

## THE EFFECT OF FEED RICH IN DHA ON EGG PRODUCTION, EGG COMPONENTS AND DHA CONTENT IN YOLK

N. GJORGOVSKA<sup>1\*</sup>, K. FILEV<sup>2</sup>, V. LEVKOV<sup>1</sup>, S. GJORGJIEVSKI<sup>2</sup>, V. KOSTOV<sup>1</sup> and R. NASTOVA<sup>1</sup>

<sup>1</sup> *Institute of Animal Science, University “Ss. Cyril and Methodius”, bul. Ilinden br. 92a, 1000 Skopje, Macedonia*

<sup>2</sup> *Faculty of Agricultural Sciences and Food, University “Ss. Cyril and Methodius”, bul. Aleksandar Makedonski bb, 1000 Skopje, Macedonia*

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### Abstract

The effect of diets rich with DHA (docosahexaenoic acid) on egg production, the influence on egg structural components and DHA deposition in egg yolk were investigated on Hisex Brown laying hens. The experimental groups were fed with diets rich with 792 mg DHA kg<sup>-1</sup> and 1180 mg DHA kg<sup>-1</sup>. The control group received commercial diets with no content of DHA. Throughout the experiment there were no statistically significant differences in live weight of laying hens (g) and feed conversion ratio (FCR:g) between control and experimental groups ( $P>0.05$ ). The structural components of eggs (egg weight, white weight, yolk weight and shell weight) were also not statistically significant ( $P>0.05$ ), but only the egg yolk:white ratio was statistically different ( $P<0.01$ ). The concentration of DHA in 100 g yolk was 818 mg and 974 mg in group 2 and 3, respectively. The transfer of DHA was significantly higher in the experimental groups fed with supplemented diet with higher amount of DHA ( $P<0.01$ ).

*Key words:* DHA, egg production, egg structure, DHA content.

### Introduction

Polyunsaturated n-3 fatty acids have an undesirable effects on productive performance of laying hens, egg weight and yolk weight (Sari et al., 2002). Laying hens strain does not have any influence on fatty acid composition of eggs yolk (Ahn et al., 1995; Grobas et al., 2001), but the hens' age has an important role in deposition of n-3 PUFA in egg yolk (Nielsen, 1998; Yannakopouls et al., 2005). The content of fatty acids in feed of added oils or fats for laying hens, as a higher energy value, would not adversely affect the production and quality of eggs (Goencueoglu and Erguen, 2004; Sari et al. 2001). Gonzalez-Esquerra and Leeson (2000) investigated the influence of different quantity of fish oil (0%, 2%, 4% and 6%) in production results and the composition of fatty acids in eggs. Egg weight linearly decreased, and the content PUFA n-3 increased linearly with increasing content of fish oil in feed. Whitehead et al. (1993) and Marshall and

Van Elswyk (1994) highlighted the negative impact of fish oil on production results, especially in the reduced weight of eggs. Abril et al. (2000) used marine microalgae (up to 4.3% included in hens' diet) and they didn't determine negative impact on health and production parameters of laying hens. Gonzalez-Esquerra and Leeson (2000) also confirmed the above data in their works. The aim of this research was to evaluate the effect of the supplemented DHA in the diet production parameters, egg components and DHA deposition in egg yolk.

### Material and Methods

Fifty Hisex Brown hens (80 weeks old) were housed in laying cages (2 birds per cage) in standard poultry house with a light regime of 16H and 8H darkness. The hens were assigned in control group (10 birds) and two experimental

\*E-mail: natashagjorgovska@gmail.com

groups (20 birds per group). The experiment lasted 45 days. The body weight of hens was measured at the beginning and at the end of the experiment. The egg production was controlled daily and the egg mass was controlled weekly. The feed consumption of hens was restricted to 120g/day, but water consumption was provided ad libitum by 2 nipple drinkers in every cage. The ingredients and nutrient composition of the experimental diets were presented in Table 1.

Egg samples were collected every 10th day, 6 eggs per group. The eggs were measured, cracked, and the shells were discharged and measured. The separation of the yolk from

the albumen was performed manually and also measured. The albumen residuals were eliminated from the yolk using blotting paper, viteline membrane was removed using tweezers, then mixed manually with a spatula and stored at -20°C prior to analyses. The total fat in the yolk was measured using Soxhlet extraction method. Concentrations of docosahexaenoic (DHA, C22:6n-3) fatty acid was measured in egg yolk. Six yolks were mixed, then dried with sodium sulphate, mixed with DI (deionized) water and hexane and centrifuged 2-3 minutes at 2500 rpm. Fatty acid was determined by gas chromatography (AOCS –Ce 1f – 96) adapted by Abril and

**Table 1**  
**Ingredients and nutrient composition of experimental diets**

Content, %	Group 1 Basal Feed (BF)	Group 2 792 mg kg <sup>-1</sup> DHA	Group 3 1180 mg kg <sup>-1</sup> DHA
Ground yellow corn	51.96	51.96	50.34
Wheat middlings	10.00	10.00	10.00
Sunflower meal	16.64	13.00	13.00
Soybean meal	10.18	10.18	10.86
Fish oil	-	1.93	2.9
Fish meal	-	1.71	1.69
DL methionine	0.08	0.08	0.08
L lysine	0.06	0.06	0.04
Choline chloride	0.05	0.05	0.05
Salt	0.23	0.23	0.24
Limestone	9.00	9.00	9.00
Dicalcium phosphate	1.3	1.3	1.3
Premix <sup>1</sup>	0.50	0.50	0.50
Total	100.0	100.0	100.0
Calculated composition of diets			
ME, Kcal/kg	2700	2700	2722
Crude proteins	15.00	15.00	15.00
Crude fibre	4.05	4.05	4.08
Fat	5.48	5.48	5.4
Ash	12.47	12.47	13
Lysine	0.80	0.80	0.80
Methionine	0.40	0.40	0.40
DHA, g/kg	0.792	0.792	43101
Calcium	3.8	3.8	3.89
Nonphytate phosphorus	0.38	0.38	0.38

<sup>1</sup> Premix (1 kg) contains: vitamin A 3,000,000 i.u., vitamin D<sub>3</sub> 700,000 i.u., vitamin E 6,000 i.u., vitamin K<sub>3</sub> 600 mg/kg, vitamin B<sub>1</sub> 800 mg/kg, vitamin B<sub>2</sub> 1,200 mg/kg, nicotinic acid 8,000 mg/kg, calcium pantothenate 2,400 mg/kg, vitamin B<sub>6</sub> 1,000 mg/kg, vitamin B<sub>12</sub> 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, cantaxantin 6,000 mg/kg, ethoxyquin 24,000 mg/kg and plant base up to 1 kg.

Barclay (1999), with identification of fatty acids by comparing of their retention times and quantified by areas standardization. Statistical analysis was performed by Statgraph 3 software package. One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, the Duncan's Multiple Range Test was performed.

## Results and Discussion

The effects of feed rich in DHA on the production parameters of the laying hens were presented in Table 2.

During the experiment there were no health disorders, and mortality was ranged in the technological norms, while differences between groups were not significant. Differences in starting and ending body weights between investigated groups were not statistically significant ( $P > 0.05$ ). The absence of a response to the dietary inclusion of DHA, on the live weight of laying hens ( $P > 0.05$ ) in the present study (Table 2) confirmed the findings of studies conducted on laying hens (Skrtic et al., 2006; Ceylan et al., 2011).

The intensity of egg production was similar, 77.89% and 84.22% in groups fed with feed rich in DHA in comparison with the control group. The feed consumption was restricted (120 g feed/day), but the feed consumption per egg was the lowest in group 3 – 142.5 g, then in group 1, 144.5 g, and group 2 149.5 g per egg, respectively. The feed conversion efficiency was the lowest in group 3, (2.08 g/g egg mass), the

highest was in group 1, (2.14 g/g egg). The daily consumption of DHA was 95 mg for experimental groups fed with 0.792 g DHA/kg rich feed and for experimental groups fed with 1.18 g DHA/ kg rich feed the daily consumption of DHA was 142 mg. The highest DHA consumption per egg was recorded in groups 2 (169 mg) and in group 3 (122 mg).

The results reported in Table 3 show that of all the egg structure parameters analysed, only the value of egg yolk and white ratio was statistically different ( $P < 0.05$ ). Several factors affect total edible portion and egg yolk:white ratio. Age of hen, size of eggs, and strain can affect these parameters (Ahn et al., 1997; Ahmadi and Rahimi, 2011; Roberts, 2004; Galea, 2011). The other results of the egg structure parameters were not significantly affected ( $P > 0.05$ ). These findings were similar to the reports published by Csuka et al. (2008) and Laca et al. (2009).

The content of yolk fat and DHA deposition of control and experimental diets are shown in Table 4. Diet treatments had no significant effect on the ( $P > 0.05$ ) fat content of the yolks. These findings are in agreement with the results reported by Coorey et al. (2015), King et al. (2012) and Balevi and Coskun (2000). The content of DHA, in a gram of yolk in the control group was 2.15 mg, and in the groups of laying hens fed with feed rich with 792 and 1180 mg DHA in kg feed were 8.18 and 9.74 mg in group 2 and 3, respectively. The f-test showed significant differences in respect to the control group in DHA (C22:6n-3) yolk contents (confidence interval of 95%).

**Table 2**  
**Effects of feed rich in DHA on the production parameters of experimental laying hens**

Specification	Group 1 Basal Feed (BF)	Group 2 792 mg kg <sup>-1</sup> DHA	Group 3 1180 mg kg <sup>-1</sup> DHA
Number of hens	10	20	20
Hen's weight, g			
- at the beginning	2180.0	2044.5	2063.5
- at the end	2260.0	2002.0	2120.5
- change in body weight	+ 80.0	-42.5	+ 57.0
Egg production			
- laying intensity,%	82.00	77.89	84.22
Feed consumption			
- daily consumption, g	120	120	120
- per egg, g	144.5	149.5	142.5
- per gram egg mass, g	2.14	2.10	2.08
DHA consumption			
- per hen, mg/day	2.5	95	142
- per egg, mg	3.01	122	169

**Table 3**  
Effects of feed rich in DHA on egg structure parameters

Specification	Group 1 Basal Feed (BF)	Group 2 792 mg kg <sup>-1</sup> DHA	Group 3 1180 mg kg <sup>-1</sup> DHA
Egg weight, g	67.53±3.91	71.20±5.15	68.41±4.18
Egg white weight, g	40.11±3.73	42.96±4.21	40.62±3.85
Egg yolk weight, g	19.28±2.14	19.55±1.74	19.82±2.14
Egg shell weight, g	8.14±0.67	8.36±0.81	8.13±0.59
Egg white, %	59.33±3.43	60.28±3.76	59.07±3.15
Egg yolk, %	28.61±3.35	27.52±2.60	29.04±3.12
Egg shell, %	12.06±0.65	11.75±0.93	11.89±0.65
Edible portion, %	87.94±0.65	87.80±3.37	88.11±0.65
Yolk:white	48.69±8.70 <sup>a</sup>	45.96±6.18 <sup>b</sup>	49.56±7.79 <sup>a</sup>

DHA - docosahexaenoic (C22:6n-3) fatty acid Values are means ± S.D

<sup>a,b</sup> – Values in the same row with no common superscript differ significantly (p<0.05)

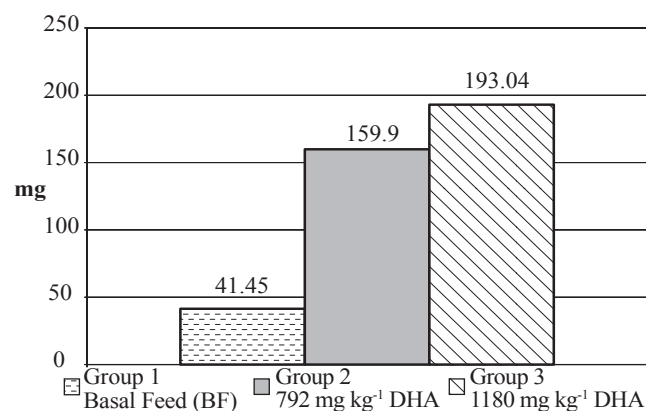
**Table 4**  
DHA deposition in egg's yolk from hens fed with feed rich in DHA

Specification	Group 1 Basal Feed (BF)	Group 2 792 mg kg <sup>-1</sup> DHA	Group 3 1180 mg kg <sup>-1</sup> DHA
Content of total fat in the egg yolk, %	28.30±0.80	27.75±0.83	28.03±1.20
Daily consumption of DHA, mg kg <sup>-1</sup>	2.5	95	142
DHA content in gram egg yolk, mg	2.15	8.18	9.74
DHA content in one yolk, mg	41.45 <sup>B</sup>	159.9 <sup>A</sup>	193.04 <sup>A</sup>

DHA - docosahexaenoic (C22:6n-3) fatty acid Values are means ± S.D

<sup>A,B</sup> – Values in the same row with no common superscript differ significantly (p<0.01)

The content of DHA, in average yolk, was the highest in the group 3 fed with 1180 mg DHA in kg feed (193.04 mg), in group 2 fed with 792 mg DHA in kg feed (159.9 mg) and low-



**Fig. 1. DHA content in egg yolk**

est in group 1 (control) 41.45 mg. The results are presented in Figure 1. Although with the diet rich with DHA (group 2 and 3) the amount of DHA in yolk was four times higher in comparison with DHA in the control. These levels are higher than the reported by other authors (Laca et al., 2009; Skrtic 2006; Skrtic et al., 2008).

## Conclusion

There are no significant differences among investigated parameters (P>0.05), except on the obtained results about the yolk and white ratio (P<0.05). DHA enriched hen egg can be produced by supplementation with 792 and 1180 mg DHA in kg feed without affecting laying performance and mostly egg structure parameters. The transfer of DHA was significantly higher in the experimental groups fed with supplemented diet with higher amount of DHA (P<0.01). These eggs may serve as dietary alternatives to provide significant amounts of n-3 PUFAs in our daily diet.

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