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THE INCREASE OF THE N-3 PUFA CONTENT IN EGGS

Z. Škrtić, Gordana Kralik, Zlata Gajčević, I. Bogut, Danica Hanžek

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SUMMARY

The research on effects of different oils on the profile of fatty acids in egg yolks was performed on 80 ISA Brown laying hens (40 in control and 40 in experimental group). Hens were 50 weeks old. The control group of hens was fed diets supplemented with sunflower oil (6%), and the experimental group was given a combination of rapeseed oil (4%) and fish oil (2%). Hens' diets contained 16.8% of crude protein and 11.57 MJ ME. The research lasted for 28 days. Content of fatty acids in yolk lipids was determined on 10 samples taken from each group. Supplemented oils (sunflower, rapeseed and fish oil) in hens' diets had significant effect on the profile of fatty acids in yolk lipids. Compared to the control group, yolk lipids of eggs produced by hens in the experimental group contained significantly higher (P<0.001) portion of favorable fatty acids (linolenic, C18:3n-3; eicosapentaenoic, C20:5n-3 and docosahexaenoic, C22:6n-3). Moreover, the experimental group of hens laid eggs that contained less unfavorable saturated fatty acids (P<0.001) and less linoleic (C18:2n-6) and arachidonic acid (C20:4n-6) in their lipids. Content of oleic acid (C18:1) and total monounsaturated fatty acids (MUFA) was significantly higher (P<0.001) in yolk lipids of the experimental group. The experimental group contained less unfavorable polyunsaturated fatty acids of the n-6 type (n-6 PUFA, P<0.001), more favorable fatty acids of the n-3 type (n-3 PUFA, P<0.001) and more favorable n-6 / n-3 PUFA ratio (P<0.001) in yolk lipids than the control group.

Key-words: laying hen, egg, yolk, fatty acid profile, EPA, DHA, n-3 PUFA

INTRODUCTION

Polyunsaturated n-3 fatty acids (n-3 PUFA) have beneficial effect on the lowering of plasmatic triglycerides and blood pressure, on healing tumors, diabetes, blood coagulation, thrombosis, as well as on the enhancement of immunity (Barlow and Pike, 1991, Simopoulos, 2000). Favorable effect on human health could be achieved by consuming only 0.5 g of n-3 LCPUFA (Mantzioris, 2000). Consumption of n-3 PUFA in developed countries is insufficient mainly because of low intake of fish. This is also worsened through feeding of animals with feeds rich in n-6 PUFA, as this leads to production of meat rich in n-6 PUFA and poor in n-3 PUFA. Similar situation occurs in industrial production of pigs, fish and chicken eggs (Lugasi et al., 2006; Simopoulos and Salem, 1989). Even cultivated plants contain less n-3 PUFA than wild plants (Simopoulos and Salem, 1989). Some 10 000 years ago, the n-6 / n-3 PUFA ratio in human nutrition was 1-4:1. Nowadays, this ratio is 20-30:1 (Calvani and Benatti, 2003). Content of n-3 PUFA in poultry meat and eggs can be increased through the use of feeds originating from plants or sea organisms (algae, fish). Dietary supplementation with feeds originating from plants (linseeds, rapeseed and linseed oil) results in the increase of α -linolenic acid (LNA, C18:3n-3) (Caston and Leeson, 1990; Cherian and Sim, 1991; Kovács et al 2000), while feeds originating from sea organisms affect the increase of eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids (Hargis et al.: 1991. Meluzzi et al., 2000). However, many authors pointed out higher biological value of

DSc Zoran Škrtić, Assistant Professor, Prof.DSc.Dr.h.c. Gordana Kralik, MSc Zlata Gajčević, Prof.DSc. Ivan Bogut and BSc Danica Hanžek - Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture, Trg sv. Trojstva 3, 31000 Osijek

EPA and DHA than of LNA (Ollis et al, 1999; Simopoulos, 2000). The most favorable profile of n-3 PUFA is achieved when combining forages of plant and animal origin. Sunflower oil or soybean oil are the most used in Croatia as supplements to hens' diets. These oils have bad influence on the profile of fatty acids in poultry products (Kralik et al., 2004, 2006). The authors assume that replacement of one part of sunflower oil in hens' diets with a combination of fish and rapeseed oil can significantly alter the profile of fatty acids in yolk lipids. The aim of this research was to investigate the influence of different oils (sunflower, fish and rapeseed oil) on the profile of fatty acids contained in egg yolks.

MATERIAL AND METHODS

The research was performed on 80 ISA Brown laying hens being 50 weeks old. Hens were divided into two equal groups (40 in control and 40 in experimental group). One cage housed four hens, and each group had ten repetitions. The research lasted for 28 days. Laying hens were given food and water ad libitum. During the experiment, a lightning regime of 16h light and 8h darkness was applied. Hens in the control group were given sunflower oil supplemented to diets (6%), and the experimental group was fed a combination of rapeseed oil (4%) and fish oil (2%) (Table 1). In the total of fatty acid content, sunflower and rapeseed oil contained 12% and 7% saturated fatty acids (SFA) and 13% and 59% monounsaturated fatty acids (MUFA), respectively. As expected, neither sunflower nor rapeseed oil contained eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). Fish oil contained 37% SFA, 21% MUFA and 30% EPA+DHA. Feeding mixture fed to the control group of hens contained 34% less oleic acid (OA, C18:1) and 42% more linoleic acid (LA, C18:2n-6) than the mixture fed to the experimental group (Table 2). Almost triple content of α -linolenic acid (LNA, C18:3n-3) was determined in the mixture fed to the experimental group, in comparison to the control group (4.46%:1.53%). Diets fed to the experimental group contained 1.52% eicosapentaenoic (EPA, C20:5n-3) and 2.91% docosahexaenoic (DHA, C22:6n-3) acids in the total of fatty acid content. EPA and DHA in diets fed to hens in the experimental group originated from fish oil. Unlike diets of the experimental group, those fed to the control group of laying hens contained neither desirable n-3 PUFA (EPA and DHA) nor lauric (C12:0), heptadecenoic (C17:1), y-linolenic (C18:3n-6), and arachidonic (AA, C20:4n-6) acids.

Table 1. Feed mixtures

Tablica 1. Sastav smjesa

Content - <i>Sadržaj,</i> %	Control group Kontrolna skupina	Experimental group <i>Pokusna</i> skupina		
Corn – Kukuruz	44.85	44.85		
Soybean cake – Sojina sačma, 46%	16.50	16.50		
Toasted soybean – Tostirana soja	6.50	6.50		
Sunflower cake – Suncokretova pogača	9.00	9.00		
Dehydrated alfalfa – Dehidrirana lucerka	2.00	2.00		
Limestone – Stočna kreda	12.50	12.50		
Phosphonal – Fosfonal	1.55	1.55		
Sunflower oil – Suncokretovo ulje	6.00	0.00		
Rapeseed oil – Repičino ulje	0.00	4.00		
Fish oil – <i>Riblje ulje</i>	0.00	2.00		
Salt – Sol	0.35	0.35		
Methionine – Metionin	0.15	0.15		
Premix ¹ - Premiks	0.60	0.60		
Total – Ukupno	100.00	100.00		
Calculated composition of	Calculated composition of diets – Kalkuliran sastav obroka			
Crude protein – Sirovi protein, %	16.80	16.80		
Crude fat – Sirova mast, %	9.42	9.42		
Crude fibre – Sirova vlakna, %	4.73	4.73		
Ash – Pepeo, %	15.49	15.49		
Lysine – <i>Lizin</i> , %	0.80	0.80		
Methionine – Metionin, %	0.42	0.42		
Triptophan – Triptofan, %	0.21	0.21		
Arginine – Arginin, %	1.11	1.11		
Ca, %	4.10	4.10		
P total – P ukupni, %	0.60	0.60		
P usable – P iskoristivi, %	0.36	0.36		
Na, %	0.16	0.16		
Cl, %	0.21	0.21		
Linoleic acid – Linolna kiselina, %	4.80	4.80		
ME (MJ/kg)	11.57	11.57		

¹ Premix (1 kg) contains: vitamin A 2,200.000 i.u., vitamin D₃ 400.000 i.u., vitamin E 3.000 mg/kg. vitamin K₃ 400 mg/kg, vitamin B₁ 400 mg/kg, vitamin B₂ 800 mg/kg, nicotinic acid 6.000 mg/kg, calcium panthotenate 1.600 mg/kg, vitamin B₆ 700 mg/kg, vitamin B₁₂ 4.000 mcg/kg, folic acid 150 mg/kg, biotin 10 mg/kg, colin chloride 80.000 mg/kg, vitamin C 4.000 mg/kg, methionine 40.000 mg/kg, iodine 160 mg/kg, manganese 13.600-18.400 mg/kg, zinc 12.000 mg/kg, cobalt 48 mg/kg, iron 5.000 mg/kg, copper 500 mg/kg, selenium 30 mg/kg, β-apo ester carotene acid 200 mg/kg, cantaxantin 600 mg/kg and a plant base up to 1 kg.

¹ Premiks (1 kg) sadrži: vitamin A 2.200.000 i.u., vitamin D_3 400.000 i.u., vitamin E 3000 mg/kg, vitamin K_3 400 mg/kg, vitamin B_1 400 mg/kg, vitamin B_2 800 mg/kg, nikotinska kiselina 6000 mg/kg, kalcijev pantotenat 1600 mg/kg, vitamin B_6 700 mg/kg, vitamin B_{12} 4000 mcg/kg, folna kiselina 150 mg/kg, biotin 10 mg/kg, kolin klorid 80.000 mg/kg, vitamin C 4000 mg/kg, metionin 40.000 mg/kg, jod 160 mg/kg, mangan 13.600-18.400 mg/kg, cink 12.000 mg/kg, kobalt 48 mg/kg, željezo 5000 mg/kg, bakar 500 mg/kg, selen 30 mg/kg, β -apo ester carotene acid 200 mg/kg, cantaxantin 600 mg/kg i biljni nosač do 1 kg.

Table 2. Content of fatty acids in feed mixtures (% of total fatty acids)

Tablica 2. Sadržaj masnih kiselina u smjesama	(% od ukupnih masnih kiselina)
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Fatty acid – Masna kiselina, %	Control group	Experimental group	
-	Kontrolna skupina	Pokusna skupina	
Lauric - Laurinska (C12:0)	0,00	0,03	
Myristic – Miristinska (C14:0)	0,12	1,40	
Pentadecanoic – Pentadekanska (C15:0)	0,03	0,23	
Palmitic – Palmitinska (C16:0)	12,40	13,22	
Heptadecanoic – Heptadekanska (C17:0)	0,07	0,25	
Stearic – Stearinska (C18:0)	3,34	3,20	
Arachidic – Arahidonska (C20:0)	0,43	0,59	
Behenic – Behenska (C22:0)	0,15	0,35	
∑SFA	16,54	19,27	
Palmitoleic – Palmitoleinska (C16:1)	0,11	1,22	
Heptadecenoic – Heptadekenska (C17:1)	0,00	0,08	
Oleic – Oleinska (C18:1)	27,95	37,61	
Eicosenoic – Eikozenska (C20:1)	0,23	0,84	
∑MUFA	28,29	39,75	
Linoleic – Linolna (C18:2n-6)	53,38	31,15	
γ-linolenic – γ-linolenska (C18:3n-6)	0,00	0,09	
Eicosadienoic – Eikozadienska (C20:2n-6)	0,06	0,52	
Eicosatrienoic - Eikozatrienska (C20:3n-6)	0,20	0,16	
Arachidonic – Arahidonska (C20:4n-6)	0,00	0,15	
∑ n-6 PUFA	53,64	32,07	
α-linolenic - α-linolenska (C18:3n-3)	1,53	4,46	
Eicosapentaenoic - Eikozapentaenska (C20:5n-3)	0,00	1,52	
Docosahexaenoic – Dokozaheksaenska (C22:6n-3)	0,00	2,91	
∑ n-3 PUFA	1,53	8,89	
∑PUFA	55,17	40,96	
∑SFA/MUFA	0,58	0,48	
∑SFA/PUFA	0,30	0,47	
∑ n-6 / n-3 PUFA	35,06	3,61	

The content of fatty acids in the volk lipids was determined on 10 samples from each group by using Chrompack CP-9000 chromatograph equipped with flame ionization detector (Csapo et al., 1986). Portion of particular fatty acids in diets, as well as in yolk lipids was shown as a percentage of total fatty acids. The following acids were determined: myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0), stearic (C18:0), behenic (C22:0), heptadecenoic (C17:1), oleic (C18:1), eicosenic (C20:1), linoleic (LA, C18:2n-6), ylinolenic (C18:3n-6), eicosadienoic (C20:2n-6), eicosatrienoic (C20:3n-6), arachidonic (AA, C20:4n-6), α -linolenic (LNA, C18:3n-3), eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids. The sum of SFA was presented as C14:0+C15:0+C16:0+C17:0+C18:0+C22:0, and Σ MUFA was presented as C17:1+C18:1+C20:1. The sum of n-6 PUFA was presented as C18:2n-6+ C18:3n-6+C20:2n-6+C20:3n-6+C20:4n-6, and Σ n-3 PUFA was presented as C18:3n-3+C20:5n-3+C22:6n-3. The content of fatty acids in egg yolks was obtained by calculating arithmetic means (\bar{x}) and

standard deviation (s). Differences in investigated characteristics were determined by the ttest at three significance levels (5% P<0.05, 1% P<0.01 and 0.1% P<0.001) by using Statistica v7.1.30.0 software (STATSOFT.INC 1984-2005).

RESULTS AND DISCUSSION

The profile of fatty acids is presented in Table 3. Supplemented oils (6% of sunflower oil supplemented to diets of the control group, 4% of rapeseed oil and 2% of fish oil supplemented to diets of the experimental group) had significant effect on the profile of fatty acids in egg yolks. The control group exhibited significantly higher (P<0.001) portion of stearic acid (C18:0) in yolks than the experimental group (9.10%:7.53%). Higher portion (P<0.001) of myristic (C14:0), pentadecanoic (C15:0) and heptadecanoic (C17:0) acids, as well as of palmitic acid (C16:0, P<0.05) was determined in egg yolks produced by laying hens of the experimental group.

Supplementation of rapeseed oil (4%) and fish oil (2%) in diets fed to hens of the experimental group affected the increase of the MUFA content in yolks. Eggs laid in the experimental group had higher content of palmitoleic (C16:1, P<0.05) and heptadecanoic (C17:1, P<0.001) acids than eggs produced in the control group. There was no difference determined between investigated groups (P>0.05) with respect to the content of myristoleic (C14:1) and eicosenic (C20:1) acids in yolk lipids. In both groups the most represented fatty acid in egg yolks was oleic acid (OA, C18:1). The content of OA in egg yolks of the experimental group was significantly higher (P<0.001) than in yolks of the control group (45.40%:38.56%).

When compared to yolks of the experimental group, yolks of the control group (supplemented 6% of sunflower oil) had significantly higher content of all n-6 PUFA. The most represented n-6 PUFA in yolks of both groups was linoleic acid (LA, C18:2n-6). The control group had for 24.59% higher content of LA than the experimental group. Differences in the content of LA in yolk lipids between the investigated groups were statistically significant (P<0.001). Moreover, there was statistically higher portion of γ -linolenic (C18:2n-6, P<0.05), eicosadienoic (C20:2n-6, P<0.001) and eicosatrienoic (C20:3n-6, P<0.001) acids determined in yolk lipids of the control group than of the experimental one.

Fatty acid – <i>Masna kiselina</i> %	Control group Kontrolna skupina (x ±s)	Experimental group Pokusna skupina (x̄±s)	t-test
Myristic – Miristinska (C14:0)	0.27±0.04	0.34±0.03	***
Pentadecanoic – <i>Pentadekanska</i> (C15:0)	0.07 ± 0.01	0.13 ± 0.02	***
Palmitic – Palmitinska (C16:0)	25.73±1.08	23.19±0.79	*
Heptadecanoic – Heptadekanska (C17:0)	0.23±0.03	$0.34{\pm}0.04$	***
Stearic – Stearinska (C18:0)	9.10±0.37	7.53±0.26	***
Behenic – Behenska (C22:0)	0.02 ± 0.01	0.03 ± 0.01	n.s.
\sum SFA	35.42±1.14	31.56±0.79	***
Myristoleic - Miristoleinska (C14:1)	0.03±0.02	$0.04{\pm}0.01$	n.s.
Palmitoleic – Palmitoleinska (C16:1)	1.50±0.36	1.83±0.29	*
Heptadecenoic – Heptadekenska (C17:1)	0.11±0.01	$0.20{\pm}0.01$	***
Oleic – Oleinska (C18:1)	38.56±1.52	45.40±1.42	***
Eicosenoic – Eikozenska (C20:1)	0.23±0.05	0.26 ± 0.04	n.s.
\sum MUFA	40.43±1.72	47.73±1.58	***
Linoleic – Linolna (C18:2n-6)	21.27±2.13	16.04±1.62	***
γ-linolenic – γ-linolenska (C18:3n-6)	0.16±0.07	0.09±0.04	*
Eicosadienoic – Eikozadienska (C20:2n-6)	0.26±0.06	0.14±0.02	***
Eicosatrienoic - Eikozatrienska (C20:3n-6)	0.17±0.01	0.13±0.01	***
Arachidonic – Arahidonska (C20:4n-6)	1.94±0.11	1.00±0.11	***
∑ n-6 PUFA	23.80±2.26	17.40±1.70	***

Table 3. Fatty acid profile of yolk (% of total fatty acids)

Tablica 3. Profil masnih kiselina u žumanjku (% od ukupnih masnih kiselina)

α-linolenic - α-linolenska (C18:3n-3)	0.38±0.06	1.06±0.11	***
Docosahexaenoic – Dokozaheksaenska (C22:6n-3)	-	2.14±0.34	***
\sum n-3 PUFA	0.38±0.06	3.20±0.37	***
\sum PUFA	24.18±2.32	20.60±1.71	***
SFA/MUFA	0.88±0.04	0.66±0.03	***
SFA/PUFA	1.46±0.15	1.53±0.16	*
n-6 / n-3 PUFA	62.63±6.32	5.44±0.78	***

n.s. P>0.05. * P<0.05; ** P<0,01; *** P<0.001

The greatest differences between investigated groups related to fatty acid profile in egg yolks were determined for some acids of the n-3 PUFA group. The experimental group had 2.79 times higher content of α -linolenic (LNA, C18:3n-3) acid in yolks than the control group (1.06%:0.38%). Differences in the content of LNA between the investigated groups were statistically significant (P<0.001). In egg yolks produced of the experimental group, the most represented acid of omega-3 type was docosahexaenoic acid (C22:6n-3, 2.14%), and the content of DHA was 2.02 times higher than the content of LNA. According to the described method (Csapo et al., 1986), presence of DHA was not detected in egg yolks of the control group. By means of the same method no presence of eicosapentaenoic acid (EPA, C20:5n-3) was determined in yolks of both groups. Van Elswyk et al (1992) did not determine presence of EPA in yolks of control group either, while the DHA content was very low. Low content of EPA (0.1%) in yolk lipids of eggs produced by control group was also pointed out by Kralik et al (2005). In researches of Jiang et al. (1991), content of EPA+DHA in yolk lipids was 0.5-3.0%. Higher content of EPA+DHA (3.15%) was determined by Kralik et al (2005). According to Simopoulos (2000), content of EPA+DHA in consumable eggs bought on the market was 1.1 mg/g of yolk. Meluzzi et al. (2000) stated that addition of 3% of fish oil in hens' diets affected the content of EPA to be 19.53 mg/egg, and content of DHA 143.70 mg/egg.

Compared to the group of hens fed with sunflower oil, combination of fish and rapeseed oils in hens' diets affected the yolk lipids content of SFA (31.56%:35.42%, P<0.001) is lower and the content of MUFA (47.73%:40.43%, P<0.001) is higher. Statistically significantly higher content of PUFA (24.18%:20.60%, P<0.001) was observed in yolks of hens fed with supplemented sunflower oil than in hens fed with combination of fish and rapeseed oil. Differences in the content of stearic acid (C18:0) between groups had an effect on the differences in total SFA in yolk lipids. Higher content of OA in rapeseed oil, while higher content of PUFA in yolk lipids of the control group was a consequence of high content of OA in rapeseed oil, while higher content of PUFA in yolk lipids of the control group was caused by high content of LA in sunflower oil. Jiang et al. (1991) stated that the portion of SFA in yolk lipids was 30.2-35.5% and of MUFA 34.5-55.4. According to Simopoulos (2000), content of SFA and MUFA in consumable eggs bought on the market was 80.7 and 115.4 mg/g of yolk. More favorable ratio of SFA/MUFA was determined in egg yolks of the experimental group than of the control group (0.66:0.83, P<0.001). The SFA/PUFA ratio was more favorable in the control group than in the experimental one (1.46:1.53, P<0.05).

The group of hens fed with combination of rapeseed and fish oil had lower content of n-6 PUFA (17.4%:23.8%, P<0.001), higher content of n-3 PUFA (3.2%:0.38%, P<0.001) and more favorable ratio of n-6 / n-3 PUFA (5.44:62.63, P<0.001) in yolk lipids than the group of hens fed with supplemented sunflower oil. Gonzales-Esquerra and Leeson (2000) determined the content of n-3 PUFA in eggs from 53 mg (0% of fish oil) to 246 mg (6% of fish oil added to hens' diet). Galobart et al. (2002) pointed out more favorable ratio of SFA (29.83%:32.27%), MUFA (43.36%:38.80%) and EPA+DHA (1.57%:0.73%) in hens fed diets supplemented with linseed oil, which was not the case in diets supplemented with sunflower oil.

CONCLUSION

Oils (sunflower, rapeseed and fish oil) supplemented to laying hens' diets had significant effect on the profile of fatty acids in egg yolk lipids. Hens fed diets supplemented with combination of fish oil (4%) and rapeseed oil (2%) laid eggs that had significantly higher content (P<0.001) of favorable fatty acids (OA, LNA, EPA and DHA) in yolk lipids than eggs laid by hens fed with supplemented sunflower oil (6%). Supplementation of fish and rapeseed oil in hens' diets resulted in lowering of the content (P<0.001) of unfavorable SFA, as well as LA and AA in yolk lipids. The experimental group of laying hens (fed diets supplemented with 4% of rapeseed oil and 2% of fish oil) had lower content of n-6 PUFA (P<0.001), higher content of n-3 PUFA (P<0.001) and more favorable ratio of n-6 / n-3 PUFA (P<0.001) in egg yolk lipids than the control group (fed diets supplemented with 6% of sunflower oil). Combination of fish and rapeseed oils added to hens' diets resulted in production of eggs that had significantly more favorable profile of fatty acids in yolks, if compared to eggs produced by hens fed with supplemented sunflower oil.

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POVEĆANJE SADRŽAJA N-3 PUFA U JAJIMA

SAŽETAK

Istraživanje utjecaja različitih ulja na profil masnih kiselina u žumanjcima jaja obavljeno je na 80 ISA Brown nesilica (40 u kontrolnoj i 40 u pokusnoj skupini) starih 50 tjedana. Kontrolna skupina nesilica dobivala je u hrani suncokretovo ulje (6%), a pokusna skupina kombinaciju repičinog (4%) i ribljeg (2%) ulja. Smjese za nesilice sadržavale su 16,8% sirovih bjelančevina i 11,57 MJ ME. Istraživanje je trajalo 28 dana. Sadržaj masnih kiselina u mastima žumanjka određen je na 10 uzoraka iz svake skupine. Korištena ulja (suncokretovo, repičino i riblje) u hrani za nesilice imala su značajan utjecaj na profil masnih kiselina u mastima žumanjaka jaja. U mastima žumanjaka kod pokusne skupine nesilica utvrđen je značajno veći (P<0,001) sadržaj poželjnih masnih kiselina (linolenske, C18:3n-3; eikozapentaenske, C20:5n-3 i dokozaheksaenske, C22:6n-3) u odnosu na kontrolnu skupinu. Osim toga, pokusna skupina nesilica imala je manji sadržaj (P<0,001) nepoželjnih zasićenih masnih kiselina te linolne (C18:2n-6) i arahidonske kiseline (C20:4n-6) u mastima žumanjaka. Sadržaj oleinske kiseline (C18:1) i ukupnih mononezasićenih masnih kiselina (MUFA) bio je značajno veći (P<0,001) u mastima žumanjaka pokusne skupine. Pokusna skupina nesilica imala je manji sadržaj nepoželjnih polinezasićenih masnih kiselina n-6 tipa (n-6 PUFA, P<0,001), veći sadržaj poželjnih masnih kiselina n-3 tipa (n-3 PUFA, P<0,001) i povoljniji omjer n-6 / n-3 PUFA (P<0,001) u mastima žumanjaka jaja odnosu na kontrolnu skupinu.

Ključne riječi: kokoš nesilica, jaje, žumanjak, profil masnih kiselina, EPA, DHA, n-3 PUFA

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