Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans

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Abstract

The aim of the research was to enrich eggs with n-3 polyunsaturated fatty acids by using plant oils and fish oil as dietary supplements in laying hens' feed. The focus was put on the effect of the daily consumption of 100 g of egg yolk, i.e. 100 g of egg mass, on the human health. The 1st group of laying hens was fed a diet containing soybean and fish oil, and the 2nd group was given feed containing a combination of linseed, rapeseed, soybean, and fish oils. Eggs laid by the 2nd group contained 4.73% α-linolenic acid, 0.20% eicosapentaenoic acid and 2.37% docosahexaenoic acid (% of total fatty acids in yolk lipids, $P < 0.001$), which marks an increase of × 4.04 for α-linolenic acid, × 3.33 for eicosapentaenoic acid, and × 1.75 for docosahexaenoic acid compared to eggs laid by the 1st group. Total n-3 polyunsaturated fatty acids in eggs of the 2nd group were × 2.8 higher than in the 1st first group. Calculated per 100 g of eggs of the 2nd group, the intake for the human body corresponds to 435 mg α-linolenic acid, 18.43 mg eicosapentaenoic acid, and 218.2 mg docosahexaenoic acid.

Deposition, ALA, EPA, DHA, consumption

Nutritionists recommend consumption of eggs enriched with n-3 polyunsaturated fatty acids (n-3 PUFA), having in mind the necessity of preventing various diseases and strengthening the immune system in humans. The benefit for human health is estimated on the basis of n-3 PUFA content in egg yolk lipids, as well as on the ratio of n-6 / n-3 PUFA (Okuyama et al. 1997; Simopoulos 2000). Omega-3 fatty acids prevent the occurrence of rheumatoid arthritis and cancer (Aydin and Dogan 2010), as well as cardiovascular diseases (Van Elswik 1997; Raes et al. 2002; Pita et al. 2010), and have positive effects on the immune system (He et al. 2007). Rašić et al. (2014) determined that consumption of eggs enriched with n-3 PUFA over a period of three weeks had a positive effect on microvascular reactivity, on the lowering of blood pressure and triglycerides, so they concluded that omega-3 eggs could be efficient in preventing cardiovascular diseases. Laying hens have a limited ability to convert alpha-linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in their organism, and it is recommended that these fatty acids are taken in through feed, because in that way the enrichment of eggs with n-3 PUFA was more effective (Kralik 2007; Škrtić 2007, 2008). Oils (linseed, fish, rapeseed, soybean) are generally used as a source of energy from the laying hens' feed. Various studies have shown that the type of oil could have a significant impact on the profile of fatty acids in egg yolks (Rowghani et al. 2007; Aydin and Dogan 2010; Herkel et al. 2016). Fish oil is a rich source of essential n-3 PUFA, but if it is used in large amounts in feeding mixtures for poultry, then it has a negative effect on the organoleptic characteristics of meat and eggs (Scaife et al. 1994; Scheideler 1997). Rapeseed and linseed oils are well known sources of ALA that can convert to long chain n-3 polyunsaturated acids, such as EPA and DHA, through the elongation and desaturation processes (Yang et al. 2000).

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The aim of this research was to enrich eggs with n-3 PUFA, especially with EPA and DHA, by using a combination of linseed, rapeseed, soybean and fish oils in laying hens' diet at the amount of 5%. Combination of different oils is interesting because of the interaction of individual fatty acids in the metabolic processes, as well as because of the impact on altering the n-6/n-3 PUFA ratio in yolk lipids. The control group of laying hens was given a diet containing 4.5% soybean oil and 0.5% fish oil. This paper aimed to investigate how and at what concentration the sources of ALA, EPA and DHA from oils, i.e. from feed, could influence their deposition in yolk lipids of table eggs. Furthermore, the intention was to determine the effects of the stated n-3 PUFA by consumption of 100 g of egg yolk, i.e. 100 g of egg mass of enriched eggs, and to compare them with conventional eggs.

Materials and Methods

The research was carried out on the Lohmann Classic laying hens aged 30–34 weeks. Laying hens were divided into two groups (control and experimental). Each group consisted of five cages, and each cage contained 40 hens (on the area of 750 cm^2/head), i.e. there were 200 hens in the 1st group and 200 hens in the 2nd group. Laying hens were fed diets of isoprotein and isocaloric composition during a period of four weeks (Table 1).

Apart from other feed ingredients, the 1st group of hens was given diets containing 4.5% of soybean oil and 0.5% of fish oil (total 5%), whereas the 2nd group was fed diets composed of a combination of the following oils: linseed 1%, fish 0.75%, rapeseed 2%, and soybean 1.25%, which amounts to the same total of 5%. Laying hens were administered feed and water *ad libitum*. During the experiment, the lighting regime was applied according to technological standards. Eggs used for the analysis were collected on the last day of the experiment. Two eggs were collected from each cage (total n = 10 pcs).

Table 1. Composition of diets.

Ingredients, %	1st group	2nd group
Corn	49.80	48.83
Soybean cake	26.13	25.62
Sunflower cake	3.91	5.00
Dehydrated alfalfa	2.00	2.00
Livestock yeast	0.50	0.50
Limestone	10.18	10.57
Monocalcium phosphate	1.51	1.51
Salt	0.32	0.32
Methionine	0.15	0.15
Oil	5.00[1]	5.00[2]
Premix[3]	0.50	0.50
Total	100.00	100.00
Calculative values, %		
Crude protein	18.0	18.0
Crude fat	7.4	7.4
Crude fibres	4.0	3.8
Ash	13.7	13.3
ME MJ/kg	11.5	11.5

[1] soybean oil 4.5%, fish oil 0.5%

[2] oil combination: linseed oil 1%, fish oil 0.75%, rapeseed oil 2%, soybean oil 1.25%

[3]Premix contains per 1 kg: vitamin A 200,000 UI, vitamin D_3 500,000 UI, vitamin E 10,000 mg, vitamin K_3 600 mg, vitamin B_1 400 mg, vitamin B_2 1,000 mg, vitamin B_6 1,000 mg, vitamin B_{12} 3,000 µg, vitamin C 4,000 mg, vitamin H 12 mg, vitamin B_3 8,000 mg, vitamin B_5 2,400 mg, vitamin B_9 150 mg, vitamin B_4 100,000 mg, iodine 200 mg, manganese 18,000 mg, zinc 14,000 mg, cobalt 30 mg, iron 12,000 mg, copper 1,600 mg, selenium inorganic 50 mg, calcium 238 g, phytase 100,000 FYT, canthaxanthin 500 mg, beta-apo-beta carotene acid 300 mg, antioxidant (butylated hydroxytoluene) 20,000 mg

The profile of fatty acids in egg yolk lipids was determined according to the method of Csapo et al. (1986) by using Chrompack CP-9000 gas chromatograph equipped with flame ionization detector. The operating conditions of the gas chromatography were as follows: 0.35 g of dried egg-yolk was weighed into a flask, 8 cm^3 concentrated hydrochloric acid was added and it was boiled for 60 min. After cooling down, 7 cm^3 ethanol were added, then 15 cm^3 diethylether following one min of shaking. The next extraction was with 15 cm^3 benzine (b.p. < 60 °C). After phase separation, the organic phase that contains about 150–200 mg fat was separated and evaporated under vacuum on a rotadest. Then 4 cm^3 0.5 M sodium-hydroxide in methanol was added, and boiled in a water bath for 5 min. Next, 4 cm^3 14% boron-trifluoride in methanol were added and boiled for 3 min following the addition of 4 cm^3 n-hexane. It was boiled for one min after which the level of the organic phase was brought to the neck of the flask with saturated sodium-chloride solution. When the phases were separated, samples were taken for analysis from the organic phase, and dried on sodium sulphate. The fatty acid methyl esters (FAMEs) were separated on a 100 m × 0.25 mm wall coated open tubular (WCOT) column equipped with a CP-SIL 88 (FAME) stationary phase. The quantitation of FAMESs was obtained with a flame ionisation detector (FID) at 270 °C. The temperature of the splitter injector was 270 °C, the carrier gas was

helium with the head pressure of 235 kPa. The oven was temperature programmed from 140 °C (10 min) with 10 °C/min increase up to 235 °C (26 min). The injected volume varied between 0.5 and 2 µl. The instrument was a Chrompack CP 9000 gas chromatograph (Chrompack, Delft, the Netherlands). Portions of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), ALA, as well as EPA and DHA acids show as a percentage of total fatty acids contained in yolk lipids. The influence of different feeding treatments (oils) was determined by Student's *t*-test. Research results were processed by Statistica v. 8.0.

Results

Contents of fatty acids in linseed, fish, rapeseed and soybean oils (% of total FA) are presented in Table 2.

Table 2. Profile of fatty acids in oils (% of total fatty acids).

Fatty acid	Linseed oil	Fish oil	Rapeseed oil	Soybean oil
Myristic acid (C14:0)	0.11	6.14	1.41	0.06
Pentadecanoic acid (C15:0)	0.03	1.98	0.52	0.02
Palmitic acid (C16:0)	6.81	21.92	7.66	4.70
Heptadecanoic acid (C17:0)	0.16	1.31	0.32	0.04
Stearic acid (C18:0)	3.83	5.90	2.82	2.34
Arachidic acid (C20:0)	0.17	1.03	0.46	0.49
Behenic acid (C22:0)	0.13	0.62	0.00	0.24
∑SFA*	11.23	38.90	13.20	7.89
Palmitoleic acid (C16:1)	0.05	5.10	1.40	0.20
Heptadecenoic acid (C17:1)	0.00	1.27	0.00	0.00
Oleic acid (C18:1n9c)	18.78	17.34	42.86	63.89
Eicosenoic acid (C20:1n9)	0.22	2.21	4.38	0.86
Erucic acid (C22:1n9)	0.00	0.15	2.59	0.04
∑MUFA**	19.04	26.07	51.23	64.82
Linoleic acid (C18:2n6)	14.57	3.65	19.75	20.55
γ-linolenic acid (C18:3n6)	0.12	0.48	0.49	0.00
Eicosadienoic acid (C20:2)	0.06	0.68	0.43	0.03
Eicosatrienoic (C20:3n6)	0.00	0.15	0.00	0.00
Arachidonic acid (C20:4n6)	0.00	1.41	0.29	0.00
Docosadienoic acid (C22:2n6)	0.00	0.63	0.00	0.08
∑n-6 PUFA***	14.75	7.00	20.96	20.66
α-linolenic acid (C18:3n3)	54.82	2.45	14.48	6.63
Eicosatrienoic acid (C20:3n3)	0.15	0.10	0.13	0.00
Eicosapentaenoic acid (C20:5n3)	0.00	7.15	0.00	0.00
Docosahexaenoic acid (C22:6n3)	0.00	18.34	0.00	0.00
∑n-3 PUFA	54.97	28.04	14.61	6.63
∑n-6 PUFA /∑n-3 PUFA	0.27	0.25	1.43	3.12

*SFA - saturated fatty acids; **MUFA - monounsaturated fatty acids; ***PUFA - polyunsaturated fatty acids

Linseed oil contains 54.82% ALA. Rapeseed oil and soybean oil contain the most monounsaturated fatty acids (51.23% and 64.82%, respectively), of which oleic acid (OA, 42.86% and 63.89%, respectively) is the most represented. Rapeseed and soybean oils also contain linoleic acid (LA, 19.75% and 20.55%, respectively), and ALA (14.48% and 6.63%, respectively). Fish oil is rich in saturated fatty acids (SFA, 38.90%), and EPA (7.15%) and DHA (18.34%) are represented from the n-3 PUFA group.

The n-6/n-3 PUFA ratio in oils is very narrow, ranging from 0.27 (for linseed oil) to 3.12 (for soybean oil). Fatty acid profile in laying hens' feed depends on the type and concentration of supplemented oils (Table 3).

Table 3. Profile of fatty acids in laying hens' diet (% of total fatty acids).

Fatty acid	1st group	2nd group
Myristic acid (C14:0)	2.27	1.69
Pentadecanoic acid (C15:0)	0.09	0.25
Palmitic acid (C16:0)	13.07	12.73
Heptadecanoic acid (C17:0)	0.12	0.13
Stearic acid (C18:0)	3.29	3.19
Arachidic acid (C20:0)	0.55	0.41
Behenic acid (C22:0)	0.79	0.70
Lignoceric acid (C24:0)	0.26	0.17
∑SFA*	20.43	19.27
Palmitoleic acid (C16:1)	0.20	0.51
Oleic acid (C18:1n9c)	33.02	24.97
Eicosenoic acid (C20:1n9)	0.40	0.51
Erucic acid (C22:1n9)	0.04	0.06
∑MUFA**	33.66	26.05
Linoleic acid (C18:2n6)	41.49	35.98
γ-linolenic acid (C18:3n6)	0.29	0.32
Eicosadienoic acid (C20:2)	0.29	0.30
Eicosatrienoic (C20:3n6)	0.10	0.13
Arachidonic acid (C20:4n6)	0.14	0.07
Docosadienoic acid (C22:2n6)	0.26	0.48
∑n-6 PUFA***	42.57	37.28
α-linolenic acid (C18:3n3)	3.12	13.36
Eicosatrienoic acid (C20:3n3)	0.09	0.15
Eicosapentaenoic acid (C20:5n3)	0.09	1.16
Docosahexaenoic acid (C22:6n3)	0.04	2.74
∑n-3 PUFA	3.34	17.40
∑n-6 PUFA/∑n-3 PUFA	12.75	2.14

*SFA – saturated fatty acids; **MUFA - monounsaturated fatty acids; ***PUFA - polyunsaturated fatty acids

The greatest difference between laying hens' diets was noticed in the content of n-6 PUFA and n-3 PUFA. The 1st group being fed diet supplemented with 4.5% soybean oil and 0.5% fish oil contained 42.57% of n-6 PUFA and only 3.34% of n-3 PUFA, so the ratio of n-6/n-3 PUFA was 12.75. Diet given to the 2nd group contained 37.28% of n-6 PUFA and 17.40% of n-3 PUFA and their ratio was 2.14. The most represented fatty acid in n-6 PUFA group in both laying hens' diets was LA (41.49% and 35.98%, respectively). In total n-3 PUFA, ALA was present at important amounts in both diets (3.12% and 13.36%, respectively). It should be pointed out that the content of DHA in the diet of the 1st group was × 68.5 higher than in the diet given to the 2nd group.

Table 4 overviews the profile of fatty acids (% of total FA) in yolk lipids of the 1st and 2nd group. In yolk lipids, eggs laid by the 2nd group contained significantly less ∑ SFA than the 1st group (31.33% : 34.72%, $P < 0.001$). Similar results were obtained

Table 4. Fatty acid profile in lipids of egg yolk (% of total fatty acids, n = 10).

Fatty acid	1st group $\bar{x}$ ± sd	2nd group $\bar{x}$ ± sd	*P*-value
Myristic acid (C14:0)	0.34[a] ± 0.03	0.29[b] ± 0.03	0.005
Pentadecanoic acid (C15:0)	0.20 ± 0.03	0.19 ± 0.04	0.688
Palmitic acid (C16:0)	22.16[a] ± 1.13	19.86[b] ± 1.58	0.001
Heptadecanoic acid (C17:0)	0.34 ± 0.07	0.34 ± 0.04	0.759
Stearic acid (C18:0)	8.29 ± 1.13	7.69 ± 1.20	0.265
Arachidic acid (C20:0)	0.17 ± 0.04	0.17 ± 0.02	0.928
Heneicosanoic acid (C21:0)	0.12 ± 0.02	0.13 ± 0.02	0.426
Tricosanoic acid (C23:0)	3.00 ± 0.36	2.66 ± 0.61	0.144
∑SFA*	34.72[a] ± 1.13	31.33[b] ± 0.64	<0.001
Myristoleic acid (C14:1)	0.17 ± 0.04	0.17 ± 0.03	0.705
Palmitoleic acid (C16:1)	2.43[a] ± 0.35	2.00[b] ± 0.30	0.008
Elaidic acid (C18:1n9t)	0.02 ± 0.004	0.02 ± 0.002	0.151
Oleic acid (C18:1n9c)	38.67 ± 1.93	38.94 ± 1.28	0.725
Eicosenoic acid (C20:1n9)	0.22[b] ± 0.04	0.27[a] ± 0.03	0.002
Erucic acid (C22:1n9)	0.31[b] ± 0.04	0.37[a] ± 0.05	0.013
∑MUFA**	41.82 ± 2.04	41.76 ± 1.08	0.939
Linoleic acid (C18:2n6)	19.13 ± 1.87	17.83 ± 0.8	0.060
γ-linolenic acid (C18:3n6)	0.24 ± 0.03	0.22 ± 0.03	0.085
Eicosadienoic acid (C20:2n6)	0.26 ± 0.08	0.32 ± 0.08	0.158
Eicosatrienoic acid (C20:3n6)	0.36 ± 0.05	0.35 ± 0.05	0.641
Arachidonic acid (C20:4n6)	0.57 ± 0.07	0.61 ± 0.05	0.128
Docosadienoic acid (C22:2n6)	0.28 ± 0.043	0.26 ± 0.02	0.085
∑n-6 PUFA***	20.85 ± 1.91	19.59 ± 0.83	0.071
α-linolenic acid (C18:3n3)	1.17[b] ± 0.15	4.73[a] ± 0.21	<0.001
Eicosatrienoic (C20:3n3)	0.02 ± 0.01	0.03 ± 0.01	0.115
Eicosapentaenoic acid (C20:5n3)	0.06[b] ± 0.02	0.20[a] ± 0.03	<0.001
Docosahexaenoic acid (C22:6n3)	1.35[b] ± 0.22	2.37[a] ± 0.18	<0.001
∑n-3 PUFA	2.60[b] ± 0.18	7.32[a] ± 0.23	<0.001
∑n-6/∑n-3 PUFA	8.02[a] ± 0.64	2.68[b] ± 0.12	<0.001

$\bar{x}$ = arithmetic means; **s**d = standard deviation; [a,b]$P < 0.05$, $P < 0.01$ or $P < 0.001$
*SFA – saturated fatty acids; **MUFA – monounsaturated fatty acids; ***PUFA – polyunsaturated fatty acids

Table 5. Concentration of n-3 PUFA in yolks and in eggs.

Fatty Acid	1st group		2nd group	
	mg/100 g of yolk	mg/100 g of egg	mg/100 g of yolk	mg/100 g of egg
ALA*	254.2	108.0	1102.0	435.0
EPA**	14.10	5.99	46.63	18.43
DHA**	291.7	124.0	552.1	218.2

*ALA - α-linolenic acid; **EPA – eicosapentaenoic acid; *** DHA – docosahexaenoic acid

by Kralik et al. (2008) in their previous research of use of fish oil in production of table eggs. Eggs produced in the 2nd group contained significantly ($P < 0.05$) less palmitoleic acid, and more eicosenoic acid. Differences in total contents of MUFA and n-6 PUFA between eggs of both groups were not significant ($P > 0.05$).

Table 5 presents concentrations of ALA, EPA and DHA in egg yolks and in total egg mass of the 1st and 2nd group.

The content of ALA was 254.2 mg/100 g in egg yolks of the 1st group, and 1102 mg/100 g of the 2nd group. The content of EPA was increased from 14.1 mg/100 g to 46.63 mg/100 g. The content of DHA was also increased from 291.7 to 552.1 mg/100 g of yolk. In comparison to the content of FA in whole eggs of the 1st group, eggs of the 2nd group had ALA increased by × 4.02, EPA by × 3.07, and DHA by × 1.75.

Discussion

Our research results regarding the content of n-3 PUFA in oils proved that there were some differences. Linseed oil is rich in ALA (54.85%), fish oil is rich in DHA (18.34%) and EPA (7.15%). Rapeseed oil and soybean oil contain great amounts of oleic acid (42.86% and 63.89%, respectively). As of the n-3 PUFA group, ALA is the only one present in both types of oil. These results are in accordance with those published by Rowghani et al. (2007), Kralik et al. (2008), Antongiovanni et al. (2009) and Salamatdoustnobar et al. (2009), although there is very little fluctuation referring to FA, caused by the origin of oil. In general, plant oils contain less than 25% ALA, with the exception of linseed oil, which is rich in ALA (Hiltunen and Holm 2000; Herkel et al. 2016). ALA in linseed oil varies between 34.1 and 64.4% (Hiltunen and Holm 2000). The time of harvest and plant processing affects the yield and quality of linseed oil (Nykter and Kymäläinen 2006). The n-6 PUFA/n-3 PUFA ratio in linseed oil is 4:1. The diet fed to the 2nd group contained more MUFA than the diet of the 1st group (33.66% : 26.05%, respectively), but the most common one was oleic acid (33.02% : 24.07%, respectively). The diet for the 1st group was richer in linoleic acid than the diet composed for the 2nd group (41.49% : 35.98%). At the same time, the 2nd group was given a diet containing more ALA, EPA and DHA than the 1st group (13:36%, 1.16% and 2.74% compared to 3.12%, 0.09% and 0.04%, respectively).

We did not establish significant differences in LA between groups of eggs, although eggs from the 2nd group contained less LA, but slightly more arachidonic acid ($P > 0.05$), which is the opposite to our previous research results (Kralik et al. 2008), as well as to research results of Sari et al. (2002) and Husveth et al. (2003). Beynen (2004) determined that only high concentration of LNA in a diet affected the synthesis of detectable content of EPA in yolks, which was generally lower than the content of DHA. The author also stated that LA from feed incorporated more efficiently into the yolk than ALA.

Eggs from hens of the 2nd group contained more n-3 PUFA than the 1st group ($P < 0.001$). When compared to the 1st group, higher portions of ALA (4.73% : 1.17%), EPA (0.20% : 0.06%), DHA (2.37% : 1.35%) and n-3 PUFA (7.32% : 2.60%) were determined in the yolk lipids of the 2nd group. Although the diet given to the 1st group of hens contained smaller amounts of EPA and DHA, these fatty acids were detected in yolk lipids, because laying hens can synthesize EPA and DHA at a limited amount if the diet contains ALA at a sufficient amount (Kralik et al. 2008). Increased amounts of EPA and DHA in feed resulted in increased deposition of DHA in the yolks lipids of the 2nd group, which is a result of dietary supplementation with different oils. Similar results were achieved by Bavelaar and Beynen (2004) and Huyghebaert et al. (2007). Kralik et al. (2008) reported that dietary supplementation of laying hens' feed with various combinations of fish and rapeseed oils, instead of soybean oil, resulted in the increased content of n-3 PUFA for × 1.4 to 2.10.

Rowghani et al. (2007) stated that rapeseed oil (3% and 5%) increased the percentage of ALA to 3.43% and 6.02%, respectively, and also affected the increase of DHA. Linseed oil also increased Σn-3 PUFA to 4.72% and 6.80% when compared to the control (1.43%), which was also found by Herkel et al. (2016). Lewis et al. (2000) determined that supplementation of 7% rapeseed oil affected the increase of ALA by × 1.2% to 6.3%, and the increase of DHA × 4. The authors concluded that three eggs enriched with n-3 PUFA correspond to the amount of n-3 PUFA in one fish meal daily. Bearing in mind that people are more prone to consume eggs than fish, they can thus be an alternative source of n-3 PUFA. According to the Canadian standards, it is recommended to consume eggs enriched with n-3 PUFA by up to 50% of energy in a meal. Samman et al. (2009) stated that omega eggs had significantly lower percentage of myristic and palmitic acids, which resulted in a lower percentage of SFA, but significantly higher percentage of ALA, DHA and total omega-3, which is also the case in our research. Gül et al. (2012) pointed out that dietary supplementation of laying hens' feed with rapeseed oil resulted in increased MUFA, especially of oleic acid, in eggs. In this research, oleic acid in egg yolk was at approximately the same level in both groups (> 45%).

The ratio of n-6/n-3 PUFA in yolk lipids was 2.68 in the 2nd group, and 8.02 in the 1st group ($P < 0.001$). Sugano (1996) stated that the optimal ratio of n-6/n-3 PUFA in human nutrition was 3:1, however, some studies in developed industrial countries proved this ratio to range from 10:1 and to 15:1 and more (Simopoulos 2000). Our research proved that the feeding treatment in the 2nd group (combination of plant and fish oils) was very efficient in enriching yolk lipids with n-3 PUFA, and consequently in reducing the n-6/n-3 PUFA ratio, compared to the feeding treatment with soybean oil and fish oil.

In line with Meluzzi et al. (2000), Kralik et al. (2008) and Herkel et al. (2016), enrichment of eggs with the n-3 PUFA depended on the increased content of n-3 PUFA in the hens' diet, and it was connected with the lowering of n-6 PUFA in egg yolks. According to Simopoulos (2000), LA and ALA are very important as precursors of n-6 PUFA and n-3 PUFA. Linoleic acid is metabolized into other n-6 fatty acids, including AA. Alpha-linolenic acid is metabolized into the n-3 fatty acids, such as EPA and DHA. When LA is present at larger amounts, it will inhibit the transformation of ALA into EPA and DHA, and with insufficient amount of LA, lower AA concentration will be created, which was not the case in our research. Our results show that the narrowing of the n-6/n-3 PUFA ratio and the increase of the n-3 PUFA content in feed can affect the FA profile in yolk lipids directed towards the increased deposition of n-3 PUFA, which was also reported by Beynen (2004), Škrtić et al. (2007), as well as by Kralik et al. (2008). Bavelaar and Beynen (2004) proved a linear relationship between ALA and LA in feed and their contents in yolk lipids.

The recommended daily intake of n-3 PUFA is 0.5% of the total energy in a meal (Anon 1990), which is an equivalent to 1.1–1.5 g FA for adults. Ferrier et al. (1995) calculated that feeding laying hens with diets containing 10% linseed oil, which is rich in ALA, results in production of eggs, where only one egg covers 30% of the n-3 PUFA daily requirements. Such egg contains 264 mg ALA, 10 mg EPA and 82 mg DHA. Jiang et al. (1991) also reported that EPA and DHA could be increased in yolk lipids when the feed was rich in ALA that was converted to EPA and DHA in the laying hens' livers, and synthesized fatty acids were deposited in the egg yolks. That conversion may be inhibited by high LA concentration. Beynen (2004) determined that higher amounts of linseed in feed affected the increased deposition of EPA and DHA in yolk lipids, whereas the feeding of hens with soybean was less efficient. The author concluded that the diet enriched with linseed or soybean influenced the deposition of 141 mg and 98 mg of DHA, unlike in the control group, where egg yolks contained 78 mg of DHA. Favorable effects on human health can be achieved by daily consumption of 0.5 g of n-3 PUFA (Mantzioris 2000). The results

of this research proved that 100 g of egg mass from the 2nd group contained 435 mg ALA, 18.43 mg EPA and 218.2 mg DHA, which is significantly more than in the 1st group, and also more than reported by the mentioned authors.

Modified diets administered to laying hens influenced the deposition of fatty acids in eggs. The EPA content was increased from 0.06 to 0.20%; the DHA content from 1.35 to 2.37% ($P < 0.001$). Compared to the 1st group, total n-3 PUFA were increased from 2.60 to 7.32% in total fatty acids in egg yolks of the 2nd group. Eggs laid in the 2nd group of hens contained 435 ALA, 18.4 EPA, and 218.2 DHA mg/100 g of eggs, which marked an increase of × 4 for ALA, × 3 for EPA, and × 1.7 for DHA in comparison to the 1st group. Furthermore, the total n-3 PUFA was increased in the 2nd group × 2.8 compared to the 1st group. This leads to the conclusion that consumption of eggs enriched with n-3 PUFA can have an important beneficial effect on human health.

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