Multi-enriched eggs with omega 3 fatty acids, vitamin E and selenium

Natasha Gjorgovska[1†], K. Filev[2]

[1]Institute of Animal Science, Skopje, Macedonia; [2]Faculty of Agricultural Sciences and Food, Skopje, Macedonia

SUMMARY

Production of designed eggs was focused on enriching the egg yolk with n-3 fatty acids protected with antioxidants such as vitamin E and selenium.

Forty (40) molted laying hens (Hisex Brown) were divided in four groups times ten and treated with enriched feed:-0.46 mg selenium/kg, DL -tocopherol acetate 100 and 200 mg/kg, fish oil 1.93% and 2.90%.

The layers (group 3 and 4) fed with 200 mg of vitamin E/kg feed performed higher laying intensity (90.0 and 82.0%) in comparison to layers (group 1 and 2) fed with 100 mg vitamin E/kg feed which performed lower intensity (68.3% and 70.0%).

Selenium content in egg yolk was balanced between 18.36µg/100g to 21.50µg/100g, and 3.48µg to 4.14µg in one yolk.

The treatment with vitamin E increased its content in yolk, because hens receiving 100 mg vitamin E in kg feed produced eggs with 9.88 mg and 13.2 mg vitamin E/100 g yolk. Hens treated with 200 mg vitamin E in kg feed, in 100 g yolk transferred 24.03 mg and 20.50 mg vitamin E.

Omega 3 fatty acids, docosahexaenoic (DHA, C22:6n-3) and eicosapentaenoic (EPA, C20:5n-3), are highly contained in eggs of hens treated with higher level of fish oil (1180 mg DHA and EPA/kg feed) because in 100 g yolk 1050 mg and 1062 mg were found and in those treated with a lower level of fish oil (792 mg DHA and EPA/kg feed) in 100 g yolk 896 mg and 873 mg were found, respectively. In one yolk (by calculations) 169 mg and 170 mg were found in groups treated with a higher level and 139 mg to 144 mg DHA and EPA/yolk were found in the groups treated with a lower level of n-3 fatty acids.

From this study it can be confirmed that it is possible multi-enriched eggs with n-3 fatty acids, vitamin E and selenium to be produced.

Keywords: enriched eggs, omega 3 fatty acids, vitamin E, selenium

† Corresponding author e-mail: ngjorgovska@yahoo.com

INTRODUCTION

Designing table eggs and their enrichment by many essential nutrients is considered excellent food for humans called “functional food”. These eggs are a good source of lipids rich with omega 3 fatty acids, vitamins and very important essential dietary minerals such as selenium, proteins and many other essential nutrients (Stadelman, 1999; Leeson and Caston, 2003; Park et al., 2005).

Enrichement by polyunsaturated fatty acids as omega 3 was started to be realized many years ago during the 80th years in the last century. The procedure for production of this type of eggs permanently was advanced till today (Herber and Van Elswyk, 1996; Filev et al., 2001a; Filev et al., 2001b).

Vitamins enrichement becomes very important because of antioxidative properties of some of them and essential physiological characteristics important for humans. One of the most important vitamins is vitamin E because it improves the functionality of the table eggs and their important nutritive value (Meluzzi et al., 2000).

Selenium (Se) is recognized as an essential dietary nutrient for almost more than 40 years. It is required for maintenance of health, growth and physiological functions. At the beginning Se has been added to the laying hens’ diet via inorganic sources as sodium selenite, but later it has been shown that organic selenium is more bioavailable (Utterback et al., 2005; Gjorgovska and Filev, 2010).

The use of organic selenium results on less selenium transfer to the environment through feces and it is more deposited into eggs and tissues (Payne et al., 2005).

Our plan of experiment was to produce enriched eggs by polyunsaturated omega (n-3) fatty acids, expecting to be protected with DL alpha tocopherol acetate and selenium which are important antioxidative substances.

MATERIAL AND METHODS

Forty molted 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 and were divided into four experimental groups (10 birds per group). The experiment was lasting 45 days. The live 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 waterers in every cage. Egg samples were collected every 10th day, 6 eggs per group. The eggs were measured, cracked,

the shells were discharged, the yolks were separated, then mixed and homogenized, stored frozen and analyzed up to 7 days.

The hens were fed with diets 1 and 2 (Tab. 1). Both mixtures were supplemented with 0.46 mg/kg selenium, 0.30 mg/kg as sodium selenite and 0.16 mg/kg as selenium yeast. Mixture 1 was supplemented with 100 mg/kg vitamin E (DL alpha tocopherol acetate). Mixture 2 was supplemeneted with 200 mg/kg vitamin E. The needed amount of n-3 polyunsaturated fatty acids (omega 3) was provided by using 1.93% fish oil and 1.71% fish meal (herring) in mixture 1 and in mixture 2, 2.90% fish oil and 1.69% fish meal were provided. The total amount of omega 3 fatty acids in mixture 1 was 792 mg/kg and in mixture 2 it was 1180 mg/kg (by calculation).

Table 1. Composition of enriched feed mixtures with omega 3 fatty acids, vitamin E and selenium

Ingredient	Content in %	
	Mix 1	Mix 2
Maize	51.96	50.34
Wheat bran	10.00	10.00
Soybean meal	10.18	10.86
Sunflower meal (28%)	13.00	13.00
Fish meal	1.71	1.69
Fish oil	1.93	2.90
Synthetic methionine	0.08	0.08
Lysine	0.06	0.04
Choline chloride (60%)	0.05	0.05
Calcium carbonate	9.00	9.00
Di calcium phosphate	1.30	1.30
Salt	0.23	0.24
Premix	0.50	0.50
Total	100.00	100.00
ME, Kcal/kg	2700	2722
Crude protein, %	15.0	15.0
Crude fiber, %	4.05	4.08
Lysine, %	0.80	0.80
Methionine, %	0.40	0.40
Crude fat, %	5.48	5.40
Omega 3 fatty acids, g/kg	0.792	1.180
Calcium, %	3.80	3.89
Phosphorus available, %	0.38	0.38
Selenium, mg/kg (total)	0.46	0.46
- Se from sodium selenite, mg/kg	0.30	0.30
- Se from selenium yeast, mg/kg	0.16	0.16
DL α tocopherol acetate, mg/kg	100	200

Concentrations of docosahexaenoic (DHA, C22:6n-3) and eicosapentaenoic (EPA, C20:5n-3) fatty acids were 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 acids were determined by gas chromatography (AOCS –Ce 1f – 96) adapted by Abril and Barclay (1999), with identification of fatty acids by comparing of their retention times and quantified by areas standardization.

For establishing the vitamin E concentration in the egg yolk 6 yolks were used for every sample, in fresh condition, homogenized and then saponified with stirring in an alcoholic solution of potassium hydroxide. The analytes were then extracted with hexane and washed with water. The organic phase was removed by evaporation and the residue was dissolved in methanol, filtered and then injected into the chromatographic system. The mobile phase used was a 2.5 m*M* acetic acid-sodium acetate buffer. The flow rate was 1.0 ml/min (Delgado-Zamarreno et al., 2001) The High Performance Liquid Chromatography (HPLC) system used for analysis was Perkin Elmer. The results were expressed in mg/100 g yolk, and in one yolk.

Selenium content was measured in egg yolk. The egg samples were prepared by mixing 6 yolks in one sample, homogenized, and then the needed amount of yolk was used for analysis. The rest of samples were frozen and kept at -20^{o}C. Selenium content in the yolk was conducted mass-spectrophotometrically and presented in µg/100 g yolk, and in one yolk (Chinrasri et al., 2009).

Data were tested for significance using the analysis of variance, the F-test according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The obtained results from this investigation are presented in Table 2. The live weight of the hens at the beginning of the experiment and at the end was similar between the groups and did not have significant differences. Hens' performances as egg production intensity were significantly different between the groups 1 and 2, where vitamin E was supplemented at the level of 100 mg/kg, and the groups 3 and 4 on the other hand where vitamin E was supplemented at the level of 200 mg/kg ($p<0.01$).

Such kind of differences was not found between the groups 1 and 2 as well as the groups 3 and 4. These productive performances are not similar according to the results published by Maziar et al. (2008) and some other authors (Gebert et al., 1998; Qi and Sim, 1998; Meluzzi et al., 2000).

The egg weight was also significantly different between the groups 1 and 2 in relation to the groups 3 and 4, but in the opposite way because the hens fed with an increased amount of vitamin E (200 mg/kg) laid smaller eggs ($p<0.01$).

Table2. Production of enriched eggs with omega 3 fatty acids, vitamin E and selenium

Indicator	Selenium content in feed 0.46 mg/kg			
	Vitamin E content in feed 100 mg/kg		Vitamin E content in feed 200 mg/kg	
	DHA and EPA content in kg feed (calculated)			
	792 mg	1180 mg	792 mg	1180 mg
	Group 1	Group 2	Group 3	Group 4
Number of experimental hens	10	10	10	10
Hen's age, weeks	80	80	80	80
Live weight of hens				
- at the beginning, kg	2.33	2.20	2.15	2.24
- at the end, kg	2.08	2.20	2.13	2.22
Egg production				
- intensity, %	68.33^{A}	70.00^{A}	90.00^{B}	82.00^{B}
- average egg weight, g	72.23^{A}	70.48^{A}	69.06^{B}	66.97^{B}
Daily feed intake, g/hen	120	120	120	120
Se content in yolk				
- in 100 µg, g	21.14	18.57	18.36	20.50
- in one yolk, µg	4.14	3.58	3.48	4.02
Vitamin E content in yolk				
- in 100g, mg	13.20^{A}	9.88^{A}	24.03^{B}	20.50^{B}
- in one yolk, mg	2.59^{A}	1.91^{A}	4.55^{B}	4.01^{B}
DHA and EPA content in yolk				
- DHA, mg/100g	823	972	803	982
- EPA, mg/100g	73	78	70	80
- DHA + EPA, mg/100g	896^{A}	1050^{B}	873^{A}	1062^{B}
- DHA + EPA, in one egg, mg	144^{A}	169^{B}	139^{A}	170^{B}

DHA - docosahexaenoic acid ;EPA - eicosapentaenoic acid ;DHA + EPA – sum of docosahexaenoic acid and eicosapentaenoic acid; ^{A,B} – Values in the same row with no common superscript differ significantly ($p<0.01$)

Selenium content in the egg yolk in all experimental groups in 100 g yolk ranged from 18.36 µg to 21.14µg, and in one yolk from 3.48µg to 4.14µg. Se deposition in egg yolks indicated that it can be incorporated into eggs effectively. The differences of Se concentration in the yolks of different groups are the result of the amount of feed consumption because the feed of all groups were treated with the same amount of Se, but the hens were not fed individually.

The results obtained by Payne et al. (2005) who investigated the Se deposition in eggs fed with a different amount of Se from sodium selenite showed that the level of Se in eggs laid from hens fed with 0.30 ppm of Se was

0.284 ppm and the hens fed with 0.60 ppm of Se laid eggs whose concentration of Se was 0.299 ppm which transformed in µg is equal to 28.4 and 29.9 µg/100g of the egg mass.

Vitamin E content in 100g yolk was in a direct dependence on the treatment because in groups treated with 100 mg vitamin E/kg feed was lower 13.2 mg and 9.88 mg in 100g yolk, respectively, but in those treated with 200 mg vitamin E in kg feed was 24.03 mg and 20.50 mg in 100g yolk. The similar situation was with the vitamin E content in one yolk which was 2.59 and 1.91 mg in the egg yolk of hens fed with 100 mg vitamin E in kg feed, and in those which had 200 mg vitamin E in kg feed the content was higher 4.01 and 4.55 mg, respectively.

Maziar et al. (2008) established higher concentration of 485.37 µg/g yolk of vitamin E in eggs from hens fed with 200 mg/kg supplemented α-tocopherol acetate in his experiment. According to Maziar et al. (2008) the relation between the α-tocopherol acetate supplementation level in the feed and its transfer in the yolk has a linear relationship.

Omega 3 fatty acids are presented on a higher level in the egg yolk from hens which receive feed with a higher concentration of fatty acids (DHA and EPA). Because of that, hens fed with DHA and EPA in the amount of 792 mg/kg from fish oil laid eggs in which yolk contained 144 and 139 mg (DHA and EPA) in group 1 and 3, respectively, and in others in which feed contained 1180 mg/kg feed DHA and EPA (from fish oil), laid eggs in which the yolk contained 169 and 170 mg DHA and EPA in group 2 and 4, respectively.

CONCLUSIONS

This experiment confirms that it is possible to produce multi-enriched eggs with omega 3 fatty acids, vitamin E and selenium.

The higher level of vitamin E in feed enabled higher laying intensity of hens 82% in the group 4 and 90% in the group 3, but bigger egg weight of 72.23 g and 70.48 g in groups 1 and 2, respectively.

The selenium content in the egg yolk was balanced from 3.48µg to 4.14µg in one yolk.

The content of vitamin E in one yolk was in linear relationship with the content in the mixture.

The amount of selenium and vitamin E contained in one multi-enriched egg is able to satisfy 10% and 35% of the daily human requirements estimated at 45 µg/day for Se and 12 mg/day for vitamin E (USDA, Dietary Reference Intakes, 2011).

From 81% to 83% of DHA and EPA values recommended by the European Food Safety Authority (EFSA, 2010) is satisfied with one multi-enriched eggs from the experimental groups 2 and 4, respectively.

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