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# **An overview on the use of hemp (*Cannabis sativa* L.) in animal nutrition**

Upotreba konoplje (*Cannabis sativa* L.) u hranidbi domaćih životinja

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# AN OVERVIEW ON THE USE OF HEMP (*Cannabis sativa* L.) IN ANIMAL NUTRITION

Klir, Ž., Novoselec, J., Antunović, Z.

Scientific review  
Pregledni znanstveni članak

## SUMMARY

**The aim of the paper was to research the possibility of using hemp (*Cannabis sativa* L.) in animal nutrition. In animal nutrition, hemp seeds can be used, as well as hempseed cake, and hemp oil as supplement in feed mixtures. Hemp seeds are rich in crude protein and crude fat with adequate proportions of linoleic (LA, C18:2 n-6) and linolenic (ALA, C18:3 n-3) acid. The addition of hemp oil in diets of dairy goats increased milk fat with increasing conjugated fatty acid (CLA) and polyunsaturated fatty acids (PUFA) proportions. When feeding ewes with addition of hemp seeds (180 g/day) or hempseed cake (480 g/day), higher milk fat content was observed with higher proportions of LA, CLA and ALA. Addition of hempseed cake (143 g/kg DM) in diets of cows increased milk yield, compared to the control group and the group with higher levels of hempseed cake (233 or 318 g/kg DM). Nutrition of laying hens with hemp seeds in diet (10 and 20%) provided enrichment of yolk fat with ALA, and did not negatively influence laying performance. The aforementioned indicated possibility of using hemp in diets of animals without major changes in production, and with possible increase of desirable fatty acids in animal products.**

**Keywords:** hemp, nutrition, domestic animals, animal products

## INTRODUCTION

*Cannabis sativa* L. is a cosmopolitan species widely distributed in the world, and this common name is used for various cannabis strains (Žuk-Golaszewska and Golaszewski, 2018). Hemp is a textile plant with cortical fibres and seed rich in oil and one of the oldest non-food plants used by humans, also used for foodstuff, as a drying oil, for therapeutic purposes (Fournier, 2013), and also used in pharmaceuticals and chemistry (Callaway, 2004). Hemp varieties which are cultivated for those purposes must be listed in the European Union (EU) common catalogue of varieties of agricultural plant species, while the maximum content of tetrahydrocannabinol (THC), which is the main psychoactive substance, is limited to 0.2% (w/w) (EFSA, 2011). Although strains of hemp cultivated for food must contain less than 0.2% of THC by weight (whole plant), they may not be free of this compound entirely (Yang et al., 2017).

High and variable soybean price has led to its partial or total replacement as protein source for livestock with alternative feedstuffs (Vasta et al., 2008). Besides,

Nabradi and Popp (2011) reported that there is a presence of genetically modified soybean on the market, which is prohibited in organic farming (EC, 834/2007). The organic farming sector in the EU has been rapidly developing during past decades, therefore control over quality and production is necessary (Antunović et al., 2017a). In recent research, alternative feedstuffs were used in livestock diets to replace soybean as a protein and fat source (Klir et al., 2017, Antunović et al., 2017b; Antunović et al., 2018). In animal nutrition hemp seeds and cakes can be used in all animal species and the whole hemp plant (including stalk and leaves) may be suitable for ruminants, while hemp hurds can be used for bedding (EFSA, 2011). In the previous research, it was determined that hemp oil can be used as a supplement in feed mixtures for animals as a rich source of essential fatty acids (Cozma et al., 2015), while seeds and hempseed cake can be used as a fat and protein source in animals' diets (Mierlita, 2019). Hempseed

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oil contains up to 80% of polyunsaturated fatty acids (PUFA), in which linoleic acid (LA, C18:2 n-6) and  $\alpha$ -linolenic acid (ALA, C18:3 n-3) contents are as high as 60 and 19%, respectively (Parker et al., 2003) compared with other vegetable oils, with the exception of linseed oil (Mierlita, 2018).

Therefore, the aim of the present review paper was to assess from available scientific literature, the possibility of using hemp (*Cannabis sativa* L.) in animal nutrition.

### CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF HEMP

According to Panel on Additives and Products or Substances used in Animal Feed (EFSA, 2011) hemp seeds and hempseed cakes could be used as feedstuffs for all animal species, but do not suggest the use of whole hemp plant-derived feedstuffs in animal nutrition. In contrast, EFSA (2015) considered hemp seeds safe for the consumers exposed to milk from dairy cows fed the hemp plant-derived feed. Table 1 presents hempseed cake and meal as rich sources of crude proteins, while seeds are a very good source of crude fat. Hemp seed oil is a well-balanced source of essential amino acids and fatty acids, thus complete nutritional source (Leizer et al., 2000; Callaway, 2004). Hemp seed protein

has good amounts of the sulphur-containing amino acids and very high levels of arginine and glutamic acid (Callaway, 2004). Composition of essential amino acids of hemp seeds is presented in Table 2. As reported by Mustafa et al. (1999), hempseed meal is an excellent natural source of rumen undegraded crude protein (774.2 g/kg of crude protein), similar to heat-treated canola meal (790.8 g/kg of crude protein). In this study, the protein content of hemp meal was 320.8 g/kg DM, as reported in the Table 1. From the same research, it has been reported that hempseed meal (HM) had higher neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) compared to borage meal, canola meal (CM) and heated canola meal (HCM). Crude protein content of HM was slightly lower compared to CM and HCM, while degradable crude protein and rumen-undegraded crude protein was similar to HCM. This research showed that HM had no detrimental effect on feed intake or nutrient utilization by sheep when substituted for CM in the isonitrogenous diets, suggesting replacement up to 200 g/kg of the diet dry matter (DM).

The apparent metabolizable energy of hempseed cake for chickens is given with 10.1 MJ/kg (Kalmendal, 2008), while hemp seeds and hemp seed cake for laying hens was 3186 and 1849 kcal/kg, respectively (Mierlita, 2019).

**Table 1. Chemical composition of some parts of a whole hemp plant and its byproducts**

Tablica 1. Kemijski sastav pojedinih dijelova konoplje i njenih nusproizvoda

g/kg of DM	Seed <sup>1</sup> <i>Sjemenke</i> <sup>1</sup>	Cake <sup>2</sup> <i>Pogača</i> <sup>2</sup>	Meal <sup>3</sup> <i>Sačma</i> <sup>3</sup>	Hurds <sup>4</sup> <i>Srčika</i> <sup>4</sup>
Dry matter, g/kg <i>Suha tvar, g/kg</i>	912	937	-	963
Crude protein <i>Sirovi protein</i>	249	344	320.8	32.0
Crude fat <i>Sirova mast</i>	327	124	52.4	8.00
NDF	297	393	508	900
ADF	213	321	390	789

<sup>1</sup>Mierlita (2016); <sup>2</sup>Karlsson et al. (2010); <sup>3</sup>Mustafa et al. (1999); <sup>4</sup>EFSA (2011); NDF-neutral detergent fibre -*Neutralna deterđžentna vlakna*; ADF-acid detergent fibre - *Kisela deterđžentna vlakna*

**Table 2. Composition of essential amino acids of different food including hempseeds (Callaway, 2004)**

Tablica 2. Sastav esencijalnih aminokiselina različite hrane uključujući i sjemenke konoplje (Callaway, 2004.)

Amino acid (% of protein) <i>Aminokiselina (% proteina)</i>	Hempseeds <i>Sjemenke konoplje</i>	Soybean <i>Soja</i>	Maize <i>Kukuruz</i>	Egg white <i>Bjelanjak</i>
Histidine – <i>Histidin</i>	0.71	0.76	0.26	0.28
Isoleucine – <i>Izoleucin</i>	0.98	1.62	0.35	0.74
Leucine – <i>Leucin</i>	1.72	2.58	1.19	1.08
Lysine – <i>Lizin</i>	1.03	1.73	0.33	0.74
Methionine – <i>Metionin</i>	0.58	0.53	0.18	0.47
Phenylalanine – <i>Fenilalanin</i>	1.17	1.78	0.46	0.76
Threonine – <i>Treonin</i>	0.88	1.35	0.34	0.58
Tryptophan – <i>Triptofan</i>	0.20	0.41	0.04	0.20
Valine – <i>Valin</i>	1.28	1.60	0.46	0.98

By pressing hemp seeds, 23-28% of oil can be obtained, and by extraction 30-32% (Pasković, 1966). Hemp oil, seeds and cakes are rich in PUFA, especially LA n-6, and ALA n-3. The LA is a dominant fatty acid in hemp oil whose concentration is more than 50%, followed by ALA which is present with over 20% (Table 3), and still lower than ALA concentration in linseed oil (51.3 g/100 g; Bodas et al., 2010), but higher than ALA concentration in canola, soybean, sunflower and olive oil (Leson, 2013). Content of essential fatty acids in hemp oil content is approximately 75% and comprised

primarily of LA and ALA in a 3:1 ratio, therefore it does not promote an over-accumulation of certain metabolic products (Leizer et al., 2000).

Hemp seeds and hempseed cake contain tocopherols, especially  $\gamma$ -tocopherol (60.85 and 33.72 mg/100g DM, respectively), which increased oxidative stability of sheep milk (Mierlita, 2018). The presence of several terpenes was determined in the seed oil, the most abundant of which were  $\beta$ -caryophyllene and myrcene which have anti-inflammatory and cytoprotective activities (Leizer et al., 2000).

**Table 3. Fatty acid profile of hemp seed, hempseed cake and hempseed oil from different research**

Tablica 3. Masnokiselinski profil sjemenki, pogače i ulja konoplje iz različitih istraživanja

% of FAME	Mierlita (2016)	Mierlita (2018)		Juodka et al. (2018)	Cozma et al. (2015)
	Seed Sjemenke	Seed Sjemenke	Cake Pogača	Cake Pogača	Oil Ulje
Myristic (C14:0) <i>Miristinska (C14:0)</i>	0.04	nd	nd	0.07	0.03
Palmitic (C16:0) <i>Palmitinska (C16:0)</i>	5.89	6.20	9.30	4.46	6.54
Palmitoleic (C16:1 n-7) <i>Palmitoleinska (C16:1 n-7)</i>	0.15	nd	nd	0.15	nd
Stearic (C18:0) <i>Stearinska (C18:0)</i>	2.05	2.10	3.80	1.76	2.73
Oleic (C18:1 n-9) <i>Oleinska (C18:1 n-9)</i>	10.11	9.50	13.10	8.27	10.91
Linoleic (C18:2 n-6) <i>Linolna (C18:2 n-6)</i>	56.5	56.10	52.50	59.52	55.78
$\alpha$ -Linolenic (C18:3 n-3) <i><math>\alpha</math>-Linolenska (C18:3 n-3)</i>	21.15	22.40	19.10	15.85	20.65
$\gamma$ -Linolenic (C18:3 n-6) <i><math>\gamma</math>-Linolenska (C18:3 n-6)</i>	nd	3.70	2.20	4.52	nd

FAME-Fatty acid methyl esters - *Metilni esteri masnih kiselina*; nd-Not determined - *Nije utvrđeno*

## USE OF HEMP SEEDS AND HEMPSEED CAKE IN RUMINANTS' NUTRITION

Hemp seeds (HS) and hempseed cake (HSC) as supplements in the diets of livestock have been studied recently. Mierlita (2016) studied dietary supplementation with HS in the amount of 250 g/kg of concentrate mixtures when feeding mid-lactating ewes. Concentrate mixtures were isoproteic, while mixture with HS contained 95 g/kg DM of crude fat compared to control which contained 34 g/kg DM. In this trial, the concentration of LA and ALA of HS were 56.5 and 21.15% of fatty acid methyl esters (FAME), respectively. This feeding increased the fat content in milk and energy corrected milk yield. Authors explained that reason could be due to a slow release of unsaturated fatty acids from hemp seed in rumen, which decreased the amount of *trans* fatty acids, thus milk fat depression was avoided. Additionally, crude fat content in HS diets was much higher compared to controls (95 vs. 34 g/kg DM).

Similarly, Mierlita (2018) fed lactating ewes (DM intake 2.12 kg/d) with feed mixture containing 180 g/d (as-fed) HS or HSC with addition of 480 g/day (as-fed). All diets were isoenergetic. Beside milk fat, milk yield increased as well, when ewes were fed whether with HS or HSC compared to controls, probably due to amino acids of hemp which was balanced for milk protein synthesis. Hemp seeds in diets improved fatty acid profile of ewes' milk, especially with increased proportion of rumenic acid (conjugated linoleic acid-CLA, C18:2 *c9*  $\tau$ 11), and total n-3 fatty acids without detrimental effects on milk production in the study by Mierlita (2016). Chemical composition and fatty acid profile of milk from ewes fed HS or HSC is viewed in the Table 4. Hemp seeds increased concentration of ALA in ewes' milk by 66% and hempseed cake increased ALA by 49% in the study by Mierlita (2018). Total saturated fatty acids (SFA), short-chain fatty acids (SCFA) and medium-chain fatty acids (MCFA) decreased, while PUFA, monounsaturated fatty acids (MUFA), and long-chain fatty acids (LCFA) increased.

**Table 4. Chemical composition and fatty acid profile of milk from ewes fed feed mixtures containing hemp seeds or hempseed cake**

Tablica 4. Kemijski sastav i masnokiselinski profil mlijeka ovaca hranjenih krmnim smjesama koje sadrže sjemenke i pogaču konoplje

Traits Pokazatelj	Mierlita (2016)		Mierlita (2018)		
	Control Kontrola	HS-175g/d	Control Kontrola	HS-180 g/d	HSC-480 g/d
Milk yield (g/day) Količina mlijeka (g/dan)	769.6	784.3	728 <sup>b</sup>	781 <sup>a</sup>	767 <sup>a</sup>
Fat (%) Mast (%)	7.39 <sup>b</sup>	7.98 <sup>a</sup>	7.42 <sup>b</sup>	8.12 <sup>a</sup>	7.97 <sup>a</sup>
Protein (%) Bjelančevine (%)	6.11	6.15	5.61	5.60	5.62
Lactose (%) Laktoza (%)	5.02	5.09	5.20 <sup>a</sup>	5.10 <sup>ab</sup>	4.85 <sup>b</sup>
Fatty acids (% of FAME) - Masne kiseline (% FAME)					
C4:0-8:0	6.86 <sup>b</sup>	8.01 <sup>a</sup>	5.87 <sup>a</sup>	5.91 <sup>a</sup>	4.83 <sup>b</sup>
C10:0	8.53 <sup>a</sup>	4.09 <sup>b</sup>	9.21 <sup>a</sup>	8.39 <sup>b</sup>	7.52 <sup>c</sup>
C12:0	3.34 <sup>a</sup>	2.19 <sup>b</sup>	4.88 <sup>a</sup>	4.27 <sup>b</sup>	3.81 <sup>b</sup>
C14:0	8.64 <sup>a</sup>	6.55 <sup>b</sup>	9.73 <sup>a</sup>	9.15 <sup>b</sup>	8.98 <sup>b</sup>
C16:0	23.31 <sup>a</sup>	20.77 <sup>b</sup>	23.15 <sup>a</sup>	20.87 <sup>b</sup>	20.73 <sup>b</sup>
C16:1 n-7 t11	0.50	0.40	0.51 <sup>c</sup>	1.13 <sup>b</sup>	1.68 <sup>a</sup>
C18:0	11.15 <sup>b</sup>	14.13 <sup>a</sup>	11.12 <sup>a</sup>	10.24 <sup>b</sup>	9.79 <sup>b</sup>
C18:1 n-7	3.47 <sup>b</sup>	5.75 <sup>a</sup>	2.53 <sup>c</sup>	4.38 <sup>b</sup>	5.08 <sup>a</sup>
C18:1 n-9	22.48 <sup>b</sup>	24.66 <sup>a</sup>	22.77	21.37	21.51
C18:2 n-6	1.99 <sup>b</sup>	2.56 <sup>a</sup>	2.08 <sup>c</sup>	2.49 <sup>b</sup>	3.14 <sup>a</sup>
C18:2 n-7 c9t11	2.03 <sup>b</sup>	2.70 <sup>a</sup>	1.07 <sup>b</sup>	2.20 <sup>a</sup>	2.64 <sup>a</sup>
C18:3 n-3	2.06 <sup>b</sup>	2.09 <sup>a</sup>	1.53 <sup>c</sup>	2.54 <sup>a</sup>	2.28 <sup>b</sup>
C18:3 n-6	nd	nd	0.17	0.09	0.10
C20:4 n-6	0.20	0.24	0.21	0.19	0.20
C20:5 n-3	0.28 <sup>b</sup>	0.41 <sup>a</sup>	0.18 <sup>b</sup>	0.34 <sup>a</sup>	0.29 <sup>a</sup>
C22:6 n-3	0.39 <sup>b</sup>	0.56 <sup>a</sup>	0.28 <sup>c</sup>	0.46 <sup>a</sup>	0.37 <sup>b</sup>

FAME-Fatty acid methyl esters - Metilni esteri masnih kiselina; nd-Not determined - Nije utvrđeno; HS-175g/d-feed mixture supplemented with 175 g/day of hemp seeds - Krmna smjesa s dodatkom 175 g/dan sjemenki konoplje; HS-180 g/d-feed mixture supplemented with 180 g/day of hemp seeds - Krmna smjesa s dodatkom 180 g/dan sjemenki konoplje; HSC-480 g/d-feed mixture supplemented with 480 g/d of hempseed cake - Krmna smjesa s dodatkom 480 g/dan pogače konoplje

Mierlita (2018) reported that HSC is less efficient than whole seeds in increasing concentrations of ALA in milk, probably due to substantial biohydrogenation of ALA in rumen, which provides fatty acids from HSC directly in the rumen fluid. Besides, feeding ewes with HSC is more prone to increasing concentration of CLA and vaccenic acid (VA) in milk compared to HS diets. The efficiency of the fatty acid transfer from diet to milk depends on several factors, including nature of the fat supplementation and the interaction between fat source and rumen microbes, when fatty acids are converted in C18:0 by lipolysis and biohydrogenation process (Cremonesi et al., 2018).

Oxidative stability of milk was preserved when the sheep were fed with diets containing HS or HSC, as determined by higher  $\alpha$ -tocopherol concentration in sheep milk, higher total antioxidant capacity, as indicated by lower malondialdehyde concentration in sheep milk (Mierlita, 2018).

In the study by Karlsson et al. (2010), dairy lactating cows were fed with silage (494 g/kg DM) and concentrate mixtures (506 g/kg DM) formulated to contain increasing proportions of HSC: 0, 143, 233 and 318 g/kg DM. Milk yield was the highest when the cows were fed with addition of 143 g/kg DM compared to controls and cows fed with higher levels of HSC. Concentrations of urea increased with every additional level of HSC, while efficiency of converting dietary crude protein into milk protein decreased. Thus, inclusion of 233 or 318 g/kg DM of HSC had no benefits in milk performance.

Young calves were fed diets based with 175 mixed rations *ad libitum* and restricted supplement of protein feed made of HSC in experimental group and 50% of soybean meal and 50% of rolled barley in control group in the study by Hessle et al. (2008). Daily intake of NDF and fat were higher for calves fed HSC than for those animals fed soybean meal, with lower intake of starch. Authors did not determine any differences in liveweight gain of young calves. The NDF intake was higher by 31% when

calves were fed with HSC compared to soybean as related to higher fibre content in HSC, which was also accompanied by fewer long particles in faeces. Higher starch content in soybean meal diets reduced digestion of fibre in rumen (Russel and Wilson, 1996), leading to faster passage rates, therefore increasing number of long particles in faeces (Hessle et al., 2008). Hessle et al. (2008) suggested HSC as alternative protein feed for intensively fed growing cattle. Also, in the same research no differences were determined in carcass traits when steers were fed diets containing HSC compared to soybean meal.

In the trial by Cremonesi et al. (2018), 9.3% (on DM basis) of HS was included in diets for dairy goats with the aim to analyse the microbiome diversity of rumen liquor. The authors observed that HS inclusion in the diet promoted changes in rumen biohydrogenation pathway in dairy goats. Hemp seeds promoted an increase of C18:2 n-6 biohydrogenation intermediates, like C18:1  $\iota$ 6-8, C18:1  $\iota$ 9, C18:1  $\iota$ 10; C18:1  $\iota$ 12, compared to rumen liquor of goats fed linseeds which promoted more production of C18:3 isomers. Thus, the change of ALA and LA ratios in the diets affected the biohydrogenation pathway as reported by Shingfield (2010) as well.

In the research with steers, Turner et al. (2008) studied the use of protein supplement of 0.2-1.4 kg as-fed of HSC or 0.7 kg rolled barley and 0.7 kg of soybean meal. Results showed no differences in hot carcass weights and shear force on cooked *m. Longissimus dorsi* (MLD) from each steer. The concentration of phospholipids, cholesterol, free fatty acids and triacylglycerols were similar as in MLD from steers fed soybean meal. Total lipid fatty acid profile differed in MLD as affected by HSC supplement, which increased VA, oleic acid (OA, C18:1 n-9) and CLA concentrations, with decreasing n-6/n-3 ratio. Since PUFA proportions were not influenced by HSC, authors concluded that both diets are highly subjected to biohydrogenation in the rumen.

### USE OF HEMP SEEDS AND HEMPSEED CAKE FOR NON-RUMINANTS' NUTRITION

In the study by Halle and Schöne (2013) 10 or 15% HSC was included in diets of laying hens, and it was concluded that the proportion of up to 10% of HSC did not negatively influence the laying performance of hens, like feed intake, laying intensity, egg weight or feed conversion. However, by increasing the level of dietary HSC (5, 10 or 15%), rich in PUFA, resulted in linear increase in the concentrations of LA and ALA with decrease of SFA and MUFA. Concentrations of ALA were higher than in yolk from hens fed rapeseed, but still lower than in yolk from hens fed with addition of linseed in the diets.

Research with laying hens fed with the addition of HS or HSC was carried out by Mierlita (2019). In this research ingredients from the control group diets were corn, soybean meal and sunflower oil (2.5%) and compared to experimental mixtures that were designed to replace sunflower oil and partially soybean meal with fat from HS (8.04%) or HSC (20.32%). All concentrate mixtures were isonitrogenous with the highest ALA concentrations and the lowest LA/ALA ratio determined in HS diets. During the 10-week trial, egg weight and egg mass were the highest from laying hens fed diets containing HS (64.7 g and 59.6 g/hen/day, respectively), compared to HSC (58.3 g and 55.8 g/hen/day, respectively) or control diets (58.8 g and 56.6 g/hen/day, respectively). Performance of hens consuming experimental diets was not influenced by HS or HSC diets. In contrast, egg yolk fatty acid profile differed with addition of HSC in the mixtures, leading to increase of LA (18.62% of FAME) compared to HS and the control group (16.47 and 16.69% of FAME), while ALA (2.87% of FAME) was higher only compare to control (0.60% of FAME) and lower compared to HS (3.51% of FAME) group. Concentrations of OA were lower in yolk when hens were fed with HS or HSC, leading to lower MUFA concentrations. The LCFA concentrations, like eicosa-pentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were higher in yolk of experimental groups, as was the total PUFA. The lowest LA/ALA content was determined in HS group, followed by HSC and then control group (4.69, 6.49 and 27.81, respectively). Total cholesterol concentrations in egg and yolk were not influenced by hemp addition in the diets. Higher concentration of  $\alpha$ -tocopherol was determined in eggs from laying hens fed with concentrate mixtures containing HS or HSC, with the highest determined in HS group (Mierlita, 2019), indicating better antioxidant capacity.

Similarly, Gakhar et al. (2012) fed laying hens with the addition of 10 or 20% of HS in diet during 12 weeks. Authors determined higher egg weights in hens fed with 20% of HS, compared to other groups, with no differences in feed intake and body weight or egg quality like eggshell thickness, albumen height or specific gravity. Also, fatty acid profile of egg differed in proportions of ALA, EPA, docosapentaenoic acid (DPA, C22:5 n-3) and DHA, being the highest in group fed with 20% of HS (Table 5). In the study by Raza et al. (2016) the addition of hempseed at the level of 25% in the diet of hens improved n-3 PUFA and the n-3/n-6 ratio with decreasing SFA in the yolk of eggs stored at room temperature for 30 days.

**Table 5. Laying performance and fatty acid concentration of egg yolk from hens fed diets containing hemp seeds (Gakhar et al., 2012)**

Tablica 5. Proizvodni pokazatelji i koncentracija masnih kiselina žumanjka jajeta kokoši nesilica hranjenih obrocima koje sadrže sjemenke konoplje (Gakhar i sur., 2012.)

Traits Pokazatelj	Control Kontrola	HS-10%	HS-20%	P-values / P-vrijednost		
				Diet (D) Obrok (D)	Week (W) Tjedan (W)	D×W
Egg weight (g) Masa jajeta (g)	56.2 <sup>b</sup>	56.7 <sup>b</sup>	60.5 <sup>a</sup>	<0.01	<0.01	0.86
Feed intake (g/day) Konzumacija hrane (g/dan)	96.9	96.0	98.3	0.78	0.48	0.70
Body weight (kg) Tjelesna masa (kg)	1.52	1.52	1.56	0.57	0.66	0.99
FCE (g of feed/g of egg)	1.74	1.73	1.63	0.22	0.85	0.60
Egg mass (g/hen/day) Masa jajeta (g/nesilica/dan)	55.9	55.3	60.0	0.01	0.01	0.94
Fatty acids (mg/yolk) - Masne kiseline (mg/žumanjak)						
C16:0	728	757	737	0.87	<0.01	0.76
C16:1 n-7	38	37	31	0.19	<0.01	0.04
C18:0	264 <sup>b</sup>	299 <sup>ab</sup>	319 <sup>a</sup>	0.03	<0.01	0.63
C18:1 n-9	963	990	921	0.60	<0.01	0.78
C18:2 n-6	963	1029	1080	0.27	<0.01	0.78
C18:3 n-3	15.8 <sup>c</sup>	51.7 <sup>b</sup>	91.3 <sup>a</sup>	<0.01	0.01	0.08
C20:5 n-3	0.2 <sup>c</sup>	0.9 <sup>b</sup>	1.2 <sup>a</sup>	<0.01	0.17	0.40
C22:5 n-3	1.8 <sup>c</sup>	3.7 <sup>b</sup>	4.8 <sup>a</sup>	<0.01	0.01	0.16
C22:6 n-3	17.1 <sup>c</sup>	39.2 <sup>b</sup>	47.4 <sup>a</sup>	<0.01	0.01	0.01
n-3	34.9 <sup>c</sup>	95.5 <sup>b</sup>	144.6 <sup>a</sup>	<0.01	<0.01	0.03

FCE-Feed conversion efficiency-účinkovitost konverzije hrane; HS-10% - Feed mixture supplemented with 10% of hemp seeds - Krmna smjesa s dodatkom 10% sjemenki konoplje; HS-20%-Feed mixture supplemented with 20% of hemp seeds - Krmna smjesa s dodatkom 20% sjemenki konoplje

Št'astnik et al. (2019) studied the addition of 5 and 15% of HSC in diets for broilers which were formulated to obtain similar apparent metabolizable energy. When 15% of HSC was used in the diets, a decreased live weight of broilers was observed (2.079 vs. 2.300 g), compared to diets without HSC, while carcass weight, percentage of breast meat and thigh meat did not differ. Authors explained that this was due to differences in feed intake, due to higher content of fibre in the diet with 15% of HSC (59.75 vs 27.22 g/kg of diet), which was higher than it is recommended for broilers. The experimental diets with 5% of HSC did not influence live body weight of broilers.

Similarly, experimental diets did not lead to any differences regarding the chemical composition of breast and thigh meat. A similar study was conducted by Neijat et al. (2014), where laying hens were fed diets containing 10, 20 or 30% of HS. Overall performance of hens, like feed intake, body weight gain, rate of egg lay and egg weight did not differ between groups. Also, plasma proteins and concentration of cholesterol and glucose were not influenced by addition of HS in the diet in this research.

### ADDITION OF HEMPSEED OIL IN DIETS

Addition of hempseed oil (HSO) in the hay-based diets for dairy goats was studied in the trial by Cozma et al. (2015). Hempseed oil was added in the concentrate mixture by 4.70% during 31 days of the experiment. The addition of HSO did not influence milk yield, while milk fat content was higher compared to the control group (Table 6). This can be explained by *in vitro* study carried out by

Bernard et al. (2013), in which lipogenesis in goats were not affected by ruminal biohydrogenation products.

From the Table 6 it can be viewed that HSO added in goats' diets increased CLA, LA, MUFA and PUFA concentrations, but lowered total SFA, probably due to lowered palmitic acid which is a dominant SFA in goats' milk. At the same time, proportions of certain SCFA, such as C6:0, C8:0 were not influenced by HSO which is desirable, because these fatty acids are responsible for the particular sensory properties of goat's cheese upon lipolysis (Klir et al., 2017), although C10:0 was slightly lowered. The VA increased by 192% in the trial by Cozma et al. (2015), as a result of rumen incomplete biohydrogenation of ALA and LA, which is in agreement with Chilliard et al. (2007). According to Chilliard et al. (2003), biohydrogenation was less efficient when oil was added free than as part of the seeds, which inhibited the biohydrogenation of its own fatty acids in rumen, thus increasing the transfer of PUFA and *trans* fatty acids to milk, which means that VA and CLA were more significantly increased by free oil than by oilseeds, whereas stearic and oleic acids were less affected.

The addition of HSO did not affect goats' plasma parameters, like cholesterol, triglycerides, phospholipids, although total lipids were increased, suggesting possible hyperlipidemic effect (Cozma et al., 2015). Activities of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) were not influenced by HSO in the study by Cozma et al. (2015). Similar subject was studied by Neijat et al. (2014), where laying hens were fed diets containing 4.5 or 9% of HSO. Activity of GGT



in hens' plasma was lower when 4.5% of HSO was added in the diet (Table 7), which may indicate possible protective effect of HSO on liver damage. At the same

time, overall performance of hens and plasma proteins and cholesterol concentration were not influenced by dietary HSO addition in the study by Neijat et al. (2014).

**Table 6. Dairy performance and fatty acid profile of milk from goats fed diets containing hempseed oil (Cozma et al., 2015)**

Tablica 6. Proizvodnja mlijeka i masnokiselinski profil mlijeka koza hranjenih obrocima s dodatkom ulja konoplje (Cozma i sur., 2015.)

Traits – Pokazatelji	Diet – Obrok		SEM	P-value P-vrijednost
	Control Kontrola	Hempseed oil Ulje konoplje		
Milk yield (l/day) Količina mlijeka (l/dan)	1.28	1.33	0.041	ns
Fat (%) - Mast (%)	2.70	3.59	0.120	<0.001
Protein (%) - Bjelančevine (%)	3.16	3.28	0.034	<0.05
Fatty acids (g/100 g of total fatty acids) - Masne kiseline (g/100 g ukupnih masnih kiselina)				
C6:0	2.67	2.69	0.071	ns
C8:0	2.95	2.87	0.098	ns
C10:0	9.64	8.44	0.373	<0.05
C12:0	4.34	3.15	0.225	<0.01
C14:0	9.31	7.56	0.377	<0.01
C16:0	24.20	20.74	0.671	<0.01
C18:0	9.70	11.59	0.740	<0.10
C18:1 n-9	17.43	15.35	1.174	ns
C18:1 n-7 t11	3.06	8.95	0.724	<0.001
C18:2 n-7 c9t11	0.49	2.14	0.174	<0.001
C18:2 n-6	2.40	2.77	0.138	<0.10
C18:3 n-3	0.94	1.21	0.159	ns
SFA	70.97	64.59	0.762	<0.001
OBCFA	5.36	4.58	0.134	<0.001
MUFA	22.77	26.53	0.745	<0.01
PUFA	5.30	7.69	0.287	<0.001
Trans FA	6.63	14.85	0.925	<0.001

SEM-Standard error of the mean - Standardna pogreška srednje vrijednosti; ns-Not significant - Nije značajno; SFA-Saturated fatty acids - Zasićene masne kiseline; OBCFA-Odd and branched-chain fatty acids - Neparne i razgranate masne kiseline; MUFA-Monounsaturated fatty acids - Mononezasićene masne kiseline; PUFA-Polyunsaturated fatty acids - Polinezasićene masne kiseline; Trans FA-Trans fatty acids - Trans masne kiseline

**Table 7. Activities of enzymes and electrolyte concentration in plasma of laying hens fed diets containing hempseed oil (Neijat et al., 2014)**

Tablica 7. Aktivnosti enzima i koncentracija elektrolita u plazmi kokoši nesilica hranjenih obrocima s dodatkom ulja konoplje (Neijat i sur., 2014)

Enzymes (U/L) - Enzimi (U/L)	Control Kontrola	HSO-4.5%	HSO-9%	SEM	P-value P-vrijednost
Creatine kinase Kreatin kinaza	432	472	416	35.1	0.53
Aspartate aminotransferase Aspartat aminotransferaza	180	169	167	5.12	0.17
Gamma glutamyl-transferase Gamma glutamil-transferaza	39.8 <sup>a</sup>	32.9 <sup>b</sup>	37.9 <sup>a</sup>	1.30	<0.01
Electrolyte (mmol/L) - Elektroliti (mmol/L)					
Na	153	150	152	1.42	0.30
K	4.86 <sup>a</sup>	4.62 <sup>b</sup>	4.88 <sup>a</sup>	0.07	<0.05
Cl	122	121	122	1.03	0.58
P	1.98	1.98	2.07	0.10	<0.001
Ca	6.73	6.45	6.79	0.23	0.55

SEM-Standard error of the mean - Standardna pogreška srednje vrijednosti; HSO-4.5%-diet supplemented with 4.5% of hempseed oil - Obrok s dodatkom 4,5% ulja konoplje; HSO-9%-diet supplemented with 9% of hempseed oil - Obrok s dodatkom 9% ulja konoplje

Gakhar et al. (2012) fed laying hens with addition of 4, 8 or 12% of hemp oil in diet during 12 weeks. As the addition of HSO was higher, concentrations of LA, ALA, EPA, DPA and DHA in eggs were increasing without the effect on feed intake or production of eggs. Neijat et al. (2016) also determined increase of these fatty acids in

total lipids of egg yolk compared to controls, especially ALA, which multiplied by 5 or 12 times when 4.5 or 9% of HSO was added, respectively (Table 8). Compared to the control diet, in the trial by Gakhar et al. (2012), hepatic fatty acid desaturase-1 (encoding  $\Delta$ -5 desaturase, *FADS1*) mRNA expression was reduced by 39% in hens fed diets

containing 12% of HSO, while *FADS2* mRNA decreased by 45 and 51% when fed diets containing 8 and 12%, respectively.

Mourrot and Guillevic (2015) carried out a trial with pigs fed diets which only differed in the source of fat content; either palm oil (PO), or rapeseed oil (RO), or HSO. The PO diet contained 0.6 g of ALA/kg of feed, the RO diet contained 1.9 g and the HSO 3.4 g. Growth performance, body composition and meat quality parameters were similar

between differently fed pigs, lipid content in the MLD was higher when pigs were fed with addition of HSO. Likewise, HSO led to increased LA concentration in plasma, back fat and liver compared to other oils, and lowered OA concentration in plasma and back fat. Hempseed oil increased ALA concentration in plasma, back fat, MLD and liver, while total n-3 fatty acids were multiplied by 2.6 times. This was expected, since HSO diet contained 10.97 % of ALA compared to other diets which were lower in n-3 fatty acids.

**Table 8. Laying performance and fatty acid concentrations of egg yolk from hens fed diets containing hempseed oil (Gakhar et al., 2012)**

Tablica 8. Proizvodni pokazatelji i koncentracije masnih kiselina žumanjka jajeta kokoši nesilica hranjenih obrocima s dodatkom ulja konoplje (Gakhar i sur., 2012.)

Traits - Pokazatelji	Control Kontrola	HSO-4%	HSO-8%	HSO-12%	P-value / P-vrijednost		
					Diet (D) Obrok (D)	Week (W) Tjedan (W)	D×W
Egg weight (g) Masa jajeta (g)	56.2	55.9	54.6	55.8	0.57	0.03	0.99
Feed intake (g/day) Konzumacija hrane (g/dan)	96.0 <sup>a</sup>	81.2 <sup>b</sup>	89.6 <sup>a</sup>	88.7 <sup>a</sup>	0.03	0.88	0.37
Body weight (kg) Tjelesna masa (kg)	1.52	1.49	1.58	1.47	0.11	0.98	0.96
FCE (g of feed/g of egg)	1.74	1.70	1.83	1.66	0.31	0.73	0.13
Egg mass (g/hen/day) Masa jajeta (g/nesilica/dan)	55.9	57.0	53.9	55.1	0.25	0.21	0.50
Fatty acids (mg/yolk) - Masne kiseline (mg/žumanjak)							
C16:0	725	673	697	716	0.50	<0.01	0.28
C16:1 n-7	38 <sup>ab</sup>	34 <sup>a</sup>	42 <sup>b</sup>	38 <sup>ab</sup>	0.10	<0.01	<0.01
C18:0	264 <sup>c</sup>	273 <sup>c</sup>	314 <sup>b</sup>	353 <sup>a</sup>	<0.01	<0.01	0.12
C18:1 n-9	961 <sup>a</sup>	863 <sup>b</sup>	847 <sup>b</sup>	758 <sup>d</sup>	<0.01	<0.01	0.2
C18:2 n-6	960 <sup>b</sup>	842 <sup>b</sup>	956 <sup>b</sup>	1090 <sup>a</sup>	0.01	<0.01	0.43
C18:3 n-3	15.8 <sup>d</sup>	58.7 <sup>c</sup>	108.9 <sup>b</sup>	192.3 <sup>a</sup>	<0.01	<0.01	<0.01
C20:5 n-3	0.2 <sup>d</sup>	1.1 <sup>c</sup>	1.9 <sup>b</sup>	2.8 <sup>a</sup>	<0.01	0.01	0.53
C22:5 n-3	1.8 <sup>d</sup>	5.4 <sup>bc</sup>	5.3 <sup>b</sup>	7.3 <sup>a</sup>	<0.01	0.15	0.59
C22:6 n-3	17.1 <sup>c</sup>	40.9 <sup>b</sup>	45.6 <sup>a</sup>	48.1 <sup>a</sup>	<0.01	<0.01	0.1
n-3	34.9 <sup>d</sup>	106.0 <sup>c</sup>	161.7 <sup>b</sup>	250.6 <sup>a</sup>	<0.0001	<0.0001	<0.01

FCE-Feed conversion efficiency - Učinkovitost konverzije hrane; HSO-4%-Diet supplemented with 4% of hempseed oil - Obrok s dodatkom 4% ulja konoplje; HSO-8%-Diet supplemented with 8% of hempseed oil - Obrok s dodatkom 8% ulja konoplje; HSO-12%-diet supplemented with 12% of hempseed oil - Obrok s dodatkom 12% ulja konoplje.

## CONCLUSION

The hemp seeds and hempseed cake may be included in diets of animals, as a valuable source of crude protein and essential fat, without major changes in production traits, but with increasing beneficial fatty acids in animals' products. Hempseed oil, as a supplement in feed mixtures may be efficiently used as a source of essential fatty acids, especially in the enrichment of animal products with PUFA n-3 and n-6 fatty acids. In further studies, it is necessary to research whether the hemp derived feed affects the metabolic profile of animals with an emphasis on antioxidant status of blood and sensory properties of products, which will give a more comprehensive picture of hemp use in animal nutrition.

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## UPOTREBA KONOPLJE (*Cannabis sativa* L.) U HRANIDBI DOMAĆIH ŽIVOTINJA

### SAŽETAK

**Cilj je ovoga rada istražiti mogućnost upotrebe konoplje (*Cannabis sativa* L.) u hranidbi domaćih životinja. U hranidbi domaćih životinja mogu se koristiti sjemenke, pogača od sjemenki konoplje i ulje konoplje kao dodatak krmnim smjesama. Sjemenke konoplje su bogate sirovim bjelančevinama i sirovim mastima s odgovarajućom koncentracijom nezasićenih masnih kiselina, osobito linolnom (LA, C18:2 n-6) i linolenskom (ALA, C18:3 n-3) kiselinom. Dodatak ulja konoplje u obroke koza u laktaciji povećao je udio masti u mlijeku te je doveo do povećanja koncentracije konjugirane linolne (CLA) kiseline i polinezasićenih masnih kiselina (PUFA). Pri hranidbi ovaca krmnim smjesama s dodatkom sjemenki konoplje (180 g/dan) ili pogače sjemenki konoplje (480 g/dan) utvrđeno je povećanje količine mliječne masti, kao i povećanje koncentracije LA, CLA i ALA. Pogača sjemenki konoplje (143 g/kg ST) u obrocima krava povećala je količinu proizvedenoga mlijeka u usporedbi s kontrolnom skupinom i skupinom krava koje su konzumirale više razine pogače sjemenki konoplje (233 ili 318 g/kg ST). Hranidba kokoši nesilica pogačom sjemenki konoplje u obrocima (10 i 20%) rezultirala je obogaćivanjem žumanjka ALA-om, pri čemu nije utvrđen negativan utjecaj na pokazatelje nesivosti. Navedeno ukazuje na mogućnost upotrebe konoplje u obrocima domaćih životinja bez većih promjena u proizvodnji, uz mogućnost povećanja poželjnih masnih kiselina u animalnim proizvodima.**

**Ključne riječi:** konoplja, hranidba, domaće životinje, animalni proizvodi

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