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Production of shell eggs enriched with n-3 fatty acids

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ABSTRACT: *Unsaturated long chain fatty acids (n-3 FAs) have been proposed in a human diet to reduce the risk of atherosclerosis and therefore the risk of stroke. N-3 FAs also play an important role in retinal and brain tissue development in the neonate. The main natural source of n-3 FA is marine fish.*

The aim of this work was to create shell egg enriched with n-3 FAs using natural golden marine algae (MA) as a supplement in hen's diet. Three experiments were conducted: (1) hundred hens from the hybrid Lohmann Brown were fed with diet containing 1.27% MA; (2) hundred hens from the same hybrid were fed with diet containing 1.77% MA; (3) hundred hens were the control group. The duration of the experiments was 4 weeks.

Slight enriching of the shell egg yolk at the both groups fed with diet containing MA happened after the end of the second week. The concentration of docosahexaenoic acid (DHA; $C_{22:6,\ n=3}$) at the 1st experimental group was 90.3 mg/100 g of egg mass, and 112.1 mg/100 g of egg mass at the 2nd experimental group. The concentration of DHA at the control group was 54.5 mg/100 g of egg mass. After the 3rd week the concentration of DHA at the 1st group increased to 201.2 mg/100 g of egg mass and to 304.9 mg/100 g of egg mass at the 2nd group. At that time the concentration of the DHA at the control group remained unchanged. At the end of the 4th week the concentration of DHA reached the maximum level: 224.5 mg/100 g of egg mass at the 1st group and 328.4 mg/ 100 g of egg mass at the 2nd group. The concentration of the DHA at the control group was 51.9 mg/100 g egg mass. It is interested to note that eicosapentaenoic acid (EPA; $C_{20:5,\ n=3}$) appeared in low concentrations of 10-15 mg/100g of egg mass at the end of the 4th week of the experiment at the 2nd experimental group.

Keywords *- eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), marine algae (MA), omega 3 fatty acids (n-3 FAs),*

I. INTRODUCTION

Dietary intake of omega-3 polyunsaturated fatty acids (n-3 PUFA) decreases the risk of heart disease, inhibits the growth of prostate and breast cancer, delays the loss of immunological functions, and is required for normal fetal brain and visual development. Dietary sources of n-3 PUFA include fish, chicken, eggs, canola oil, and soybean oil [1, 2]. Increased consumption of n-3 PUFA requires identification of a food source that the public would eat in sufficient amounts to meet recommended intake.

Egg is considered a perfect natural food that has been consumed for centuries worldwide [3]. A large number of scientific trials into the feeding of enriched diets to laying hens have been carried out, demonstrating the levels of feed supplementation required to give a significant increase in the eggs Researchers found that addition of copper sulphate pentahydrate to the poultry ration has reduced the cholesterol content [4]. Addition of garlic paste to the poultry ration has also brought down the cholesterol content [5]. Focus has been given mainly to improve the omega 3 fatty acid content of egg yolk and also to reduce the cholesterol level of the yolk. According to the investigations of Kirubakaran *et al* [6] flaxseed and holy basil feeding increased α-linolenic acid (ALA) in the egg yolk. The current practice of producing n-3 fatty acid-enriched eggs is primarily achieved by feeding laying hens flaxseed rich in the n-3 fatty acid ALA [7]. However, flax based diets do not yield eicosapentaenoic acid (EPA) enriched eggs and significant enrichment of eggs with EPA entails the inclusion of marine oils in the form of fish oil or fish mil [8]. But, it can give the undesirable "fishy odours" of the yolk. Studies has shown that single cell marine *thraustochytrid algae*, such *as Schizochytrium* spp. and other close related species, are promising alternative sources of long chain PUFAs [9-12]. These micro algae can be grown heterotrophically and are commercially available as dried products.

The objective of this study was to evaluate the influence of feed supplemented with marine algae (*Schizochytrium spp*.) on DHA enrichment of the eggs of the Lohmann Brown hens 28 weeks old.

II. MATERIALS AND METHODS

Three hundred Lohmann Brown hens (28 weeks old) were housed in laying cages (4 birds per cage) in standard poultry house with a light regime of 16H and 8H darkness. The hens were assigned in three experimental groups (C-control, I experimental, II experimental) with 100 birds per group. The experiment was lasting 28 days. The feed consumption of hens was restricted to 130 g/day, but water consumption was provided *ad libitum*. The ingredients and nutrient composition of the experimental diets was presented in Table 1.

All groups of hens were fed with diets of standard ingredients. Feed of I and II experimental group of hens in relation to feed of control group of hens was supplemented with micro algae *Schizochytrium* spp. (DHA Gold®, Martek, USA) as a source of ω-3 fatty acids in amount of 1.27% and 1.77% respectively.

During the experiment eggs were collected daily, 6 eggs per group. The eggs were measured, cracked, the shells were discharged and the separation of the yolk from the albumen was performed manually. Broken and cracked eggs were selected separately for each group.

Six yolks were mixed, then dried with sodium sulphate, mixed with deionized water and hexane and centrifuged 2-3 minutes at 3000 rpm. Lipids were isolated from the yolk by Soxhlet extraction. The determination of the content of docosahexaenoic acid (DHA, C22:6, n=3) in lipid fraction was performed on gas chromatograph model Hewlett Packard 5890, series II plus equipped with flame ionization detector (FID) and HP-FFAP capillary column. Identification of DHA was made by comparison of the retention time (R_t) of DHA - methyl ester obtained after methylation of the DHA isolated from the samples with the R_t of the corresponding analytical standard. Determination of DHA was performed by comparison of the peak area obtained for DHA – methyl ester of the sample with the peak area obtained for the corresponding standard.

DHA-methyl ester analytical standard in methylene chloride was obtained from Sigma-Aldrich (USA).

III. RESULTS AND DISCUSSION

Raw and chemical composition of feed is given in Table 1.

Table 1. Raw and chemical composition of feed for control and experimental groups

Ingredients (%, w/w)	**Experimental diet**		
	C	**I**	**II**
DHA Gold®,	-	1.27	1.77
Corn	58.08	58.58	58.08
Soybean oil	2.5	2.5	2.5
Soybean meal (46% CP)	14.84	14.84	14.84
Sunflower meal (28%)	8.83	8.83	8.83
Fish meal	4.5	4.5	4.5
Wheat bran	1.77	-	-
Di-calcium phosphate	0.74	0.74	0.74
Calcium carbonate	7.66	7.66	7.66
Sodium chloride	0.23	0.23	0.23
Sodium propionate	0.30	0.30	0.30
Ropadiar solution (8%)	0.05	0.05	0.05
Vitamin-mineral premix	0.50	0.50	0.50
Total	100	100	100
Chemical composition			
Crude fibre (%)	5.01	5.01	5.01
Crude protein (CP %)	16.52	16.52	16.52
Crude fat (%)	4.13	4.13	4.13
Ash (%)	11.39	11.39	11.39
Lysine (%)	0.92	0.92	0.92
Methionine (%)	0.44	0.44	0.44
Cysteine (%)	0.22	0.22	0.22
Ca (%)	3.60	3.60	3.60
Phosphorus total	0.61	0.61	0.61
Non phytate phosphorus (%)	0.36	0.36	0.36
Metabolic energy, MJ/kg	11.51	11.51	11.51

During the experiment, physical and chemical properties of the eggs have been monitored. The obtained data are given in Table 2.

Table 2. Physical and chemical properties of the analyzed eggs

Parameter	C	I	II
Number of analyzed eggs	48	48	48
Average egg mass (g)	59.75	59.81	59.66
Average albumen mass (g)	37.08	36.93	36.76
Average yolk mass (g)	15.51	15.45	15.55
Average shell mass with membranes (g)	7.16	7.35	7.36
Yolk colour (Lash)	12.80	13.0	13.25
Number of analyzed yolks	32	32	32
Dry matter, %	48.78	48.69	49.20
Proteins, %	14.46	14.66	14.88
Fats, %	26.51	26.20	26.42

According to the obtained data, it can be observed that the consumption of DHA has no influence on physical and chemical properties of the eggs. A slight change in the yolk colour was observed in experimental group I and II *vs* control group, which was probably due to the variations in the content of pigments in the composition of the experimental diet.

Enrichment of eggs yolk with DHA starts in 2nd weeks from the beginning of the treatment of poultry with feed enriched with micro algae which provides DHA content of 1.4 mg/kg feed (group I) and 1.95 g/kg feed (group II), respectively. At that time the average concentration of DHA in egg yolk obtained from hens from group 1 is found to be 3.5 mg/kg, while 100 g egg mass contains 90.3 mg DHA. The content of DHA in single egg (average concentration) is 54.07 mg (Table 3).

In the second experimental group, the average concentration of DHA in the yolk is 4.3 mg/g or 112.06 mg in 100 g of egg mass or 66.86 mg in individual egg. Contrary to our expectations, DHA is found also in the eggs of control group (2.1 mg/g egg yolk, 54.5 mg/ 100 g of egg mass and 32.57 mg/egg).

After 3 weeks of the treatment, the concentration of DHA in the yolk from the laying hens from the first experimental group increased twice (7.8 mg/g) in comparison with the concentration of DHA after 2 weeks of the treatment (3.5 mg/g). At the same period in the second experimental group the content of DHA in the yolk increased almost 3 times (11.7 mg/g). The content of DHA in the yolk of the laying hens of the control group remained unchanged (Table 3).

After 4 weeks of the treatment, the content of DHA in the yolk of the laying hens from the first and the second experimental group reached its maximum. Namely, the content of DHA in the yolk of the laying hens from the first experimental group was 8.7 mg/g or 225 mg/100 g egg mass or 134 mg per egg with average mass of 60 g. The content of DHA in the yolk of the laying hens from the second experimental group was 12.6 mg/g or 328 mg/100 g egg mass or 196 mg per egg with average mass of 60 g. Interestingly, the content of DHA in the yolk of the laying hens from the control group remained the same (2.0 mg/g).

Table 3. DHA content in egg yolk

	C	I	II
	Content of DHA in feed, g/kg		
	-	1.4	1.95
	DHA consumption per day per hen, mg		
	-	165	230
	DHA content 14 days after the treatment		
DHA content in yolk (mg/g)	2.1	3.5	4.3
DHA content in egg mass (mg/100 g)	54.49	90.3	112.06
DHA content in egg (mg/egg)	32.57	54.07	66.86
	DHA content 21days after the treatment		
DHA content in yolk (mg/g)	1.9	7.8	11.7
DHA content in egg mass (mg/100 g)	49.3	201.25	304.92
DHA content in egg (mg/egg)	29.47	120.51	181.93
	DHA content 28 days after the treatment		
DHA content in yolk (mg/g)	2.0	8.7	12.6
DHA content in egg mass (mg/100 g)	51.9	224.47	328.38
DHA content in egg (mg/egg)	31.02	134.41	195.93

It is interesting to note that eisocapentaenoic acid (EPA, C20:5, n=5) appeared in low concentrations of 10-15 mg/100 g of egg mass at the end of the 4th week of the experiment at 2nd experimental group. Our results were in line with those of Filev *et al* [11]. Namely, the content of DHA in the yolk of laying hens (ISA Brown) 60 weeks old which were fed one month with standard feed mixture containing 1.18 mg/kg DHA was 159.41 mg per egg. In the experiment conducted by Gjorgovska *et al* [12] the content of DHA in one average egg of laying hens (Hisex Brown) 80 weeks old which were fed for one month with 1.18 g DHA/ kg supplemented feed was 170.49 mg per egg.

IV. CONCLUSION

Chicken eggs represent one of the few natural products that contain DHA ranging from 29 to 33 mg per egg. The addition of marine algae in feed increases the concentration of DHA in chicken egg, without influence on its physical and chemical properties. The presence of higher amount of DHA in feed induces egg production richer with DHA. The enrichment of eggs with DHA can be 4 to 6 times higher in treated hens in comparison with eggs obtained from hens which were fed with supplemented feed without DHA.

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