The Effect of Feeding Laying Hen Conventional and Omega-3 Enriched Diet on Fatty Acid Profiles in Egg Yolk Lipids

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Abstract: The aim of this research was to enrich table eggs with n-3 polyunsaturated fatty acids (n-3 PUFA), especially with essential eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). After five weeks of feeding of laying hens with standard and experimental diets, there was analysis carried out to determine the fatty acid profile in egg yolks of both groups. Omega-3 eggs contain statistically significantly ($P < 0.05$) less sum of saturated fatty acids (Σ SFA) and more Σ n-3 PUFA, as well as narrower ratio of Σ n-6/ Σ n-3 PUFA than conventional eggs. If referring to individual fatty acids of omega-3 eggs, there were increased portions ($P < 0.05$) of α -linolenic acid (α -LNA), docosapentaenoic acid (DPA), EPA and DHA determined in this research, which was in line with the research objective. Within the presented research, concentration of n-3 PUFA in egg yolk lipids of experimental group was two times higher than that in the control group. The ratio of Σ n-6 PUFA/ Σ n-3 PUFA was reduced from 12.04 to 6.17. Within their enzymatic system, laying hens can act as a bioreactor to naturally enrich their eggs with n-3 PUFA, especially with EPA and DHA. The results of this research proved that the fatty acid profile can be modified by designing of laying hens' diets with specific forages. Eggs were enriched with favorable fatty acids in the following order: α -LNA > DHA > DPA > EPA.

Key words: Laying hen, egg yolk, PUFA, EPA, DHA.

1. Introduction

Table eggs are considered as a functional product or food [1, 2]. Products designed to preserve human cardiovascular system should contain higher portions of n-3 polyunsaturated fatty acids (n-3 PUFA), the ratio of which shall be balanced with n-6 PUFA. Human organism needs n-3 PUFA as well as n-6 PUFA. Many health organizations recommend the n-6/n-3 PUFA ratio to range from 3:1 to 10:1, however, in reality, that ratio reaches 15:1 in USA, and 1:1 to 3:1 in Japan [3-5].

Research results obtained in the Republic of Croatia indicated that the ratio of n-6/n-3 PUFA in eggs was ranging from 11:1 [6] up to 35:1 [7]. Feng and

Scheideler [8] pointed out that PUFA were the main eight components of cellular fluid and membranes, and they were important in many biological functions by having a role of receptors, transmitters and by affecting enzymatic and immune systems. Two polyunsaturated fatty acids: linoleic (LA, C18:2n-6) from n-6 PUFA group and α -linolenic acid (α -LNA, C18:3n-3) from n-3 PUFA group are essential fatty acids, because they can not be synthesized in an organism, but need to be taken in through food. In an animal organism, LA and α -LNA can be deposited, and they can oxidize or metabolize into long chain acids in highly unsaturated fatty acids through elongation and desaturation. LA is converted to γ -linolenic acid (GLA, C18:3n-6), dihomo-gamma-linolenic acid (DGLA, C20:3n-6) and

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arachidonic acid (AA, C20:4n-6). LNA is metabolized into eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). They can not be mutually converted, but they compete for the same enzymes— $\Delta 6$ and $\Delta 5$ desaturase during desaturation of their long chain homologs [9].

AA affects growth in early life. Leaf et al. [10] reported on positive correlation between body weight and concentration of AA in plasma triglycerides. Almost one-thirds of fatty acids in human cortex refer to DHA, which has an important role in brain and retina functions. Provision of PUFA is crucial for normal development. Deficiency of PUFA can cause damages to human cells and brain. Also, provision of essential fatty acids is important for retina development in puberty [11]. Deficiency of n-3 or n-6 PUFA causes unfavorable physical or biochemical changes, however, surplus of essential fatty acids also has unfavorable effects. Balance between n-6 and n-3 PUFA depends on their content in food. Absolute amount of each acid is also important, because there is competitive inhibition between two series of fatty acids for $\Delta 5$ and $\Delta 6$ desaturases. EPA and DHA compete with AA in production of eicosanoids [12]. Food rich in n-6 fatty acid changes its viscosity, vasoconstriction and reduces bleeding time. On the other hand, large quantities of n-3 fatty acid increase the need for antioxidants and vitamin E, reduce platelet aggregation, inhibit metabolism of AA during formation of prostaglandins and cause immunosuppression [12]. According to the Health and Welfare Canada [13], recommended ratio of n-6/n-3 shall range from 4:1 to 10:1 for young people.

The aim of this research was to enrich table eggs with n-3 PUFA, especially with EPA and DHA by manipulating the feed.

2. Materials and Methods

The research was carried out on 250 laying hens of the Tetra SL line. Laying hens were kept in two groups. Each group had five replicates with 25 hens.

All were fed and watered *ad libitum*. Eggs were collected and analyzed after 21 days of feeding. Composition of diets used in production of conventional eggs (standard diet, K) and eggs enriched with n-3 PUFA (experimental diet, P) is overviewed in Table 1, and the food analysis methods are according to Refs. [14-19]. Profile of fatty acids in egg yolk lipids was determined according to the method of Csapo et al. [20] by using Chrompack CP-9000 chromatograph equipped with flame ionization detector. The analysis was performed on 10 eggs per group (two eggs per each replicate).

Research results were analyzed in STATISTICA version 8.0 [21]. Differences between tested groups were determined by using the *t*-test, and the statistically significant differences level between experimental groups were at $P < 0.05$.

3. Results and Discussion

In Table 2, there is an overview of fatty acids profile of standard diet (K) and experimental diet (P), which contains high percentage of α -LNA for 2.50 times, as well as 0.88% EPA and 1.59% DHA (in total fatty acids) in experimental diet, but it is not the case with standard diet. Experimental diet contained the mixture of oils: soybean 1.25%, rapeseed 2%, linseed 1% and fish 0.75%, so that the diet contained 0.88% EPA, 0.16% DPA, 1.5% DHA and 8.33% α -LNA. Diet fed to the control group contained only 3.33% α -LNA. In this way, modified diet affected double deposition of Σ n-3 PUFA, especially EPA and DHA in egg yolks of experimental group when compared with the control. Surai and Sparks [1], Fraeye et al. [2], Kralik et al. [6] and Sim [22] reported on the effects of modified feed on the composition of fatty acids in yolks. Due to decreased portion of Σ n-6 PUFA in experimental diet from 48.83% to 38.45%, and increased portion of Σ n-3 PUFA from 3.33% to 10.96% in total fatty acids, the ratio of Σ n-6/ Σ n-3 PUFA was also decreased from 14.66 to 3.51. After five weeks of feeding laying hens with standard and experimental

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Table 1 Composition and chemical analysis of laying hens' diets.

Ingredient (%)	Standard diet (K)	Experimental diet (P)
Corn	40.81	47.08
Soybean cake	18.50	21.66
Soybean toasted	8.30	4.04
Triticale	15.00	-
Sunflower cake	1.65	6.00
Alfalfa	1.00	2.50
Livestock yeast	0.50	1.00
Limestone	9.36	10.24
Monocalcium phosphate	1.56	1.50
Livestock salt	0.32	0.32
Synthetic methionine	0.24	0.16
Sunflower oil	2.26	-
Oil mixture	-	5.00
Premix ^{1,2}	0.50	0.50
Total	100.00	100.00
Chemical analysis of diets ³ (g/kg)		
Moisture	87	81
Crude protein	190	202
Crude fibers	40	52
Ash	139	169
Fat	52	65
Ca	41	51
Metabolizable energy (kcal/kg)	2,789	2,772

¹ Premix mixture K contains in 1 kg: vitamin A 200,000 UI, vitamin D₃ 500,000 UI, vitamin E 10,000 mg, vitamin K₃ 600 mg, vitamin B₁ 400 mg, vitamin B₂ 1,000 mg, vitamin B₆ 1,000 mg, vitamin B₁₂ 3,000 µg, vitamin C 4,000 mg, vitamin H 12 mg, vitamin B₃ 8,000 mg, vitamin B₅ 2,400 mg, vitamin B₉ 150 mg, vitamin B₄ 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.

² Premix mixture P contains in 1 kg: vitamin A 200,000 UI, vitamin D₃ 500,000 UI, vitamin E 20,000 mg, vitamin K₃ 400 mg, vitamin B₁ 420 mg, vitamin B₂ 900 mg, vitamin B₆ 540 mg, vitamin B₁₂ 2,300 mg, vitamin B₉ 170 mg, pantothenic acid 1,400 mg, vitamin B₃ 5,000 mg, vitamin H 17,000 µg, vitamin B₄ 80,000 mg, vitamin C 4,500 mg, iodine 180 mg, manganese 14,000 mg, zinc 12,500 mg, iron 6,000 mg, copper 1,000 mg, selenium inorganic 80 mg, butylated hydroxytoluene 3,400 mg, propylgallate 1,400 mg, canthaxanthin 600 mg, beta-apo-8'-carotene-acid-ethylester 200 mg.

³ Referential methods used for chemical analysis of diets: HRN ISO 6496 [14], HRN EN ISO 5983-2 [15] and HRN EN ISO 6865 [16], modified according to instructions of FOSS Fiber Cap manual; HRN ISO 5984 [17] and HRN ISO 6492 [18], modified according to instructions of extraction system ANKOM XT15; RU-5.4.2-11 (internal method); HRN EN ISO 6869 [19].

Table 2 Profile of fatty acids in laying hens' diet (% of total fatty acids).

Fatty acid	Standard diet (K)	Experimental diet (P)
ΣSFA ¹	21.05	16.38
ΣMUFA ²	26.96	34.20
Σn-6 PUFA ³	48.83	38.45
α-LNA C18:3n-3	3.33	8.33
ETA C20:3n-3	-	-
EPA C20:5n-3	-	0.88
DPA C22:5n-3	-	0.16
DHA C22:6n-3	-	1.59
Σn-3 PUFA	3.33	10.96

¹ ΣSFA: C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0 + C24:0.

² ΣMUFA: C16:1 + C18:1n-9t + C18:1n-9c + C18:1 + C20:1n-9 + C22:1n-9.

³ ΣPUFA: C18:2n-6 + C18:3n-6 + C20:2 + C20:4n-6 + C18:2A + C18:2B + C18:2C + C18:2D.

Table 3 Profile of fatty acids in yolk lipids of conventional and omega-3 eggs (% of total fatty acids).

Fatty acid	Conventional eggs	Omega-3 eggs
ΣSFA ¹	35.34 ± 1.77 ^a	32.95 ± 1.05 ^b
ΣMUFA ²	41.21 ± 2.03	42.45 ± 0.87
Σn-6 PUFA ³	21.74 ± 1.44	21.17 ± 1.33
α-LNA C18:3n-3	0.89 ± 0.18 ^b	2.03 ± 0.31 ^a
ETA C20:3n-3	0.01 ± 0.005 ^b	0.02 ± 0.004 ^a
EPA C20:5n-3	0.01 ± 0.004 ^b	0.06 ± 0.01 ^a
DPA C22:5n-3	0.09 ± 0.03	0.10 ± 0.02
DHA C22:6n-3	0.68 ± 0.22 ^b	1.22 ± 0.06 ^a
Σn-3 PUFA	1.68 ± 0.39 ^b	3.43 ± 0.28 ^a

¹ ΣSFA: C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0.

² ΣMUFA: C14:1 + C16:1 + C18:1n-9t + C18:1n-9c + C18:1 + C20:1n-9.

³ ΣPUFA: C18:2n-6 + C18:2A + C18:2B + C18:2C + C18:2D + C18:3n-6 + c9,t11-CLA + C20:2 + C20:3n-6 + C20:4n-6 + C22:4n-6.

^{a, b} Mean numbers in rows with different letters differ statistically at $P < 0.05$.

Fig. 1 The Σn-6/Σn-3 PUFA ratio in yolk lipids.

^{a, b} Mean numbers marked with different letter differ statistically at $P < 0.05$.

Fig. 2 Portion of α-LNA, EPA and DHA.

^{a, b} Mean numbers marked with different letter differ statistically at $P < 0.05$.

diets, produced eggs were collected with the aim to analyze the fatty acid profile in egg yolks of both laying hens groups.

Table 3 presents profile of fatty acids in yolk lipids of conventional and omega-3 eggs. Omega-3 eggs contain statistically significantly ($P < 0.05$) less saturated fatty acids (Σ SFA) and more Σ n-3 PUFA, as well as lower ratio of Σ n-6/ Σ n-3 PUFA than conventional eggs. If referring to individual fatty acids in omega-3 eggs, there were increased portions ($P < 0.05$) of α -LNA, EPA, DPA and DHA, which was the aim of this research. Surai and Sparks [1] claimed that eggs could be enriched with n-3 PUFA in two ways. One simpler way is to enrich eggs with α -LNA, which is a precursor of EPA and DHA, by feeding of laying hens diets with linseed oil. The other way of enriching eggs with n-3 PUFA is supplementation of fish oil to diets, because it contains desirable DHA that is along with newly synthesized EPA and DHA, deposited in egg yolks. This was the case in this research.

Fig. 1 shows the ratio of Σ n-6/ Σ n-3 PUFA in yolk lipids of analyzed eggs. Results of this research, as well as of other authors, proved that the profile of fatty acids can be modified through composition of laying hens' diets with specific forages. Hargis et al. [23] investigated the omega-3 FA in egg yolk with respect to dietary supplementation with 3% of fish oil in comparison with fat supplementation. When referring to fish oil, eggs contained 235 mg of n-3 FA, of which EPA and DHA took up 89%. In order to avoid unfavorable fishy odor, Scheideler and Froning [24] used linseed or linseed oil rich in α -LNA, because it got incorporated into egg yolk in form of n-3 PUFA. They determined that α -LNA was represented in the highest portion, but there were also long-chain n-3 PUFA, such as EPA, DPA and DHA, found in phospholipid fraction. This fact pointed out that hens fed with linseed could convert α -LNA to EPA, DPA and DHA by means of enzymes of desaturase and elongase. Hens fed with linseed produced eggs enriched with fatty acids in the

following order: α -LNA > DHA > DPA > EPA [19]. Scheideler and Froning [24] reported that α -LNA was increased linearly to supplementation of 5%, 10% and 15% of linseed, which was not the case with DHA.

Hens as bioreactors can activate the enzymatic system to naturally enrich eggs with n-3 PUFA, especially with EPA and DHA (Fig. 2).

4. Conclusions

Results of the presented research proved that modification of diet composition for laying hens can successfully alter the profile of fatty acids in yolk lipids of table eggs. Laying hens can incorporate fatty acids from feed directly in yolks and convert short-chain fatty acids into those of long carbon chain. Eggs enriched with functional ingredients, such as n-3 PUFA, represent a product of better nutritional quality. In this research, concentration of n-3 PUFA in egg yolk lipids of experimental group was two times higher than that in the control group. The ratio of Σ n-6 PUFA/ Σ n-3 PUFA was reduced from 12.04 to 6.17.

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