Romanian Biotechnological Letters *Vol. 21, No. 4, 2016*

ORIGINAL PAPER

Effect of Layer Diets Enriched in Omega-3 Fatty Acids Supplemented with Cu on the Nutritive Value of the Eggs

Received for publication, March 16, 2015
Accepted, May 17, 2016

TATIANA PANAITE[1], RODICA DIANA CRISTE[1], MARIANA ROPOTA[1], GABRIELA MARIA CORNESCU[1], DANIELA CRISTIANA ALEXANDRESCU [2], VIRGIL CRISTE[3], GABRIELA VASILE[3], MARGARETA OLTEANU[1], ARABELA UNTEA [1]

[1]National Research-Development Institute for Animal Biology and Nutrition (IBNA), 1 Calea Bucuresti, Balotesti, 077015, Ilfov, Romania

[2]Valahia University of Targoviste, Faculty of Environmental Engineering and Food Science, Sinaia Street, nr. 13, 130004, Dambovita, Romania

[3]The National Research – Development Institute for Industrial Ecology– ECOIND, 71-73 Drumul Podu Dambovitei street, District 6, 060652, Bucharest, Romania

***Corresponding Author e-mail*:** tatiana_panaite@yahoo.com

Abstract

A trial on 120 Lohmann Brown laying hens (aged 60 weeks) was conducted for a period of six weeks. The layers were assigned to 4 groups and housed in cages (2 hens/cage) on three tiers. Compared to the diet for the control group (C), the diet for the experimental groups (E1, E2, E3) included 5% flaxseed meal and 2% camelina meal. Compared to the Cu concentration in diet C (6 mg/kg), the diets for the experimental groups contained: 75 mg Cu/kg (E1), 100 mg Cu/kg (E2) and 150 mg Cu/kg (E3) from CuSO4x5H2O. Throughout the experiment the production parameters were monitored. Every two weeks, 18 eggs/group were collected for yolk fatty acids and cholesterol determinations. In the same weeks, 15 samples of excreta/ group, were collected which were assayed for Cu and Zn concentration. The use of the experimental diets produced eggs with properties of functional foods because the yolk concentration of α linolenic acid was 75.56% higher than in group C, while the cholesterol concentration was significantly (P≤0.05) lower than in group C. The 150 ppm Cu supplement (E3) produced the highest allowed load of heavy metals in the droppings according to the acting norms for environmental protection.

Keywords: *flaxseed meal, camelina meal, copper, eggs, feeding value, functional food*

1. Introduction

For the modern society, the prevention of diseases through healthy diets, major attribute of people's quality of life, has turned into a major social, political and economic concern. The consumers of our time are increasingly informed about, and interested by the foods that are beneficial to health and which can provide a better quality of life [17].

The polyunsaturated fatty acids (PUFA), particularly the omega 3 polyunsaturated fatty acids (PUFA Ω3) are among the nutrients ascertained to be beneficial for health maintenance. These fatty acids are essential to a normal growth and development. They help preventing and treating heart diseases, high blood pressure, arthritis, some cancer types [31; 32]. Although the essential role of PUFA Ω3, which are not synthesized by the organism, is known, the modern diets don't provide the daily requirements for these fatty acids [24]. The hen egg is one of the foods that contain PUFA. Fortunately the feeding value of the egg can be influenced through the formulation and composition of layer diets [16]. The enrichment of hen eggs in PUFA Ω3, compared to the standard egg, can be achieved through the use of diet formulations that include marine products (such as fish oil), oilseed crop plants (e.g., flax, rape, canola, soybean,

safflower) and by-products (such as rice bran). The flax (*Linumusitatissimum* L.) is a plant rich in PUFA Ω3 [2] and therefore it has been used under different forms (seeds, oil and meal) to enrich the egg in these nutrients [5; 10; 13]. Recently, Camelina (*Camelina sativa*), also known as the false flax, raised the interest of the nutritionists due to its high concentration of PUFA, being used in layer diets particularly as meal [6; 7] and broiler diets as oil or seeds [9]. The camelina also contains a rather large amount of tocopherols (in oil, up to 800 mg tocopherols/kg), with the γ–tocopherol predominating [38]. Researchers [1] determined 400 mg polyphenols /kg camelina oil. Even enriched in PUFA Ω3, the hen egg is regarded with reticence by the consumers due to its high cholesterol content. Although [15] considers that the cholesterol content of the egg yolk cannot be modified, there are several ways in which the researchers used to accomplish this goal, but layer feeding remains the simplest, rather cheapest and most natural way to solve this problem. Furthermore, layer feeding is preferred by the consumers, as long as the dietary ingredients are natural. Among the studied feeding solutions are those that use oleaginous plants or trace elements. Researchers [34] patented several methods to reduce egg cholesterol. When they used 4% canola oil and 7% canola meal, the cholesterol concentration decreased from 215.2 to 183.7 mg/egg (14.6%). The effect of the dietary flax seeds on egg cholesterol concentration has also been investigated by some researchers [37]. They used 4.32 and 8.64% flax seeds in layer diets for 30 and 60 days, respectively. They noticed significant decreases of the cholesterol concentration in the eggs from the group treated with 8.64% flax seeds, the decreased being ascribed to the high level of dietary fibre. Among the minerals cited in the literature as means of influencing the cholesterol concentration of the egg yolk, are the copper [23; 28; 29] and chromium [12; 36]. Some researchers [3] reported that the plasma cholesterol has been decreased by 20% when the layers were treated with 250 ppm Cu from copper sulphate. Other researchers [29] proved, in a 8-week study, that the plasma cholesterol can be decreased by about 20% compared to the control group, when the layer diets have been supplemented with 125 ppm Cu (from pentahydrate copper sulphate); however, there was no decrease in the plasma cholesterol of the layers treated with 250 ppm Cu. Within this context we conducted an experimental study trying to produce eggs with improved nutritive properties (high PUFA Ω3 level and lower cholesterol level), using layer diets enriched in omega 3 polyunsaturated fatty acids supplemented with copper.

2. Material and methods

We conducted a 6-week experiment on 120 Lohmann Brown layers (aged 60 weeks) assigned to 4 groups (C, E1, E2 and E3). The experimental house provide controlled environmental conditions (average temperature: 21.7±1.40^{0}C; average humidity 50.21±7.39 %) and light schedule 16L: 8D. The layers were kept in cages (2 layers/cage), on three tiers, according to the European norms 1999/74/CE adopted in 2012.

Table 1. Dietary formulation

Item	C	E 1	E 2	E 3
Corn, %	35.74	33.75	33.75	33.75
Rice, %	15	15	15	15
Wheat, %	10	10	10	10
Rapeseeds meal, %	15	9.5	9.5	9.5
Soybean meal, %	9	9	9	9
Gluten, %	2	2	2	2
Flax meal, %	-	5	5	5

Camelina meal, %	-	2	2	2
Oil, %	2	2.4	2.4	2.4
Monocalcium phosphate, %	1.06	1.06	1.06	1.06
Calcium carbonate, %	8.7	8.7	8.7	8.7
Salt, %	0.3	0.3	0.3	0.3
Dl-methionine,%	0.15	0.12	0.12	0.12
L-Lysine HCl, %	-	0.12	0.12	0.12
Choline, %	0.05	0.05	0.05	0.05
Vitamin-mineral premix, %	1*	1**	1***	1****
Total	**100**	**100**	**100**	**100**
Chemical composition				
Metabolisable energy (kcal/kg)	2648.69	2665.43	2665.43	2665.43
Dry matter (%)	90.79	90.42	90.25	90.31
Crude protein (%)	16.14	16.61	16.52	16.82
Ether extractives (%)	5.63	6.87	6.20	6.33
α linolenic acid (g / 100g fat)	1.38	10.33	10.30	10.26
Cu (mg/kg)	6.28	74.59	98.61	156.83
*1 kg premix contains: 1350000 IU/kg vitamin A; 300000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; **600 mg/kg copper**; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 6000 mg/kg; **Premixcontains 7500 mg Cu/kg; ***Premixcontains10000 mg Cu/kg; ****Premixcontains15000 mg Cu/kg				

The layers had free access to the feed and water. The basal formulation of the diets was similar for all 4 groups (Table 1). The difference between the control group (C) and the experimental groups came from the inclusion of PUFA Ω3-rich ingredients (2% camelina meal and 5% flax meal) in the diet formulations of the experimental groups and from the level of Cu in the compound feed (CF). Compared to the Cu concentration in diet C (6 mg/kg), the diets for the experimental groups contained the following different concentrations of Cu: E1 (75 mg Cu/kg CF); E2 (100 mg Cu/kg CF); E3 (150 mg Cu/kg CF). Copper was supplied from pentahydrate copper sulphate. The concentration of α-linolenic acid (C18:3n3) was 49.9 g/100 g fat in the flax meal and 28.37 g/100 g fat in the camelina meal. The copper was added as pentahydrate sulphate ($CuSO_4 \times 5H_2O$). Throughout the experiment we monitored the daily feed intake (g/day/layer), the feed conversion ratio (g feed/g egg), the laying percentage (%) and egg weight (g). A single batch of compound feed has been manufactured for each group. Samples have been collected from these batches and assayed for the basic chemical composition, Cu and fatty acids. The methods from Regulation (CE) 152/2009 (Methods of sampling and analysis for the official control of feed) have been used: the gravimetric method for dry matter (DM); the Kjeldahl method for crude protein (CP); extraction in organic solvents for ether extractives (EE); atomic absorption spectrometry for Cu. The fatty acids concentration has been determined by gas chromatography according to standards SR CEN ISO/TS 17764-1/ 2008 (Feeds. Determination of the fatty acids content 1. Preparation of the fatty acids methyl esters) and SR CEN ISO/TS 17764-2/ 2008 (Feeds. Determination of the fatty acids content 2. Method of gas chromatography). According to Regulation (CE) 152/2009, the metabolisable energy for poultry has been calculated using equation (1), based on the proportion of specific dietary nutrients:

$$\mathbf{ME\ (MJ/kg\ CF) = 0.1551 \times \%\ crude\ protein + 0.3431 \times \%\ ether\ extractives + 0.1669 \times \%\ starch + + 0.1301 \times \%\ total\ sugar\ (expressed\ as\ saccharose)} \quad (1)$$

Every two weeks, 18 eggs/group were collected randomly from each group and used to form 6 samples of yolk/group (3 eggs/sample), which were assayed for fatty acids and

cholesterol concentration. Fatty acids were determined by gas chromatography, whose working principle is the transformation of the fatty acids from the sample into methyl esters, followed by their separation in chromatographic column, identification by comparison with standard chromatograms and percent determination of the fatty acids esters from the sample. For the preparation of the fatty acids methyl esters (FAME) in agreement with the standard ISO 5508: 2002, we weighed a sample of about 1 g of fat extracted from the dried yolk (65^{o}C). The analysis of the methyl esters was done according to standard SR EN ISO 5509:2002. We used a Perkin Elmer-Clarus 500 chromatograph fitted with flame ionization detector (FID) and BPX70 capillary column, with medium or high polar stationary phase, 60 m in length, and 0.25mm inner diameter, 0.25µm thick film. The carrier gas was H_2, while the burning gas was air of analytical purity. The amount of fatty acids esters from the sample (fat) is calculated by the relation between the sample area, the standard area and dilution. The result is expressed in g (grams) of fatty acids in 100 g fat. The method used to determine the cholesterol was in agreement with AOAC International standard, 2002 (Cholesterol in multicomponent foods– Gas Chromatographic method. Assoc. Off. Anal. Chem. Arlington, VA). The working principle is the saponification of the sample followed by extraction is petrol ether, concentration and addition of chloroform. The sample is split in the GC, it is separated in the chromatographic column, and the results are compared with the standard chromatograms by measuring the peak area. We used a Perkin Elmer-Clarus 500 chromatograph fitted with flame ionization detector (FID) and capillary separation column HP-5, 30 in length, and 0.320mm inner diameter, 0.10µm thick film. The carrier gas was H_2, while the burning gas was air of analytical purity. The concentration of the cholesterol sample is calculated function of a sample of known concentration (standard). The result is expressed in mg cholesterol/100 mg sample. Given that EU documents on environmental protection consider that the dietary Cu and Zn added to the compound feeds are heavy metals, every two weeks we collected 15 samples of excreta/group, which were assayed by atomic absorption spectrometry for the concentration of the two trace elements. A sample (about 2 g) from excreta, dried at 65^0 C, was burnt and the solution made after cooling was filtered, the residue was rinsed in ultrapure water and the fluid was collected in a 50 mL graded flask. This solution was used to determine the total concentration of Cu and Zn, with a Thermo Scientific M6 Dual atomic absorption spectrometer, using the standardized method SR ISO 11047:1999 for Cr, Cu, Mn, Pb and Zn. In the end of the experiment we collected from 6 layers per group, 1-2 mL venous blood, for cholesterol determinations by spectrophotometry. The determination was done by the laboratory of SC SYNEVOVET SRL (office@synevovet.ro), with profile of paraclinical analyses, authorised by ANSVSA Romania (licence no. 96/8.05.2014).

3. Statistical analysis

The analytical data have been compared by variance analysis (ANOVA), using Stat View for WINDOWS (SAS, version 6.0). The intra-group differences between the mean values were considered significant for $P<0.05$. The results were expressed as mean ± SD for all measurements.

4. Results and discussions

Generally, oleaginous seeds and meals are used in poultry diets as source of essential fatty acids (PUFA Ω 3), and for their content of energy and protein [4; 6]. The feed ingredients selected with the purpose to enrich the diets for the experimental groups (E1, E2, E3) in PUFA Ω 3 (Table 1) were the flax meal (FM) and the camelina meal (CM). Flaxseed

or linseeds are the seeds of an oleaginous plant (*Linumusitatissimum L.*), which contains significant proportions of PUFA Ω 3 [2]. Some researchers [13] considered that for a dietary inclusion rate of 10-20% flaxseeds, there is a significant increase of α-linolenic acid (ALA) and docosahexaenoic acid (DHA) concentration in the egg yolk. Other researchers [10] also obtained egg yolks enriched in ALA and DHA, although they used just 5% linseeds in layer diets. Because the feeds account for over 65% of the poultry production costs, we decided to examine the effect of 7% meals from oleaginous plants (5% flaxseeds meal and 2% camelina meal). Camelina, a low-input oleaginous plant, is regarded as a viable alternative to flax in poultry diets [26; 39]. Table 2 shows the chemical composition of the two meals used to manufacture the compound feeds.

Table 2. Chemical composition of the flaxseed and camelina meals

Parameter	Flaxseed meal (FM)	Camelina meal (CM)
ME, kcal/kg	1841.23	1804.8
Crude protein, %	20.91	33.72
Fat, %	17.23	8.82
Fibre, %	7.18	10.93
Ash, %	4.12	5.69
Fatty acids, g/100 g fat		
Stearic (18:0)	2.56	2.38
Ooleic(18:1)	18.81	18.89
Linoleic (18:2ω6)	19.00	24.35
α-linolenic (18:3 ω 3)	49.90	28.37
Σ Saturates	11.29	12.29
Σ UFA	88.49	87.70
Σ PUFA of which:	69.28	56.12
Ω3	49.90	28.40
Ω6	19.38	27.72
Ω6/ Ω3	0.39	0.98

As shown in Table 2, CM was characterized by a higher content of crude protein than FM, but PUFA Ω 3 concentration in FM was 1.76 times higher than in CM. The best PUFAΩ6/ PUFA Ω3 ration was determined in FM too.

Table 3. Fatty acids profile in the compound feeds fat (g/100 g fat)

Specification	C	E 1	E 2	E 3
Stearic (18:0)	2.25	2.4	2.48	2.23
Oleic (18:1)	34.73	31.93	31.59	31.76
Linoleic (18:2ω6)	43.63	40.47	40.78	41.03
α-Linolenic (18:3 ω 3)	1.35	8.18	8.03	8.05
Σ Saturates	17.82	16.42	16.56	16.32
Σ UFA	81.98	82.88	83.09	82.91
Σ PUFA, of which:	46.67	50.51	50.95	50.61
Σ Ω3	1.96	8.81	8.68	8.72
Σ Ω6	44.68	41.70	42.27	41.89
Ω6/ Ω3	22.79	4.73	4.87	4.80

The fatty acids profile of the fat from the flaxseed and camelina meals enriched the experimental diets in PUFA Ω 3 (Table 3). ALA concentration was about 6 times higher in the experimental CF than in the CF for group C. ALA (18:3 ω -3) is a highly important unsaturated fatty acid because it is precursor for the entire chain of ω 3fatty acids, such as the eicosapentaenoic acid (20:5 ω -3) and the docosahexaenoic acid (22:6 ω -3), which are of great interest as functional nutrients due to their beneficial health effects [6]. Because of the

fatty acids profile of the compound feeds fat, the best PUFA Ω6/ PUFA Ω3 ratio was determined in the CF of the experimental groups, 80% lower, in average, than in the CF for group C (Table 3).

Table 4. Layer performance (average values/ group/ experimental period)

Item	C	E1	E2	E3
Average daily feed intake (g/layer/day)	109.49±6.53^{b,c}	121.85±7.6 ^{a,d}	119.04±6.01^{a,d}	107.90±4.64^{b,c}
Laying percentage (%)	81.70±4.70 $^{b, c,d}$	89.90±4.40 ^{a}	90.90±4.01 ^{a,d}	85.5±4.40^{d}
Average egg weight (g)	63.52 ±0.85^{b,c}	65.77±0.93^{a,c,d}	64.96±1.18 ^{a,b,d}	63.43±0.81^{b,c}
Feed conversion ratio (kg CF/kg egg)	2.18±0.26 ^{b,c,d}	2.07±1.75^{a}	2.05±0.21^{a}	2.03± 0.24^{a}

Where: a, b, c, d show significant (P≤0.05) differences compared to C, E1, E2, E3

The data on layer performance (Table 4) show that groups E1 and E2 had higher average daily feed intakes ($P \leq 0.05$) than groups C and E3. On the other hand, in all three experimental groups, feed conversion ratio was lower ($P \leq 0.05$) than in group C. Egg weight was lower ($P \leq 0.05$) in group C than in groups E1 and E2, but it was not different from that of the eggs from group E3. Group C had the lowest laying percentage, statistically significant ($P \leq 0.05$) different from groups E1 and E2. The presence of flaxseed meal (5%) and camelina meal (2%) in the diets of the three experimental groups didn't affect adversely their performance, which is in agreement with the data reported by [6] and [10]. Our interpretation of layer performance, particularly in groups E1 and E2 (Table 4) is similar with that of [35], who considered that the Cu supplementation of the diets reduces the ideal concentration of glucosinolates by absorption from the intestinal lumen or by their transformation in other by-products. The presence of FM and CM in the diet for the experimental groups increased the concentration of glucosinolates more than in a conventional diet, such as the diet for group C. The lower performance of the layers from group E3, compared to the performance of the layers from groups E1 and E2, seems to indicate that the dietary level of 150 ppm Cu has a moderate beneficial effect. Although [19] showed that the use of Cu supplementations in excess of 200 ppm may cause gizzard erosion and decreased feed intake. Some researchers [3] reported that the dietary supplements of 250 ppm Cu from copper sulphate decreased the egg production, while [29] reported a higher production of eggs using dietary supplements of 250 ppm Cu from copper sulphate.

Table 5. Profile of the unsaturated fatty acids profile in the dried egg yolk (g/100 g fat)
- average values/ group/ experimental period-

Item	C	E1	E2	E3
Stearic, C18:0	10.50±1.39	10.52±0.64	9.86±1.73	11.14±0.50
Oleic, C18:1	34.45±2.70	33.63±0.86	34.62±1.20	33.24±0.82
Linoleic, C18:2n6	20.90±0.85^{b,c}	22.22±0.52^{a,d}	21.87±0.75^{a}	21.01±0.78^{b}
Linolenic, α C18:3n3	0.50±0.09^{b,c,d}	1.91±0.11^{a}	2.01±0.44^{a}	1.77±0.17^{a}
Eicosatrienoic, C20:3n3	0.23±0.03	0.21±0.01	0.20±0.03	0.23±0.04
Docosapentaenoic, C22:5n3	0.12±0.02^{b,c,d}	0.18±0.04^{a,c}	0.22±0.03^{a,b}	0.22±0.03^{a}
Docosahexaenoic, C22:6n3	1.21±0.33^{b,c,d}	2.8±0.18^{a}	2.62±0.71^{a}	2.95±0.31^{a}
Σ SFA	33.94±1.15^{c}	32.45±0.76	31.76±2.11^{a,d}	33.44±0.71^{c}
Σ UFA	66.05±1.09^{b,c}	67.56±0.76^{a}	68.16±2.00^{a,d}	66.55±0.71^{c}
Σ PUFA, of which:	28.46±2.12^{b,c,d}	31.21±0.58^{a}	30.58±0.43^{a}	29.50±0.93^{a}
Ω3	2.06±0.36^{b,c,d}	5.10±0.10 ^{a}	5.05±0.34^{a}	5.17±0.32^{a}
Ω6	26.40±1.86	26.11±0.54	25.53±0.31	25.33±0.72
Ω6/Ω3	12.82±1.77^{b,c,d}	5.12±0.13 ^{a}	5.06±0.37^{a}	4.92±0.26^{a}

Where: a, b, c, d show significant (P≤0.05) differences compared to C, E1, E2, E3

The fatty acids profile of the yolk dried at 65 ^{0}C (Table 5) shows that ALA (PUFA Ω-3) concentration was higher ($P \leq 0.05$) in the yolk from the eggs harvested from the

experimental groups, than in the yolk of the eggs from the control group. DHA concentration in the yolk was also higher ($P \leq 0.05$) in the experimental groups than in the control group. These data on the higher PUFA Ω-3 concentration in the yolk of the eggs from the experimental groups are also reflected in the PUFA Ω-6/ PUFA Ω-3 ratio, which was significantly ($P \leq 0.05$) lower in the experimental groups than in the control group. Table 5 data show that the improvement of the fatty acids profile in the eggs from the experimental groups is due, as expected, to the presence of the two meals of oleaginous plants in the diet. Some researchers [6] also reported a PUFA Ω-6/ PUFA Ω-3 ratio of 14.8, 5.6, 4.6, and 4.3, for the eggs harvested from layers fed with CF as follows: control, 5% CM, 10% CM and 15% CM, respectively. Researchers [5] showed that it is well established that the use of flax seeds in layer diets changes the fatty acids profile of the egg yolk. Some researchers [29] showed that [26] observed changes in hen body lipids, 17b-estradiol, and hepatic lipogenic enzyme concentrations due to supplementing 250 to 1,000 mg Cu/kg diet. These changes in lipid metabolism likely result in increased metabolism of cholesterol by cholesterol 7a-hydroxylase, as in the chicken [20], and result in decreased egg cholesterol [29].

Table 6. Cholesterol concentration determined in the serum and in dried yolk

Item	**C** ***6 mg Cu/kg CF***	**E1** ***75 mg Cu/kgCF***	**E2** ***100mgCu/kgCF***	**E3** ***150 mgCu/kgCF***
Serum,				
mg cholesterol /dL	99.50 ± 66.53	88.80 ± 12.16	87.50 ± 11.92	87.00 ± 10.50
Egg yolk (average values/ group/ experimental period)				
g /100g fat	$249.29 \pm 15.78^{b,c,d}$	$230.06 \pm 14.56^{a,d}$	$224.28 \pm 19.62^{a,d}$	$211.31 \pm 19.54^{a,b,c}$

Where: a, b, c, d show significant ($P \leq 0.05$) differences compared to C, E1, E2, E3

The use of experimental diets decreased ($P \leq 0.05$) the cholesterol concentration in the yolk compared to the control group (Table 6). The cholesterol concentration in the yolk of the experimental groups decreased by 17.97 % (E3), 11.15 % (E2) and 8.36 % (E1) compared to the control group. Our study didn't determine statistically significant differences in the serum cholesterol concentration from the three experimental groups and the control group (Table 6). Other researchers [30] consider that the 125 ppm Cu supplementation decreases the cholesterol concentration in the yolk, but the further increase of the dietary Cu level to 250 ppm didn't produce a significant decrease of the cholesterol concentration in the yolk. Other authors too [3; 8; 22], reported decreased cholesterol concentration in the egg using 250 ppm Cu supplements. On the other hand, [27] didn't find differences in yolk cholesterol. Although [21] showed that the diet for hens consisting of high-polyphenols level from extra-virgin olive oil can improve the fatty acid quality of egg-yolk while lowering the egg-yolk cholesterol level which could be a beneficial functional food for human health. Such eggs have also been obtained by feeding the laying hens with the three experimental diets used in this study enriched in PUFA Ω-3 and supplemented with Cu: 75 ppm (E1), 100 ppm (E2) and 150 ppm (E3). They are functional foods because they contain nutrients that have a positive influence on one or several target functions of the organism [33]. Because fecesare the main way of excreting the Cu (EFSA opinion, 9 Feb. 2003), and because Cu and Zn are heavy metals, we determined their concentration in the excreta (Table 7). We noticed a very good correlation between the dietary Cu concentration and the excreted amount of Cu ($R^2 = 0.976$). The excreted concentration of Cu increased proportionally with the dietary amount of Cu. Thus, in group 3 (150 ppm supplemental Cu), the excreted Cu reached the maximal value (500 mg/kg DM) allowed by the soil and water protection norms from Romania. Zn concentration didn't exceed these norms.

Table 7. Concentration of Cu and Zn in the droppings (average values/ group/ experimental period)

Item	C	E1	E2	E3
Copper, (mg/kg DM)	26.60±3.64^{b,c,d}	177.00±9.54^{a,c,d}	277.67±27.47^{a,b,d}	505.67±63.31^{a,b,c}
Zinc, (mg/kg DM)	297.00±31.80	300.00±23.39	329.00±12.17	335.00±40.04

5. Conclusions

In average, ALA concentration in the yolk of the eggs from the three experimental groups was 75.56% higher than in the eggs from the control group, while PUFA Ω6/ PUFA Ω3 ratio in the yolk of the eggs from the three experimental groups was 158.93% lower than in the eggs from the control group. The cholesterol concentration decreased significantly ($P \leq 0.05$) in the yolk of the eggs from the three experimental groups, the rate of reduction increasing with the dietary Cu supplement. The eggs produced by the hens fed with CF enriched in PUFA Ω-3 and supplemented with Cu (75, 100 and 150 ppm), have the character of functional food because, besides their basic nutritional impact, they also have beneficial effects which influence positively one or several target functions of the organism. The use of the supplemental 150 ppm Cu in the diet for laying hens resulted in maximal allowed heavy metals outputs in laying droppings.

References

1. ABRAMOVIC H., &ABRAM V., Physico-chemical properties, composition and oxidative stability of Camelina sativa oil; Food technol. biotechnol, 43(1), 63-70, (2005).
2. AJUYAH, A. O., K. H. LEE, R. T. HARDIN, and J. S. SIM, Changes in the yield and in the fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full-fat oil seeds. Poult. Sci. 70:2304–2314, (1991).
3. Al ANKARI A AL, NAJIB H, HOZAB A AL,Yolk and serum cholesterol and production traits, as affected by incorporating a supra optimal amount of copper in the diet of the leghorn hen. British Poultry Science. Volume 39, no. 3, pp. 393-397, (1998).
4. AZIZA, A. E., QUEZADA, N., &CHERIAN, G., Antioxidative effect of dietary Camelina meal in fresh, stored, or cooked broiler chicken meat. Poultry Science, 89(12), 2711-2718, (2010).
5. CASTON, L., and S. LEESON, Dietary flax and egg composition. Poultry Sci. 6 9:1617-1620, (1990).
6. CHERIAN G, CAMPBELL A, PARKER T., Egg quality and lipid composition of eggs from hens fed Camelina sativa. J Appl. Poult. Res., 18: 143-150, (2009).
7. CHERIAN, G., Camelina sativa in poultry diets: opportunities and challenges; BIOFUEL CO-PRODUCTS AS LIVESTOCK FEED, Editor Harinder P.S. Makkar 303, (2012).
8. CHIOU, P. W. S., K. L. CHEN, B. YU, Toxicity, tissue accumulation and residue in egg and excreta of copper in laying hens. Anim. Feed Sci. Technol. 67:49–60, (1997).
9. CIURESCU G., ROPOTA M, TONCEA I., HABEANU M., Camelia (*Camelina sativa* L. Crantz variety) oil and seeds as n-3 fatty acids rich products in broiler diets and its effects on performance, meat fatty acid composition, immune tissue weights, and plasma metabolic profile, Journal of Agricultural Science and Technology (JAST), 18 (2): 315-326, ISSN: 1680-7073, (2016).
10. CRISTE R.D., PANAITE T.D., CIURESCU C, ROPOTA M, RACHIERU D., Effects of moderate (5%) levels of linseed in layer diets, Archiva Zootehnica 12:3, 11-21, 2009, pg 11-21, (2009).
11. EFSA – Opinion of the Scientific Committee for Animal Nutrition the use of Cu in feeding stuffs. 9. Feb. (2003).
12. ESECELI H., DEGIRMENCIOGLU N., BILGIC M., The effect of ınclusion of chromium yeast (Co-fator II, Altech Inc.) and folic acid to the rations of laying hens on performance, egg quality, egg yolk cholesterol, folic acid and chromium levels, J . Vet. Adv., 9, 384-391, (2010).
13. FERRIER L.K., CASTON L.J., LEESON S., SQUIRES J., WEAVER B.J., HOLUB B.J, – alpha – Linolenic acid-and docosahexaenoic acid enriched eggs from hens fed flaxseed, influence ou blood lipids and platelet phospholipids fatty acids in humans – Am.J.Clin.Nutr. Jul; 62 (1):81-6, (1995).
14. GRIFFIN H.D., Manipulation of egg yolk cholesterol: a physiologist's view. World's Poultry Science Journal, 48, pp 101-112, (1992).

15. HARGIS, P. S., and M. E. VAN ELSWYK, Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. World's Poult. Sci. J. 49:251–264, (1993).
16. HUYGHEBAERT G., Incorporation of polyunsaturated fatty acids in egg yolk fat at varying dietary fat livels and compositions. – Archiv. FűrGellűgelkde, 59, 145-152, (1995).
17. KAPSAK W.R., RAHAVI, E.B., CHILDS, N.M. and WHITE, C., Functional Foods: Consumer attitudes, Perceptions and behaviors in a growing market; Journal of the American Dietetic Association, 804-810, (2011).
18. LIM H. S, PAIK I. K., Effects of Dietary Supplementation of Copper Chelates in the Form of Methionine, Chitosan and Yeast in Laying Hens, Asian-Aust. J. Anim. Sci. Vol. 19, No. 8: 1174 – 1178, August (2006).
19. KIM, S., P. Y. CHAO, and G. D. A. ALLEN, Inhibition of elevated hepatic glutathione abolished copper deficiency cholesterolemia. FASEB J. 6:2467–2471, (1992).
20. KONJUFCA, V.H., PESTI G.M., BAKALLI R.I., Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poult. Sci. 76:1264–1271, (1997).
21. LAUDADIO V., CECI E., LASTELLA N.M.B., TUFARELLI V., Dietary high-polyphenols extra-virgin olive oil is effective in reducing cholesterol content in eggs. Lipids in Health and Disease. https://lipidworld.biomedcentral.com/articles/, (2015).
22. LIEN, T. F., K. L. CHEN, C. P. WU, and J. J. LU, Effects of supplemental copper and chromium on the serum and egg traits of laying hens. Br. Poult. Sci. 45:535–539, (2004).
23. LIM H. S, PAIK I. K., Effects of Dietary Supplementation of Copper Chelates in the Form of Methionine, Chitosan and Yeast in Laying Hens, Asian-Aust. J. Anim. Sci. Vol. 19, No. 8: 1174 – 1178, August (2006).
24. MEYERB. J., MANN N.J., LEWIS J.L., MILLIGAN G.C., SINCLAIR A.J., HOWE P.R.C., Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids, Volume 38, no.4, pp 391-398, (2003).
25. PEARCE, J., N. JACKSON, and M. H. STEVENSON, The effects of dietary intake and of dietary concentration of sulphate on the laying domestic fowl: Effects of some aspects of lipid, carbohydrate and amino acid metabolism. Br. Poult. Sci. 24:337–348, (1983).
26. PEIRETTI, P. G., and G. MEINERI, Fatty acids, chemical composition and organic matter digestibility of seeds and vegetative parts of false flax (Camelina sativa L.) after different lengths of growth. Anim. Feed Sci. Technol. 133:341–350, (2007).
27. PEKELA. Y., ALEP M., Effects of different dietary Cu sources on laying hen performance and egg yolk cholesterol. J. Appl. Pult. Res. 20:506-513, (2011).
28. PESTI, G. M., and R. I. BAKALLI, Studies on the Feeding of Cupric Sulfate Pentahydrate and Cupric Citrate to Broiler Chickens, Poultry Science 75:1086-1091, (1996).
29. PESTI, G. M., and R. I. BAKALLI, Studies on the Effect of Feeding Cupric Sulfate Pentahydrate to Laying Hens on Egg Cholesterol Content, Poultry Science 77:1540-1545, (1998).
30. Regulation (CE) 152/2009 (Methods of sampling and analysis for the official control of feed)/26.02.2009
31. SIM J.S., SUNWOO H.H., The amazing egg: nature's perfect functional food for health promotion. Ed. by: Edmonton: Dept. of Agricultural, Food and Nutritional Science, University of Alberta, (2006).
32. SIMOPOULOS A.P., Human requirement for n-polyunsaturaled fatty acids, Poult. Sci., 79:961-970, (2000).
33. SIRÓ I, KÁPOLNA E, KÁPOLNA B, LUGASI A., Functional food. Product development, marketing and consumer acceptance-a review. Appetite, 51(3):456-67, (2008).
34. STOCK R, COMPTON J., Poultry egg with beneficial health and nutritive values, USA, nr. 6316041, (2001).
35. TRIPATHI, M. K., and A. S. MISHRA, Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132:1–27, (2007).
36. UYANIK, F., KAYA, S., KOLSUZ, A.H., EREN, M., SAHIN, N., The effect of chromium supplementation on egg production, egg quality and some serum parameters in laying hens, Turkish J. of Animal Sciences, 26, 379-387, (2002).
37. YALÇYN, H., KEMAL ÜNAL, M., &BASMACYOOLU, H., The fatty acid and cholesterol composition of enriched egg yolk lipids obtained by modifying hens' diets with fish oil and flaxseed. Grasas y Aceites, 58(4), 372-378, (2007).
38. ZUBR J., MATTHAUS B., Effects of growth conditions on fatty acids and tocopherols in Camelina sativa oil. Ind Crops. 15:155-162, (2002).
39. ZUBR J., Oil-seed crop: Camelina sativa. Ind. Crops Prod. 17:161–169, (1997).