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Dietary Enrichment of Eggs with DHA Using Different Sources of Fatty Acids

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Abstract: Ω-3 polyunsaturated fatty acids (PUFAs), first of all α-linolenic (ALA, C18:3), eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids are necessary for the brain development, the eye function, the prophylaxis of cardiovascular diseases etc. One hundred and forty ISA Brown hens (n=20/treatment), 60 weeks old were fed with diets containing sunflower (2.5%), soybean (2.5%), fish oil (1.5 and 2.5 %) or marine algae (1.5 and 2.5 %) in a period of 45 days. There was a significant ($P<0.05$) influence of the fatty acids' sources (fish oil and marine algae) on the total egg weight, albumen weight and yolk weight. DHA content in one yolk was significantly higher ($p<0.01$) in the groups fed on diet supplemented with 1.50% of fish oil (120.94 mg), 2.5% of fish oil (162.91mg), 1.50% of marine algae (130.73 mg) and 2.5% of marine algae (243.44 mg). Although with the diet supplemented with 2.5 % marine alga the amount of DHA in yolk was eight times higher (15.58 mg/g yolk) in comparison with DHA in the control (1.70 mg/g yolk).

Keywords: laying hen, fatty acids' sources, egg's structure, DHA deposition, yolk

Introduction

Of special interest for the dietologists are the alimentary products rich in Ω-3 polyunsaturated fatty acids (PUFAs), first of all α-linolenic (ALA, C18:3), eicosapentaenoic (EPA, C20:5) and docosahexaenoic (DHA, C22:6) acids, which are necessary for the brain development (Swanson et al., 2012), the eye function, the prophylaxis of cardiovascular diseases (Van Elswik 1997; Mata Lopez and Ortega 2003), etc. Some physiologically important fatty acids – docosahexaenoic (DHA, C22:6Ω-3) and γ-linolenic (GLA, C18:3Ω-6) belong in the conditionally essential PUFAs because they are not synthesized in the organism, except in a certain stage of the ontogenesis or during some illnesses (Kavtarashvili et al. 2017).

Generally, the recommended minimal daily intake/dose for healthy adults is 250–500 mg of EPA and DHA together. However, higher amounts are often recommended for certain health conditions (Global Recommendations for EPA and DHA Intake (Rev 16 April 2014). In most countries in the world, including Macedonia, Omega 3 fatty acids are deficient in the human diet, including the insufficient consumption of fish which are an important source of these acids but are an expensive nutritional product (https://ec.europa.eu/fisheries/6-consumption_en).

Laying hens have limited ability to convert alpha-linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in their organism, and for this reason it is recommended to supplement the feed with these fatty acids in order to enrich the egg n-3 PUFA content effectively (Kralik et al. 2007; Kralik et al. 2008; Škrtić et al. 2007; Škrtić et al. 2008).

The aim of our research was to evaluate the effect of different sources and dietary level of PUFA in the laying hen's diet on the eggs' quality and yolk DHA content.

Material and Methods

Animals, housing and diets

One hundred and forty ISA Brown hens, 60 weeks old, were housed in laying cages (2 birds per cage (400x350x380mm) with average live weight (1960 g) in a standard poultry house with a light regime of 16H light and 8H darkness and feed according the requirements of the hybrid before the experiment. The hens were divided in one control group (20 birds) and six experimental groups (20 birds per group). The experiment lasted 45 days. Feed consumption was 120g/day/bird. Water was supplied by 2 nipple drinkers in each cage. The test ingredients composition and the composition and nutrient content of the experimental diets are presented in Table 1 and 2.

Table 1. Lipids' and fatty acids' content of the test ingredients

Content (%)	*Sunflower oil*	*Soybean oil*	*Fish oil*	*Marine algae*
ME, KJ/kg	36990	36990	37740	
Total lipids (fat)	100	100	100	45.3
Fatty acids, saturated, total	9.75	15.34	21.29	
Fatty acids, monounsaturated, total	83.59	21.71	56.56	
Fatty acids, polyunsaturated, total	3.80	58.21	15.60	

Table 2. Composition and nutrient content of experimental diets

Ingredient (%)	*Treatments*						
	Control Basal Feed (BF)	Sunflower oil 2.5	Soybean oil 2.5	Fish oil 1.50	Fish oil 2.50	Marine algae 1.50	Marine algae 2.50
Corn	51.96	51.96	51.96	51.96	51.28	51.96	51.28
Wheat middlings	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sunflower meal	16.64	14.14	14.14	15.14	14.14	15.14	14.14
Soybean meal	10.18	10.18	10.18	10.18	10.86	10.18	10.86
Fish oil	-	-	-	1.50	2.50	-	-
Sunflower oil	-	2.50	-	-	-	-	-
Soybean oil	-	-	2.50	-	-	-	-
Marine algae	-	-	-	-	-	1.50	2.50
DL methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.08
L lysine	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Limestone	9.00	9.00	9.00	9.00	9.00	9.00	9.00
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Premix [1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Premix (1 kg) contains: vitamin A 3,000,000 i.u., vitamin D_3 700,000 i.u., vitamin E 6,000 i.u., vitamin K_3 600 mg/kg, vitamin $B_1$800 mg/kg, vitamin B_2 1,200 mg/kg, nicotinic acid 8,000 mg/kg, calcium pantothenate 2,400 mg/kg, vitamin B_6 1,000 mg/kg, vitamin B_{12} 2,000 μg/kg, folic acid 200 mg/kg, biotin 40 mg/kg, iodine (I)160 mg/kg, manganese (Mn) 16,000 mg/kg, zinc (Zn) 16,000 mg/kg, cobalt (Co) 50 mg/kg, iron (Fe) 12,000 mg/kg, copper (Cu) 1,800 mg/kg, selenium (Se) 60 mg/kg, canthaxanthin 6,000 mg/kg, ethoxyquin 24,000 mg/kg and plant base up to 1 kg.

Egg's quality parameters

The egg's structure parameters (eggwhite/albumen weight, yolk weight and eggshell weight) and their percentage in the total egg weight was measured in 6 randomly selected eggs, 3 times during the experiment (every 15th day) on a scale with 0.1 g accuracy. The eggs were weighed, then the yolks were separated with an egg separator. The albumen residuals were eliminated from the yolk using blotting paper and then weighed. The shell was wiped clean and weighed. The albumen weight was calculated by subtracting yolk and shell weight from total egg weight. The viteline membrane of the yolk was removed using tweezers, then mixed manually with a spatula and stored at –20°C prior to analyses.

Total lipids and DHA content in the yolk

The total fat in the yolk was measured using Soxhlet extraction method. The concentrations of docosahexaenoic (DHA, C22:6n-3) fatty acid in egg's yolk were measured. Six yolks were mixed, then dried with sodium sulphate, mixed with DI (deionized) water and hexane and centrifuged 2–3 minutes at 2500 rpm. DHA was determined using gas chromatography (AOCS –Ce 1f – 96) adapted by Abril and Barclay (1999), with identification of fatty acids by comparing their retention times and quantified by area's standardization.

Statistical analysis

Statistical analysis was performed using Statgraph 3 software package. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, Duncan's Multiple Range Test was performed. All results are presented as means with their standard deviations (SD).

Results and discussion

The eggs' parameters, such as total egg weight, shell weight, albumen weight, yolk weight, proportion and edible portion and yolk:white ratio are presented in Table 3. The egg weight, albumen weight and yolk weight were significantly affected by the supplementation of different sources of fatty acids in different doses (P<0.05).

The egg weight of the group fed with a supplement of marine algae (1.5 and 2.5%) and soybean oil significantly decreased (59.85 g, 60.10 g and 60.29 g, respectively) in comparison to the control group. In addition, the lower egg weight found/measured in some investigations may be a consequence of the lower feed consumption (Gonzalez-Esquerra and Leeson 2000). In our investigation the feed was limited on 240 per cage (2 hens in cage), so the intake maybe was lower on one hen then the other in the same cage. This conclusion is in accordance with the results obtained regarding egg's albumen weight. The albumen weight and yolk weight of the aforementioned experimental groups have significantly (P<0.05) lower values in comparison to the control group.

Table 3. Egg's quality parameters at the end of the supplementation period

	Control Basal Feed (BF)	*Sunflower oil 2.5*	*Soybean oil 2.5*	*Fish oil 1.50*	*Fish oil 2.50*	*Marine algae 1.50*	*Marine algae 2.50*
Egg weight, g	67.74±3.95a	63.73±3.11a	60.29±4.83b	70.46±4.42a	68.30±4.25a	59.85±5.18b	60.10±5.35b
Albumen weight, g	40.51±3.48a	41.16±2.14a	37.59±3.57b	42.93±4.13a	40.81±3.91a	36.62±4.31b	36.96±4.35b
Yolk weight, g	19.07±2.03a	17.72±1.56a	15.74±1.27b	19.35±1.46a	19.51±1.83a	15.75±1.06b	15.63±1.25b
Shell weight, g	8.16±0.69	8.48±0.54	6.93±0.60	8.32±0.81	8.14±0.61	7.56±0.67	7.51±0.57
Albumen weight %	59.77±3.06	61.11±1.65	62.28±1.56	60.80±2.95	59.41±2.97	61.04±2.36	61.37±2.25
Yolk weight %	28.19±3.00	26.28±1.64	26.14±1.46	27.54±2.58	28.66±2.85	26.41±1.67	26.07±1.73
Shell weight %	12.04±0.67	12.61±0.87	11.51±0.70	11.81±0.93	11.93±0.65	12.67±1.11	12.55±1.02
Edible portion, %	87.96±0.67	73.72±1.64	73.43±1.34	88.34±1.61	88.07±0.65	72.88±1.16	73.36±1.17
Yolk : white	47.52±7.61	43.09±3.70	42.85±2.84	45.57±6.22	48.58±7.04	45.36±2.41	43.65±3.10

The values are means ± S.D
a, b - Significant differences respect to the control for the f-tests run to compare means (P<0.05)

Total lipids and DHA content of the eggs are shown in Table 4. The total lipids' content varied from 26.09 to 27.86% in the examined eggs. There were no significant differences between the group fed with basal feed and the groups fed with supplemented feed regarding lipids' content in the eggs (P>0.05). Our results are in accordance with the results obtained by Grigorova et al. (2006) on Bovans brown laying hens fed on diet supplemented with 2% and 10% of dry biomass from green algae of Chlorella genus.

Table 4. Lipids and DHA content in experimental eggs at the end of the supplementation period

	Control Basal Feed (BF)	*Sunflower oil*	*Soybean oil*	*Fish oil 1.50*	*Fish oil 2.50*	*Marine algae 1.50*	*Marine algae 2.50*
Lipid content in egg, %	26.09±0.60	27.30±0.27	26.59±0.21	27.82±0.98	27.86±1.36	26.85±0.91	27.42±0.89
Lipid content in yolk, g	4.97±0.11	4.84±0.05	4.18±0.03	5.38±0.19	5.47±0.25	4.21±0.14	4.28±0.14
DHA mg/g yolk	1.70±0.28A	2.05±0.07A	2.05±0.07A	6.25±0.14B	8.35±0.07B	8.30±1.44B	15.58±0.8B

The values are means ± S.D

A, B – Significant differences respect to the control for the f-tests run to compare means (P<0.01).

DHA content in the egg yolk was significantly different (P<0.05). The highest content was noticed in the group fed with supplemented feed with 2.50 marine algae (15.58 mg/g egg yolk) in comparison with the control group (1.70 mg/g egg yolk). The content of DHA was also significantly affected by the nutrition supplemented with 1.50% of fish oil, 2.50% of fish oil and 1.50% of marine algae. The calculated content of DHA in one average egg yolk was significantly (P<0.05) highest (243.44 mg/yolk) in the group/fed with 2.50% of marine algae. These results are presented in figure 2.

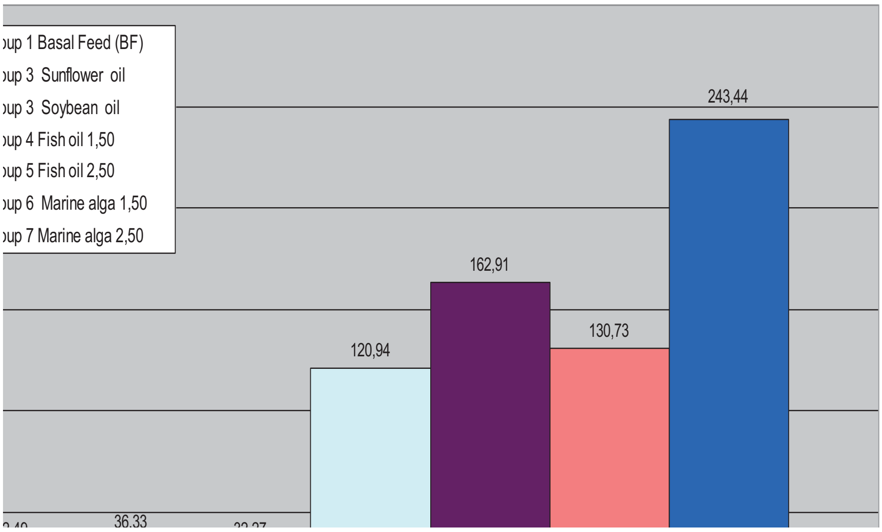

Figure 2. DHA content in yolk, mg

Researchers, during the last decade, have elucidated the importance of Omega 3 fatty acids for the human health. In most countries in the world, including Macedonia, Omega 3 fatty acids are deficient in the nutrition, including the insufficient fish consumption (https://ec.europa.eu/fisheries/6-consumption_en). Also, there are some discussions about the safety of the fish consumption as a good source of Omega 3 fatty acids. The pollution results in an increase in exposure to methyl-mercury and other contaminants in the fish tissue. (Castano et al., 2015; Taylor et al., 2016). The price of fish meat, its availability on the market, consumers' habits, make the researchers take into consideration other modes for satisfying the requirements for PUFA in the human nutrition. One mode is foodstuffs enrichment with PUFAs, and the most used animal product with low price and enriched with PUFAs is eggs enriched with Omega 3 fatty acids (Kralik et al. 2017). Further studies are required to investigate the effect of enriched eggs, on one hand, and fish with high content of Omega 3, on the other hand, as sources of Omega 3 fatty acids, on its digestibility and absorption and also on some biochemical parameters related to human health.

Conclusions

Supplemented diets with different sources of fatty acids fed to ISA Brown laying hens have produced eggs enriched with DHA fatty acid. Our results show that the diet supplemented with 1.50% of fish oil, 2.5% of fish oil, 1.50% of marine algae or 2.5% of marine algae as a sources of omega-3 fatty acids obtain acceptable level of DHA in table eggs. These table eggs are products with a significantly higher DHA content. It is possible to produce health promoting enriched eggs by manipulation of hens' diet.

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