovoljniju kvalitetu lipida. U pokusu su korištene tri skupine nesilica (tretmani A, B i C) koje su hranjene smjesama modificiranoga sastava s obzirom na korištena ulja i mikroalge. Smjese su bile uravnotežene na razini 17 % sirovih proteina i 12,10 MJ/kg ME. Proizvedena jaja korištena su za analizu sadržaja masnih kiselina (SFA, MUFA, n-6 PUFA i n-3 PUFA), na temelju čega su izračunati lipidni indeksi. Rezultati istraživanja jaja iz hranidbenih tretmana A, B i C pokazali su da su AI 0,485, 0,476 i 0,453; TI su 0,802, 0,681 i 0,653, a HH indeksi 2,976, 2,983 i 3,068. Aktivnosti $\Delta 5$ - i $\Delta 6$ desaturaza u sintezi n-6 PUFA i n-3 PUFA u jajima razlikovale su se između hranidbenih tretmana. Utvrđen je utjecaj sastava krmnih smjesa u hranidbi kokoši nesilica na kvalitetu lipidnih indeksa, a najpovoljnije zdravstvene lipidne indekse imala su jaja tretmana C.

Ključne riječi: jaja, masne kiseline, AI, TI, HHI

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