Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2022 / v.24 / n.2 / 001-006

http://dx.doi.org/10.1590/1806-9061-2020-1412

Original Article

Cholesterol Content and Fatty Acids Profile in Conventional and Omega-3 Enriched Eggs

■**Author(s)**

Kralik G[I,II] https://orcid.org/0000-0003-1603-3440
Kralik Z[I,III] https://orcid.org/0000-0001-9056-9564
Galović O[I,IV] https://orcid.org/0000-0001-8038-5745
Hanžek D[I,III] https://orcid.org/0000-0002-6577-5873

[I] Josip Juraj Strossmayer University of Osijek, Scientific Center of Excellence for Personalized Health Care, Osijek, Croatia.
[II] Nutricin j.d.o.o. Darda, Darda, Croatia.
[III] Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, Osijek, Croatia.
[IV] Josip Juraj Strossmayer University of Osijek, Department of Chemistry, Osijek, Croatia.

■**Mail Address**

Corresponding author e-mail address
Zlata Kralik
Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia.
Phone: (00385)31554867
Email: zlata.kralik@fazos.hr

■**Keywords**

Egg, fatty acids, n-3 PUFA, cholesterol.

Submitted: 14/August/2021
Approved: 19/November/2021

ABSTRACT

This study aimed to produce table eggs enriched with n-3 polyunsaturated fatty acids by diet modification and at the same time, determine the fatty acid profile and cholesterol content in egg yolk, and compare them with that of conventionally produced eggs. Eighty laying hens of Tetra SL hybrids were allocated into two experimental groups. The diets were balanced at the level of 11.60 MJ/kg ME and 16.63% crude protein. The experiment lasted 5 weeks.

In the n-3 PUFA eggs, yolk cholesterol levels were 1179.25 mg/100 g compared to the 1287.46 mg/100 g measured in the control eggs. The difference of 108.21 mg/100 g i.e. 8.4% was not statistically significant (p>0.05). The fatty acid profile in n-3 PUFA versus control eggs was as follows: SFA 30.55 vs 35.89% (p<0.001), MUFA 42.61 vs 42.28%, n-6 PUFA 22.10 vs 21.40% (p>0.05), and n-3 PUFA 4.74 vs 0.82% (p<0.001). Furthermore, ALA content was 2.28 vs 0.26%, EPA 0.31 vs 0.14%, and DHA 2.20 vs 0.43% (p<0.001) in n-3 PUFA vs control eggs, respectively. The n-6/n-3 PUFA ratio in the enriched eggs was 5.11 and in the conventional ones 23.73 (p<0.001). Based on the results of our study, it can be concluded that in n-3 PUFA-enriched eggs, the n-6/n-3 PUFA ratio is significantly improved compared to the conventional eggs. Thus, n-3 PUFA eggs are nutritionally and functionally more favorable than conventional eggs for human nutrition.

INTRODUCTION

Eggs are a source of protein, fat and micro-nutrients having a significant role in the basic human diet. Ceylan *et al.* (2011) reported that eggs contain 195-230 mg of cholesterol in average, a compound that is linked to an atherosclerosis development. That is why some people do not want to consume eggs. However, eggs are a valuable source of essential amino acids and other biological ingredients that, apart from nutrition, also have a biological function or effect physiological processes (Mazalli *et al*., 2004; Laca *et al.*, 2010; Liu *et al.*, 2010; Kralik *et al.*, 2020). Eggs from conventional farming are poor in α-linolenic acid (ALA, 18:3n3), and contain neither eicosapentaenoic (EPA, 20:5n3) nor docosahexaenoic (DHA, C20: 6n3) fatty acids (Souza *et al.,* 2008). In the last decade, beneficial changes have been achieved in egg yolks fatty acid profile by adding fish and flaxseed oil or seeds to laying hens (Cachaldora *et al.*, 2006). In detail, n-6/n-3 PUFA ratio in designed eggs (canola and fish oil) is reduced to the desired range of 4 to 10, in comparison with 35 in regular eggs (sunflower oil) (Mazalli *et al*., 2004). Cholesterol in eggs is often noted as a debatable ingredient. There are several researches that have been successful in efforts to reduce egg yolks cholesterol by using modified diets in the laying hens feeding (Sari *et al*., 2002; Sujatha & Narahari, 2011; Promila *et al.*, 2017). There are data in the literature that state that the use of linseed oil and seeds, as well as fish oil in diets, increases the levels of n-3 polyunsaturated fatty acids in egg yolks and reduces cholesterol content at the same time (Basmacioglu *et al.*, 2003; Loh *et al.*, 2009; Mattioli *et al.,* 2016). The aforementioned authors assume that the egg yolks cholesterol content is related to the n-3 polyunsaturated fatty acids (n-3 PUFA) concentration. Namely, the aim of this research was to design a laying hens diet supplemented with fish, rapeseed and flaxseed oil, leading to the production of eggs enriched with omega 3 fatty acids. Also, the aim was to determine the effect of this diet on fatty acid profiles and cholesterol content of the enriched eggs.

MATERIAL AND METHODS

Animals and diets

All procedures involving work with animals used in the research were carried out according to protocols approved by the Bioethics Committee for Animal Research, Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strosmayer University of Osijek. The study was conducted on a total of 80 laying hens of Tetra SL hybrids being 31 weeks old at the beginning of the study. The hens were randomly allocated into 2 experimental groups (conventional-control (C) and n-3 PUFA - experimental (E)), and each group was further divided into 5 replicants of 8 hens in enriched cages. The laying hens were kept in the same microclimatic conditions while feed and water were provided ad libitum. The diets for laying hens differed in the oil content. The C group of laying hens consumed conventional diet with 5% soybean oil whereas the E group of hens fed diet with 1.5% fish oil, 1.5% rapeseed oil and 2% linseed oil. Table 1 shows the diets composition, and Table 2 the fatty acids profile in diets, which were analyzed in triplicate.

Table 1 – Composition and chemical analysis of basic diet.

Ingredients	g/kg	Chemical analysis[3]	%
Corn	494.7	Moisture	9.30
Soybean cake	223.3	Crude protein	16.63
Toasted soybean	30.0	Crude fat	7.30
Sunflower cake	50.0	Crude fiber	4.00
Alfalfa	16.7	Crude ash	16.54
Calcium granules	103.3		
Monocalcium phosphate	13.3		
Yeast	5.0		
Salt	3.3		
Poultry acidifer	3.3		
Nanofeed	3.3		
Methionine	1.5		
Premix[1]	12.0		
Oils[2]	50.0		
Total	1000.0	ME (MJ/kg)	11.60

[1]Premix (1 kg) contained: vitamin A 834000 IU, vitamin D_3 208500 IU, vitamin E_3 2085 mg, vitamin K_3 167 mg, vitamin B_1 150 mg, vitamin B_2 374 mg, vitamin B_6 200 mg, vitamin B_{12} 918 µg, vitamin C 1860 mg, niacin 2085 mg, pantothenic acid 584 mg, folic acid 75 mg, biotin 7 mg, choline chloride 33600 mg, iron 2520 mg, iodine 76 mg, copper 425 mg, manganese 5640 mg, zinc 5175 mg, canthaxanthin 300 mg, selenium 30 mg

[2]C group = 5% soybean oil; E group = 1.5% fish oil+1.5% rapeseed oil + 2% linseed oil

[3]Reference methods applied for chemical analysis of feed: moisture HRN ISO 6496; ash HRN EN ISO 5984; crude protein HRN ISO 5983-2; fat HRN ISO 6492; crude fiber HRN EN ISO 6865, modified (Croatia standards, 2001; 2004; 2010)

Table 2 – Fatty acids composition in diets (% FA in total fatty acids, x ± s).

Fatty acid	C	E	*p* value
Myristic (C14:0)	0.17 ± 0.03	1.68 ± 0.03	*
Pentadecanoic (C15:0)	0.31 ± 0.01	0.29 ± 0.09	n.s.
Palmitic (C16:0)	14.26 ± 0.45	14.13 ± 0.44	n.s.
Heptadecanoic (C17:0)	0.13 ± 0.06	0.31 ± 0.05	*
Stearic (C18:0)	5.36 ± 0.16	4.38 ± 0.06	n.s.
Arachidic (C20:0)	0.45 ± 0.01	0.47 ± 0.01	n.s.
Heneicosanoic (C21:0)	-	0.23 ± 0.01	*
Behenic (C22:0)	0.49 ± 0.02	0.39 ± 0.02	n.s.
ΣSFA	21.17 ± 0.61	21.88 ± 0.59	n.s.
Palmitoleic (C16:1)	-	1.74 ± 0.02	*
Heptadecenoic (C17:1)	-	0.14 ± 0.04	*
Oleic (C18:1cis9) + elaidic (C18:1trans9)	30.54 ± 0.42	35.48 ± 0.35	*
Eicosenoic (C20:1n9)	0.17 ± 0.01	0.98 ± 0.01	*
Erucic (C22:1)	1.13 ± 0.01	1.01 ± 0.01	n.s.
ΣMUFA	31.84 ± 0.40	39.35 ± 0.39	*
Linoleic (C18:2 n6)	42.88 ± 0.01	32.46 ± 0.16	*
Σn-6 PUFA	42.88 ± 0.15	32.46 ± 0.16	*
α-linolenic (C18:3 n3)	4.12 ± 0.39	7.87 ± 0.40	*
Eicosapentaenoic (C20:5 n3)	-	1.06 ± 0.01	*
Docosahexaenoic (C22:6 n3)	-	1.39 ± 0.01	*
Σn-3 PUFA	4.12 ± 0.51	10.32 ± 0.45	*
n-6 PUFA/n-3 PUFA ratio	10.41 ± 0.21	3.14 ± 0.20	*

C = 5% soybean oil; E = 1.5% fish oil+1.5% rapeseed oil + 2 % linseed oil; SFA = saturated fatty acids; MUFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids; n.s.= non significant; *$p<0.05$.

The eggs were sampled for the fatty acids and cholesterol analysis after 5 weeks of feeding the laying hens. For the purposes of these analyses, 40 eggs were collected, i.e. 20 eggs per group of laying hens (10 eggs for fatty acid analysis, 10 eggs for cholesterol analysis).

Fatty Acids Analyses

The fatty acid profile in egg yolk and diets was analyzed as follows: the fat of the homogenized samples was extracted using the method of Folch *et al*. (1957) All solvents used were of ultrapure grade from Sigma-Aldrich (Schnelldorf, Germany). Butylated hydroxytoluene (100 mg/L) was added to the extraction mixture (chloroform: methanol, 2:1 v/v) as an antioxidant. Subsequently, fatty acid-containing lipids were transmethylated by the base-catalyzed sodium methoxide. Gas chromatography was conducted on a Bruker 430-GC apparatus (Billerica, MA, USA), equipped with a FAMEWAX (RESTEK, Bellefonte, PA, USA) type capillary column (30 m x 0.32 mm internal diameter, 0.25 µm film) and flame ionization detector. The characteristic operating conditions were as follows: temperature injector, 220 °C; temperature detector, 230 °C; helium flow, 25 ml/min. The oven temperature was graded from 50 to 225 °C at 6 °C/min and held for 21 min at 225 °C. A fatty acid standard mixture (Supelco 37 Component FAME Mix, SUPELCO® Analytical, Bellefonte, PA, USA) was used to identify the individual fatty acids in the chromatogram. Portions of individual and total fatty acids were shown as the percentage of total fatty acids in the diets, and in eggs in mg/100 g edible part.

Cholesterol Analysis

Cholesterol content was determined by the modified method of Albuquerque *et al*. (2016) as follows: 5 mL of 0.4 M KOH in ethanol were added to 0.5 g of egg yolk and the solution was shaked well in a vortex. The samples were incubated in a 50 °C water bath for 30 minutes. After cooling at room temperature, the cholesterol extraction was done twice with 10 mL of n-hexane. The extracts were combined and an aliquot of 3 mL was dried and replenished with 3 mL of mobile phase. Shimazdu HPLC system equipped with UV-VIS detector SPD-10AV VP and SIL-10AD VP auto-injector, Shimadzu Shim-pack GIST (250 x 4.6 mm I.D., 5 µm particle size) column were used for separation and quantification of cholesterol. The mobile phase was the solution of isopropanol: acetonitrile (50:50 v/v). Prior using, the mobile phase was filtered through a 0.20 µm membrane filter and degassed in an ultrasonic bath. The column temperature was 37 °C, the flow rate 1.2 mL/min and the injected volume 10 µL. Cholesterol was detected using a UV-VIS detector set at a wavelength of 210 nm. Total analysis time was 10 minutes.

Statistical Analyses

The research results were processed using TIBC Statistica™ version 13.4.0.14. (Soft Inc., © 1984-2018). Descriptive statistics and analysis of variance were implemented (ANOVA). If the P value for the analysis of variance was statistically significant, differences between the groups were tested by the Fisher LSD test.

RESULTS

The fatty acid profile of C and E diets is shown in Table 2. The main differences were found in the proportion of MUFA, n-6 PUFA and n-3 PUFA ($p<0.05$). The n-6/n-3 PUFA ratio was 3.14 to 10.41 i.e. three times lower ($p<0.05$). Table 3 shows the effect of feeding treatment on the fatty acid profile in the yolks of both C and E eggs. Designing the hens diet by using a mixture of oils (fish, flaxseed and rapeseed oil) increased the content of ALA, EPA and DHA in hen diet which resulted in a beneficial fatty acid profile in the yolks of E group eggs compared to conventionally produced eggs. Conventional eggs contained more palmitic acid (C16:0) and total saturated fatty acids (ΣSFA, $p<0.001$). Enriched eggs with n-3 PUFAs contained more heptadecenoic acid (C17:1, $p<0.05$) and less palmitoleic (C 16:1, $p<0.001$). Differences in MUFA between the egg groups were not statistically significant ($p>0.05$). The arachidonic content (C20:4n6) was higher in the C than in the E eggs ($p<0.01$) whereas the difference between the groups in Σn-6 PUFA was not statistically significant either ($p>0.05$).

Table 3 – Effect of feeding treatment on fatty acid profile in yolks of both C and E eggs (% FA in total fatty acids, x ± s).

Fatty acid	C	E	P value
Myristic (C14:0)	0.31±0.02	0.28±0.03	n.s.
Pentadecanoic (C15:0)	0.07±0.01	0.09±0.01	n.s.
Palmitic (C16:0)	26.96±0.42	21.93±0.31	***
Heptadecanoic (C17:0)	0.18±0.01	0.24±0.02	n.s.
Stearic (C18:0)	8.17±0.18	7.91±0.15	n.s.
Arachidic (C20:0)	0.02±0.01	0.02±0.01	n.s.
Tricosanoic (C23:0)	0.09±0.03	0.08±0.01	n.s.
ΣSFA	35.80±0.78	30.55±0.46	***
Myristoleic (C14:1)	0.09±0.03	0.04±0.01	n.s.
Palmitoleic (C16:1)	2.57±0.01	1.89±0.06	***
Heptadecenoic (C17:1)	0.05±0.01	0.24±0.01	*
Oleic (C18:1n 9c)	39.38±1.06	40.36±0.58	n.s.
Eicosenoic (C20:1n9)	0.19±0.02	0.20±0.01	n.s.
ΣMUFA	42.28±1.10	42.61±0.57	n.s.
Linoleic (C18:2 n-6)	19.45±1.00	20.51±0.89	n.s.
γ-linolenic (C18:2n6)	0.11±0.01	0.14±0.01	n.s.
Eicosadienoic (C20:2n-6)	0.15±0.01	0.15±0.01	n.s.
Arachidonic (C20:4n6)	1.70±1.07	1.30±0.06	**
Σn-6 PUFA	21.41±0.92	22.10±0.92	n.s.
α-linolenic (C18:3 n-3)	0.26±0.01	2.28±0.26	***
Eicosapentaenoic (C20:5 n-3)	0.14±0.01	0.31±0.02	***
Docosahexaenoic (C22:6 n-3)	0.43±0.02	2.20±0.14	***
Σn-3 PUFA	0.82±0.02	4.74±0.43	***
n-6 PUFA/n-3 PUFA ratio	23.73±1.40	5.11±0.59	***

C = 5% soybean oil; E = 1.5% fish oil+1.5% rapeseed oil + 2 % linseed oil; n.s.= non significant; *p<0.05; **p<0.01; ***p<0.001; SFA = saturated fatty acids; MUFA = unsaturated fatty acids; PUFA = polyunsaturated fatty acids

A significant difference was found between E and C group of eggs in the content of ALA, EPA and DHA ($p<0.001$). The content of Σn-3 PUFA in E group was 5.7 times higher than in the C group ($p<0.01$). The n-6/n-3 PUFA ratio was significantly more favorable in the E (5.11) compared to C (23.73).

Figure 1 shows the content of cholesterol in the E and C group of eggs (mg/100 g of egg yolk). Eggs supplemented with n-3 PUFA contained on average 1179 mg and control eggs 1287 mg cholesterol per 100 g of yolk. According to our results the eggs enriched with n-3 PUFAs contained less cholesterol than the control eggs by 108.21 mg/100 g of egg yolk i.e. 8.4%, although this difference was not significant ($p>0.05$).

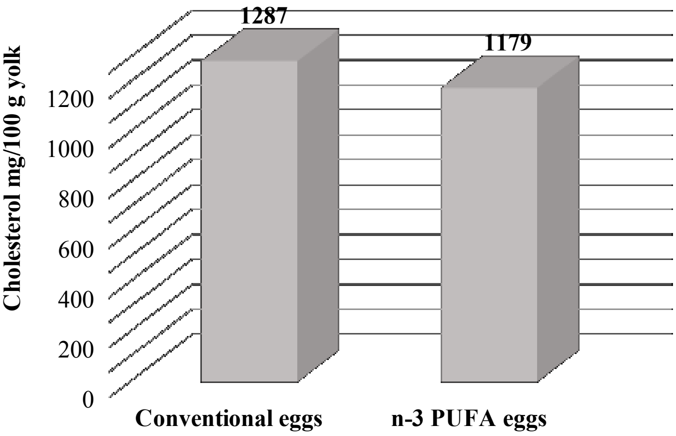

Figure 1 – Cholesterol content in the control and n-3 PUFA groups. Conventional eggs = 5% soybean oil; n-3 PUFA eggs = 1.5% fish oil+1.5% rapeseed oil + 2 % linseed oil

DISCUSSION

Omega-3 fatty acids are desirable in the functional products food sector. This has prompted egg producers to increase the content of omega-3 fatty acids in table eggs, a very important component of the food chain, by using innovative technologies. The content of some fatty acids in diets, especially of the long chain was affected by the type and amount of added oils. Although the diet for conventional egg production did not contain EPA and DHA (Table 2), these fatty acids were found in the eggs lipids of this group (Table 3). The reason for this is that hens can synthesize EPA and DHA in limited amounts if there is enough ALA in the feed. This has been affirmed by our former researches (Kralik *et al.*, 2008; Kralik *et al.*, 2017). By adding the fish oil in the diet, containing EPA and DHA as well as rapeseed and linseed oils, being the important ALA sources, are reabsorbed from the feed by the laying hens. On the other hand they formed long chain n-3 PUFAs via their metabolism through the elongation and desaturation processes depositing them in the egg yolks, a fact that is in line with Yang *et al.* (2000) findings. Increasing of blood LD cholesterol content by consuming food rich in saturated fatty acids is the risk factor for cardiovascular diseases occurance in humans. During the fatty acid profile analysis in the egg yolks of our investigated groups it was noticed that palmitic, stearic and oleic saturated fatty acids were mostly present. Palmitic fatty acid is generally believed to raise blood cholesterol more than stearic fatty acid (Fattore *et al.* 2014). Thus, the combination of oils used in laying hens diets of the omega-3 PUFA group in our study is justified since the eggs yolk of this group significantly ($p<0.001$) reduces the palmitic fatty acid proportion compared to conventionally produced eggs. In our study, the arachidonic fatty acid content in the E group was significantly reduced ($p<0.05$) compared to eggs from C group (E = 1.30 % i.e. C = 1.70 %). This was also stated by Baucells et al. (2000) and Cachaldora et al. (2006). The phenomenon is attributed to the greater use of Δ-6 saturase in the synthesis of n-3 PUFA compared to n-6 PUFA. This enzyme acts in both directions (Ayerza and Coates, 2000) and there is competition between n-3 PUFAs and n-6 PUFAs in their biosynthesis (Mazalli et al. 2004). High levels of ALA limit the synthesis of arachidonic fatty acid from linoleic since ALA competes with linoleic for the same enzyme Δ-6 desaturase as reported by Ceylan et al. (2011). These authors consider that the fish oil addition into the laying hens diets reduces the synthesis of arachidonic from linoleic fatty acid. They also state that EPA and DHA can reduce the arachidonic fatty acid production. The observations of these authors are also noticed in our study, because in eggs from E group, where laying hens consumed a diet with the addition of fish, flaxseed and rapeseed oil combination, the content of arachidonic fatty acid decreased with increasing ALA. The results of the fatty acid profiles analysis (Table 3) showed a statistically significant lower ∑SFA content in yolk lipids of eggs from E group compared to the C group (30.55 : 35.80 %, $p<0.001$). However, differences in the content of ∑MUFA and ∑n-6 PUFAs between the egg groups were not statistically significant ($p>0.05$).

The trend of cholesterol reduction in eggs enriched with n-3 PUFA was also shown by the results of Loh *et al.* (2009) and Promila *et al.* (2017). The results of our study also show lower cholesterol content in n-3 PUFA eggs compared to the eggs from the conventional production, although differences were not significant ($p>0.05$).

Sari *et al.* (2002) found that the addition of flaxseed to laying hens increased the n-3 PUFA content linearly ($p<0.001$), the n-6/n-3 PUFA ratio was decreased, as did the cholesterol level ($p<0.05$). Similar results were obtained by Promila *et al.* (2017) using flaxseed oil in diets for laying hens. They found a reduction in cholesterol of 13.57-12.05 mg/g yolk ($p<0.05$), an increase in n-3 PUFA and a decrease in n-6/n-3 PUFA ratio from 8.46 to 1.79. Since the designed diet in our study had a combination of three oils, one of which was flaxseed, we can assume that contributed to ALA increase and thus reduced the n-6/n-3 PUFA ratio in n-3 PUFA eggs.

Ansari et al. (2010) found that linseed oil enriched with copper resulted in the cholesterol reduction ($p<0.05$). At the same time, the SFA content was reduced and n-3 PUFA content increased, especially ALA. Basmacioglu *et al.* (2003) used fish oil in combination with linseed in laying hens feeding. They found lower levels of yolk cholesterol in enriched eggs compared to the control ones (12.56 and 12.97: 13.71 mg/g). Our results are consistent with the results of the aforementioned authors for the content of SFA and cholesterol in the yolks of eggs from E group whereas the content of total n-3 PUFA in these eggs increased.

Poultry are capable of producing 10 times more cholesterol per kg of liver than humans. Due to the intensive production of cholesterol, treatments related to the reduction of cholesterol in eggs through feeding are not always effective (Faitarone *et al.*, 2013). According to Shafey & Cham (1994) cholesterol in eggs is a change resistant, and essential for embryo development. Milinks *et al*. (2003) and Mattioli *et al.* (2016) stated that the cholesterol modulation in the yolk depends on feeding, i.e. concentration of the structural ingredients in eggs. However, laying hens are capable of changing the profile of polyunsaturated fatty acids relative to the source of fat in diet. Unlike mammals poultry can absorb fat and transport it through the portal bloodstream to the liver where lipogenesis takes place (Van Elswyk *et al.*, 1994). Unlike the abovementioned authors, the addition of vegetable oils to laying hens diets cannot reduce eggs cholesterol content (Faitarone *et al.*, 2013). Hargis (1988) pointed out that the cholesterol content of eggs can be altered up to 25% by the diet. Addition of copper at 150-250 mg/kg in the diets of laying hens resulted in the insignificant cholesterol content decrease in eggs (Balevi & Coskun, 2004). Eliat-Adar *et al.* (2013) considered that egg cholesterol has a very limited effect on blood cholesterol content. Keum *et al.* (2018) did not find that a fat source (lard, flaxseed oil or conjugated linolic acid - CLA) affected the egg yolks cholesterol content. However, CLA lowers cholesterol in eggs if added in laying hens diet (Yin *et al.*, 2008). From the aforementioned studies, it can be concluded that the researchers' opinions in terms of the effect of feeding the designed diets on the fatty acid profile are consistent (omega-3 fatty acids increase and the n-6/n3 PUFA ratio decreases). However, they differ in cholesterol content. Today, the lifestyle of people often involves the so-called fast food consumption being adverse from a nutritional point of view as it is rich in saturated fatty acids and has an unfavorable n-6/n-3 PUFA ratio. All the above mentioned affects the occurrence of various diseases of the cardiovascular system (Simopoulos, 2002). Simopoulos (2009) pointed out that the n-6/n-3 PUFA ratio is significant for the cardiovascular diseases prevention recommending 4:1 ratio. An acceptable 3:1 n-6/n-3 PUFA ratio by up to 5:1 was stated by Griffin (2008). The American Heart Association (AHA) recommended that n-6 PUFAs participate with 5-10% of total meal energy consumed (Harris *et al.*, 2009). Some authors recommend reducing n-6 PUFA consumption while others recommend increasing n-3 PUFA consumption, especially EPA and DHA (Candela *et al.,* 2011).

In our research, eggs from E group contained 4.73% and eggs from C group 0.82% of the n-3 PUFA in total fatty acids ($p<0.001$). The n-6/n-3 PUFA ratio in E eggs was 5.11 and C eggs 23.73 i.e. 4.6 times higher ($p<0.001$). At the same time the E eggs contained cholesterol of 1179 mg/100 g of yolk and C eggs of 1287 mg/100 g of yolk ($p>0.05$), indicating a numerical decrease by 8.4%. The analysis of all results justifies the use of this diet aiming to produce eggs enriched with n-3 PUFA that have a more favourable fatty acid profile.

ACKNOWLEDGEMENTS

This study is supported by the European Structural and Investment Funds grant for the Croatian National Scientific Center of Excellence for Personalized Health Care (grant #KK.01.1.1.01.0010) and by the Ministry of Science and Education of the Republic of Croatia.

REFERENCES

Albuquerque TG, Oliveira MBP, Sanches-Silva A, Costa HS. Cholesterol determination in foods: comparison between high performance and ultra-high performance liquid chromatography. Food Chemistry 2016;15(193):18-25.

Ansari R, Azarbayejani A, Ansari S, Asgari S, Gheisari A. Production of egg enriched with omega-3 fatty acids in laying hens. ARYA Atherosclerosis 2010;1(4):242-246.

Ayerza R, Coates W. Dietary levels of chia: influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. Poultry Science 2000;79(5):724-739.

Balevi T, Coskun B. Effects of dietary copper on production and egg cholesterol content in laying hens. British Poultry Science 2004;45(4):530-534.

Basmacioglu H, Cabuk M, Unal K, Ozkan K, Akkan S, Yalcin H. Effects of dietary fish oil and flax seed on cholesterol and fatty acid composition of egg yolk and blood parameters of laying hens. South African Journal of Animal Science 2003;33(4):266-273.

Baucells MD, Crespo N, Barroeta AC, Ferrer SL, Grashorn MA. Incorporationa of different poly unsaturated fatty acids into eggs. Polutry Science 2000;79(1):51-59.

Cachaldora P, García-Rebollar P, Alvarez C, Blas JD, Méndez J. Effect of type and level of fish oil supplementation on yolk fat composition and n-3 fatty acids retention efficiency in laying hens. British Poultry Science 2006;47(1):43-49.

Candela CG, López LB, Kohen VL. Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Nutricion Hospitalaria 2011;26(2):323-329.

Ceylan N, Ciftçi I, Mizrak C, Kahraman Z, Efil H. Influence of different dietary oil sources on performance and fatty acid profile of egg yolk in laying hens. Journal of Animal and Feed Science 2011;20(1):71-83.

Eilat-Adar S, Sinai T, Yosefy C, Henkin Y. Nutritional recommendations for cardiovascular disease prevention. Nutrients 2013;5(9):3646-3683.

Faitarone ABG, Garcia EA, Roça RDO, Ricardo HDA, De Andrade EN, Pelícia K, et al. Cholesterol levels and nutritional composition of commercial layers eggs fed diets with different vegetable oils. Brazilian Journal of Poultry Science 2013;15(1):31-37.

Fattore E, Bosetti C, Brighenti F, Agostoni C, Fattore G. Palm oil and blood lipid–related markers of cardiovascular disease:a systematic review and meta-analysis of dietary intervention trials. The American Journal of Clinical Nutrition 2014;99(6):1331–1350.

Folch J, Lees M, Stanley GS. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 1957;226(1):497-509.

Griffin BA. How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. Current Opinion in Lipidology 2008;19(1):57-62.

Hargis PS. Modifying egg yolk cholesterol in the domestic fowl:a review. Worlds Poultry Science Journal 1988;44(1):17-29.

Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, et al. Omega-6 fatty acids and risk for cardiovascular disease:a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, physical activity, and metabolism; council on cardiovascular nursing; and council on epidemiology and prevention. Circulation 2009;119(6):902-7.

Keum MC, An BK, Shin KH, Lee KW. Influence of dietary fat sources and conjugated fatty acid on egg quality, yolk cholesterol, and yolk fatty acid composition of laying hens. Revista brasileira de zootecnia 2018;47:e20170303.

Kralik G, Gajčević Z, Škrtić Z. The effect of different oil supplementions on laying performance and fatty acid composition of egg yolk. Italian Journal of Animal Science 2008;7(2):173-183.

Kralik G, Kralik Z, Strakova E, Grčević M, Hanžek D. Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans. Acta Veterinaria Brno 2017;86(3):293:301.

Kralik G, Kralik Z, Grčević M, Hanžek D, Margeta P, Galović O. Enrichment of table eggs with lutein. Poljoprivreda 2020;26:56-63.

Laca A, Paredes B, Díaz M. A method of egg yolk fractionation. Characterization of fractions. Food Hydrocolloids 2010;24(4**)**:434-443.

Liu X, Zhao HL, Thiessen S, House JD, Jones PJH. Effect of plant sterol-enriched diets on plasma and egg yolk cholesterol concentrations and cholesterol metabolism in laying hens. Poultry Science 2010;89(2):270-275.

Loh TC, Law FL, Goh YM, Foo HL, Zulkifli I. Effects of feeding fermented fish on egg cholesterol content in hens. Animal Science Journal 2009;80(1):27-33.

Mattioli S, Dal Bosco A, Martino M, Ruggeri S, Marconi O, Sileoni V, et al. Alfalfa and flax sprouts supplementation enriches the content of bioactive compounds and lowers the cholesterol in hen egg. Journal of Functional Foods 2016;22:454-462.

Mazalli MR, Faria DE, Salvador D, Ito DT. A comparison of the feeding value of different sources of fat for laying hens:2. Lipid, cholesterol, and vitamin E profiles of egg yolk. Journal of Applied Poultry Research 2004;13(2):280-290.

Milinsk MC, Murakami AE, Gomes STM, Matsushita M, De Souza NE. Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids. Food Chemistry 2003;83(2):287-292.

Promila, Kishore N, Verma R, Shunthwal J, Sihag S. Influence of linseed oil supplementation on egg cholesterol content, fatty acid profile, and shell quality. The Pharma Innovation Journal 2017;6(11):174-178.

Sari M, Aksit M, Ozdogan M, Basmacioglu H. Effects of addition of flaxseed to diets of laying hens on some production characteristics, levels of yolk and serum cholesterol, and fatty acid compositions of yolk. Archiv fur Geflugelkunde 2002;66(2):75-79.

Shafey TM, Cham BE. Altering fatty acid and cholesterol contents of eggs for human consumption. In: Sim JS, Kanai S, editors. Eggs uses and processing technologies: new developments. Washington: CAB International; 1994. p.374-385.

Simopoulos A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy = Biomedicine & Pharmacotherapie, 2002;56(8):365–379.

Simopoulos AP. Omega-6/omega-3 essential fatty acids: biological effects. World Review of Nutrition and Dietetetics 2009;99(1):1-16.

Souza JG, Costa FGP, Queiroga RCRE, Silva JHV, Schuler ARP, Goulart CC. Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil. Brazilian Journal of Poultry Science 2008;10(1):37-44.

Sujatha T, Narahari D. Effect of designer diets on egg yolk composition of White Leghorn hens. Journal of Food Science and Technology 2011;48(4):494-497.

Van Elswyk ME, Hargis BM, Williams JD, Hargis PS. Dietary menhaden oil contributes to hepatic lipidosis in laying hens. Poultry Science 1994;73(5):653-662.

Yang SC, Chen KH. The oxidation of cholesterol in the yolk of selective traditional chinese egg products. Poultry Science 2000;80(3):370-375.

Yin JD, Shang XG, Li DF, Wang FL, Guan YF, Wang ZY. Effects of dietary conjugated linoleic acid on the fatty acid profile and cholesterol content of egg yolks from different breeds of layers. Poultry Science 2008;87(2):284-290.