JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.2024010

Antioxidant Status, Lipid Metabolism, Egg Fatty Acids, and Nutritional Index of White-Egg Laying Hens Fed Flaxseed Cake

Youssef A. Attia[1], Ahmed A. Al Sagan[2], El-sayed O. S. Hussein[3], Marai J. Olal[4], Tarek A. Ebeid[5], Rashed A. Alhotan[3], Mohammed M. Qaid[3], Fulvia Bovera[6], Heba A. Shehta[7] and Vincenzo Tufarelli[8]

[1]Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Damanhour 22516, Egypt
[2]King Abdulaziz City for Science and Technology, Riyadh 12354, Saudi Arabia
[3]Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[4]Admin & Animal Nutrition Technical Services, Danisco Animal Nutrition & Health (IFF), Riyadh 11411, Saudi Arabia
[5]Department of Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[6]Department of Veterinary Medicine and Animal Production, University of Napoli Federico II,, Napoli 80137, Italy
[7]Regional Centre for Food and Feed, Agricultural Research Center, Giza 12619, Egypt
[8]Department of Precision and Regenerative Medicine and Jonian Area, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro',, Bari 70010, Italy

Flaxseed cake contains high levels of phenolic compounds, which have numerous biological activities, as well as a considerable amount of omega-3 fatty acids, such as α-linolenic acid, which remains after oil extraction. In this study, we examined the effects of flaxseed cake meal (FSCM) on the antioxidative status, lipid metabolism, egg fatty acid profile, and egg health index of white-egg laying hens. A total of 63 Hisex White laying hens were divided into three experimental treatment groups and fed diets containing 0, 5, or 10% FSCM from 48 to 58 weeks of age. Feeding with 5 and 10% FSCM did not significantly (p>0.05) influence total lipid, triglyceride, total cholesterol, very low-density lipoprotein-cholesterol, or low-density lipoprotein-cholesterol concentrations, or the high-/low-density lipoprotein ratio in the serum and egg yolk; however, 10% FSCM significantly (P<0.05) increased serum high-density lipoprotein. Dietary FSCM also did not affect (P>0.05) antioxidant markers in the eggs and blood plasma. Notably, dietary inclusion of FSCM significantly increased (P<0.05) total n-3 polyunsaturated fatty acids (PUFAs), α-linolenic acid, docosahexaenoic acid, and eicosapentaenoic acid levels in egg yolk, whereas the *n*-6:*n*-3 PUFAs ratio was markedly (P<0.05) decreased in a dose-dependent manner. Moreover, including 5–10% FSCM improved (P<0.05) egg health indices, with 10% being the most beneficial. Together, these findings indicated that the inclusion of up to 10% FSCM in laying hen diets improved egg yolk lipid and fatty acid profiles, as well as egg quality and nutritional and metabolic indices.

Key words: antioxidant status, fatty acids, flaxseed cake, health index, laying hens

J. Poult. Sci., 61: jpsa.2024010, 2024

Introduction

Feed resources for livestock production, such as soybean meal, have been limited owing to increasing demand, recent health crises, and warfare, leading to increased feeding costs and threats to livestock production[1–3]. However, in countries that do not grow soybeans, a trend exists to substitute soybeans with alternative sources of plant protein and agro-industrial co-products for livestock nutrition[1]. Crop co-products are also utilized to reduce feeding costs and environmental pollution. Moreover, co-products can serve as a vital source of bioactive molecules and fatty acids with significant advantages for animal and human health, as well as environmental sustainability[2,3].

In particular, vegetable sources of omega-3 fatty acids are beneficial because they enhance the nutritional value of eggs and meat[4–7]. Flax (*Linum usitatissimum* L.) is a critical crop worldwide as a food and feed source owing to its oil and fiber components, including numerous omega-3 fatty acids[8,9]. Notably,

Received: December 1, 2023, Accepted: March 4, 2024
Available online: April 4, 2024
Correspondence: Prof. Vincenzo Tufarelli, Department of Precision and Regenerative Medicine and Jonian Area, Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Valenzano, Bari 70010, Italy (E-mail: vincenzo.tufarelli@uniba.it)

the global production of flax reached 8.7 million tons in 2016, which was priced at about 70.2 $ (https://www.statista.com). Cold-pressed flaxseed meal is a valuable byproduct for animal and poultry breeding, as it contains substantial levels of residual oil (approximately 10%) and crude protein (approximately 30 to 35%) when extracted below 35°C[9]. However, flaxseeds contain non-starch polysaccharides, such as mucilage, along with other anti-nutritional factors including cyanogenic glycosides, tannins, phytic acid, trypsin inhibitors, and anti-vitamin B_6, which are concentrated in flaxseed meal[8–10]. Accordingly, various methods have been employed to reduce antinutritional factors, enhance nutrient digestibility, and decrease digesta viscosity in the jejunum of broiler chickens fed flaxseed cake[4,11–13].

Flaxseed, flaxseed meal, or flaxseed oil is a principal source of α-linolenic acid (ALA, C18:3, *n*-3) for broiler chickens[4], laying hens[3], and Japanese quails[14,15]. Dietary intake of omega-3-enriched meat and eggs is an appropriate strategy for lowering the incidence of lifestyle-related diseases and enhancing the human health index[16]. The inclusion of flaxseed cake meal (FSCM) in laying hen diets enhances the performance, egg quality, blood and egg yolk lipid profiles, antioxidant status, and immune response of laying hens[3]. Dietary incorporation of 10 to 20% whole flaxseeds in laying hen diets elevated the ALA concentration in egg yolk by 10 to 20-fold, respectively[17]. Similarly, dietary whole flaxseeds (15%) increased yolk ALA content to 7.07 g/100 g of fatty acids, as compared with 0.26 g/100 g of fatty acids in the control diet[18]. Alternatively, the inclusion of 10% flaxseed did not significantly affect feed intake, growth, or feed conversion ratio (FCR) in broilers[19] or laying hens[20]. However, the recommended level of FSCM in poultry feed varies across studies depending on the type of FSCM and strain of chicken evaluated, including 2–15% for broiler chickens[4,13,21] and 6–15% for laying hens[3,20,22–24]. Therefore, the objective of the present study was to investigate the effect of FSCM on the antioxidant status, mineral profile, lipid metabolism, egg fatty acid profile, and egg health index in laying hens.

Materials and Methods

Ethical statement

This study was conducted following the general guidelines for animal experimentation by Royal Decree (number M59, dated 14/9/1431H), and was approved by King Abdulaziz University (KAU) (institutional code ACUC-22-1-2).

Preparation of FSCM

The FSCM (brown variety) was obtained from a commercial supplier after cold oil extraction using an electric screw-press machine at approximately 40°, and ground into fine particles to pass through a 2 mm sieve.

Experimental design

A total of 63 Hisex White (white egg) laying hens (48 weeks of age), with an average body weight of 1712 ± 43.6 g, were used in this study. The feeding trial lasted for 10 weeks from 48 to 58 weeks of age. The initial two weeks, termed the preliminary trial period, served as a period of adaptation and was not included in the study analyses. The experimental design included three treatments, each containing seven replicates (three hens per replicate), housed in cages providing 0.36 m^2 area per hen. Each cage had a 50 cm long tube feeder and a stainless-steel nipple drinker. Laying hens were fed diets containing 0, 5, and 10% FSCM. These levels were selected based on previous studies[17,18,20,25,26]. However, to compensate for the presence of antinutritional factors in the raw, untreated flaxseed cake, we selected lower levels among the published range for use in the present study[10,27]. The dietary FSCM influences on the performance, quality, and sensory attributes of the eggs, serum, and egg trace minerals of laying hens during 48 to 58 weeks of age have been recently published[28]. In addition, the blood properties and egg yolks of laying hens were sampled at 58 weeks of age (at the end of the trial). The composition of the experimental diets is reported in Table 1. The nutritional profile and fatty acid content of the experimental diets were computed based on the analytical values of the feedstuffs[29] and chemical analyses of the FSCM.

Antioxidant content and activity of whole flaxseed phytoconstituents

The antioxidant scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined according to the method described by Aklet al[30]. The tannin content in the flaxseed cake was determined using the Folin–Denis reagent according to the method of Schanderl[31], using gallic acid as a standard. P-coumaric and ferulic acids were quantified using high-performance thin-layer chromatography, as described in Kannanet al[32]. Vitamin C content was determined according to the method described by Schanderl[31] and Kannan et al.[32]. Total antioxidant activity was standardized to that of ascorbic acid and expressed as milligrams of ascorbic acid equivalents per gram of sample on a dry weight basis.

Yolk lipid profile, fatty acid profile, and chemical composition

Seven yolk samples were randomly collected per treatment (one egg per replicate) and used to determine the chemical composition of the yolk. Yolk lipids were extracted to determine the levels of triglycerides and different cholesterols, such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol, as described in Attia et al.[3]. The hypercholesterolemia index (RHCH) was calculated as the ratio of LDL to total cholesterol.

Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in the eggs were measured using seven eggs per treatment. After lipid extraction, lipid methylation was performed using 50 mg of the extracted lipids by adding 5 mL of methanolic sulfuric acid (1 mL concentrated sulfuric acid and 100 mL methanol) and 2 mL of benzene to the tube. Then, the tube was sealed and placed in a water bath at 90°C for 90 min. Subsequently, the tube was cooled, 5 mL of petroleum ether and 8 mL of distilled water were added, the tube was shaken vigorously, and the ethereal layer was separated into a dry tube and dried by evaporation. Fatty acid profiles were determined according to the methods published by Radwan[33] by coupling two-dimensional thin-layer gas chro-

Table 1. **Composition and chemical analysis of the experimental diets fed to laying hens.**

Ingredients	Flaxseed cake (%)		
	0	5	10
Yellow corn	64.50	61.50	58.47
Soybean meal, 48% crude protein	24.00	22.00	20.00
Calcium carbonate	9.00	9.00	9.00
Flax seed cake	–	5.0	10.0
Calcium diphosphate	1.30	1.30	1.30
Sodium chloride	0.30	0.30	0.30
Vitamin-Mineral premix*	0.50	0.50	0.50
DL-methionine	0.15	0.15	0.18
L-Lysine	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10
Choline chloride 50%	0.05	0.05	0.05
Determined analysis			
Dry matter %	93.39	94.24	94.54
Crude protein %	16.69	16.74	17.03
Crude fat %	2.58	3.93	5.16
Crude fiber %	3.21	3.61	4.13
Ash,%	17.43	15.90	15.53
Calculated analysis			
Metabolizable energy, kcal/kg	2731	2722	2700
Calcium %	4.06	4.05	4.0
Phosphorus (available) %	0.351	0.367	0.344
Se, ppm	0.367	0.424	0.465
Zn, ppm	93.5	97.8	102.7
Fe, ppm	33.4	44..8	58.0
Methionine %	0.412	0.424	0.503
Lysine,%	0.885	0.886	0.892
C18-2 PUFAs, %	0.486	0.467	0.447
C18-3 PUFAs, %	0.020	0.045	0.069
C18:2/C18:3	24.1	10.5	6.50

*Three kg of vitamin–mineral premix per ton of feed supplied each kg of diet with vitamin A 12,000 IU; vitamin D3 2000 IU; vitamin E 10 mg; vitamin K3 2 mg; vitamin B1 1 mg; vitamin B2 4 mg; vitamin B6 1.5 mg; pantothenic acid 10 mg; vitamin B12 0.01 mg; folic acid 1 mg; niacin 20 mg; biotin 0.05 mg; choline chloride, 500 mg; Zn 55 mg; Fe 30 mg; I 1 mg; Se 0.1 mg; Mn 55 mg; ethoxyquin 3000 mg.

matography (GC) for the quantitative analysis of lipid classes and their fatty acid constituents using a Hewlett–Packard (HP) 6890 GC with Flame Ionization Detector (FID detector) (Palo Alto, CA, USA) (detector temperature: 250°C; injector temperature: 220°C; and injection volume: 2 µL, split fewer modes). An HP-5 column (5% diphenyl, 95% dimethyl polysiloxane), 30 m, 0.32 mm ID, and 0.25 µm film thickness was used. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The initial oven temperature was 150°C × 2 min.

Blood biochemical constituents and antioxidative status

Seven blood samples were randomly collected from each treatment group to represent all treatment replicates for the hens at 58 weeks of age. Blood serum was separated by centrifugation at 1500 × *g* for 10 min. Serum lipid profiles were then determined, including triglycerides, total serum cholesterol, plasma LDL cholesterol, and serum HDL cholesterol, as described by Attia et al.[3]. Digenetic kits purchased from Diamond Diagnostics (Cairo, Egypt; http://www. diamond diagnostics. com) were used to determine the lipid profiles. Moreover, very-low-density lipoprotein (vLDL)-cholesterol was measured[34] in (mg/dL) according to the following equation: vLDL = triglycerides/5. The HDL:LDL ratio was also calculated.

The risk of hypercholesterolemia was computed using the following equation[35]: Risk of hypercholesterolemia = LDL cholesterol/total cholesterol. The serum total antioxidant capacity (TAC) and malondialdehyde (MDA) were assayed as previously described[22,23]. The antioxidant balance was calculated as the TAC:MDA ratio[16].

Egg qualitative, nutritive, and metabolic indices

The qualitative, nutritive, and metabolic indices of eggs and their desirable relationships with human health were estimated (n = 7/treatment)[36]. The qualitative indices were estimated as follows:

PUFA/SFA ratio =(Σ PUFAs)/(Σ SFAs); Σ *n*-6 PUFAs/Σ *n*-3 PUFAs ratio = (C18:2*n*-6 + C20:2*n*-6 + C20:3*n*-6 + C20:4*n*-6)/(C18:3*n*-3 + C20:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3) [37,38].

Linoleic acid/α-linolenic acid ratio = (C18:2*n*-6)/(C18:3*n*-3) [39,40].

Sum of eicosapentaenoic acid and docosahexaenoic acid (EPA+ DHA) = % C20:5*n*-3 + % C22:6*n*-3[40,41].

Unsaturation index (UI) = (% monoenoic) + (2 × % dienoic) + (3 × % trienoic) + (4 × % tetraenoic) + (5 × % pentaenoic) + (6 × % hesaenoic)[41].

As nutritional indices, the following formulas were used:

Nutritional value index (NVI) = (C18:0 + C18:1*n*-9)/(C16:0) [42].

Atherogenic index (AI) = (C12:0 + 4 × C14:0+C16:0)/ ∑ UFAs,

where UFAs is unsaturated fatty acids.

Thrombogenic index (TI) = (C14:0+C16:0+C18:0)/[(0.5×∑MUFAs) + (0.5×∑*n*-6 PUFAs) + (3×∑*n*-3 PUFAs) + (∑*n*-3 PUFAs /∑*n*-6 PUFAs)].

Platelet aggregation and thrombus formation can occur when AI and TI levels are elevated. Consequently, lower values are beneficial for human health[43].

Egg health index (EHI) = (C18:0+C18:1)/C16:0.

Desirable fatty acids (DFAs) have a beneficial, neutral hypocholesterolemic effect in humans: DFA = ∑MUFAs+∑PUFAs +C18:0).

Undesirable fatty acids (UDFAs) have a hypercholesterolemic effect on humans: UDFAs = C14:0 + C16:0.

The lipid quality parameter expresses the percentage correlation between the main n-3 PUFAs [EPA + DHA) and total lipids. Higher values of this index are synonymous with higher-quality

Table 2. **Amount of antioxidants in flaxseed cake.**

Flaxseed cake	Mean[1] ± SD
DPPH %	36.32±6.12
Ascorbic acid %	4.14±2.90
Tannic acid %	0.13±0.01
Ferulic acid (mg/g)	5.38±0.71
p-Coumaric acid (mg/g)	3.07±0.56

DPPH, 1,1-diphenyl-2-picryl-hydrazil; SD, standard deviation. [1]Mean of nine replicates.

dietary lipid sources[44]. The equation used to calculate lipid quality was:

Egg lipid quality index (ELI) = 100 × [EPA + DHA]/ [% of total fatty acids].

Hypocholesterolemic/hypercholesterolemic ratio (HHR) = (C18:1 + Σ PUFAs)/(C12:0 + C14:0 + C16:0).

Health-promoting index (HPI) = (ΣUFAs)/[C12:0 + (4 × C14:0) + C16:0].

Fish lipid quality/Flesh lipid quality (FLQ) = 100 × (C20:5*n*-3 + C22:6*n*-3)/(Σ SFAs)[45].

As metabolic indices, the following formulas can be utilized as surrogates for real desaturase activity[46–48].

Elongase index = (C18:0/C16:0) × 100

Thioesterase index = (C16:0/C14:0) × 100[49].

Δ9-desaturase (C16:1 + C:18:1) = (C16:1*n*-7 + C18:1*n*-9/(C16:0 + C18:0 + C16:1*n*-7 + C18:1*n*-9) × 100.

Δ5-desaturase + Δ6-desaturase = (C20:2*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3)/(C18:2*n*-6 + C18:3*n*-3+ C20:2*n*-6 + C20:4*n*-6 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3).

Activity index of n-3 β-oxidation in the yolk eggs = (Σ n-3 PUFAs)/(C18:3n-3).

Statistical analysis

The normality of data and differences among treatments were statistically examined using Statistical Analysis Software[50]. All percentages were transformed to log10 to normalize the data distribution before performing statistical analysis. The statistical model used was a one-way analysis of variance (ANOVA), and the replicate was the experimental unit. Tukey's post hoc test was used to compare significant differences among the means of the treatments. Pearson's correlation test was employed to estimate the correlation coefficient between FSCM and fatty acid profiles in the egg yolk and related indices.

Results

The antioxidant levels of FSCM are listed in Table 2. FSCM is a rich source of antioxidants. The DPPH, ascorbic acid, tannic acid, ferulic acid, and *p*-coumaric acid values were: 36.32±6.12%, 4.14±2.90%, 0.13±0.01%, 5.38±0.71 mg/g, and 3.07±0.56 mg/g, respectively.

The fatty acid and health index profiles of FSCM are shown in Table 3. The results indicated that flaxseed cake is a suitable source of omega-3 fatty acids (11.12), with the following profiles: omega-6:omega-3 (1.44), atherogenic index (0.271), thrombogenic index (3.42), hypocholesterolemic index (3.98), lipid quality index (1.30), and health index (2.97).

The influence of dietary FSCM on the blood serum and egg lipid fractions of laying hens is shown in Tables 4 and 5, respectively. Feeding of 5 to 10% FSCM did not significantly affect the serum concentrations of total lipids, triglycerides, total cholesterol, vLDL-cholesterol, LDL-cholesterol, HDL/LDL, or RHCH. However, dietary 10% FSCM increased the concentration of serum HDL cholesterol compared to that in the control. Table 5 presents the egg yolk lipid profiles of laying hens fed 5 or 10% FSCM. All lipid fractions, were unaffected by the FSCM levels.

Evaluation of the effect of FSCM levels on serum antioxidant indices of Hisex laying hens indicated that 5–10% FSCM did not significantly affect the blood serum content of glutathione peroxidase (GPX), TAC, vitamin E, MDA, or the MDA:TAC ratio (Table 6). As shown in Table 7, feeding with 5 or 10% FSCM did not significantly influence the antioxidative markers in eggs, including TAC, MDA, the MDA:TAC ratio, and vitamin E.

Tables 8–10 show the effects of dietary inclusion of 5 or 10% FSCM on yolk fatty acid profiles, including SFAs, MUFAs, and PUFAs. As shown in Table 8, increasing dietary FSCM significantly reduced hexadecanoic acid (C16:0) and total SFAs in the egg yolk. Table 9 reveals that 5% dietary FSCM increased the yolk concentrations of 9-octadecenoic acid (C18:1, *n*-9) and total MUFAs. Notably, feeding FSCM to laying hens resulted in a proportional increase in the total *n*-3 PUFA concentration in egg yolk lipids, whereas the *n*-6:*n*-3 PUFAs ratio decreased significantly as the dietary FSCM increased (Table 10). Data from the current trial showed that the incorporation of ALA, EPA, and DHA into the egg yolk via dietary FSCM was successful (Table 10).

Based on the fatty acid profile of egg yolks, the qualitative, nutritional, and metabolic indices of eggs were significantly affected by feeding FSCM, except for the elongase and thioesterase indices (Table 11). The qualitative and nutritional indices of the egg yolks of the 5 and 10% FSCM groups were considerably better than those of the control group. The metabolic indices in the egg yolk of the 5 and 10% FSCM hens were significantly lower than those in the control (Table 11).

The correlations between FSCM, fatty acid profiles, and health indices are presented in Table S1. The FSCM exhibited significant ($P<0.05$) positive correlations with egg PUFA:SFA ratio ($R^2=0.999$), PUFAs ($R^2=0.998$), NVI ($R^2=0.991$), HHR ($R^2=0.995$), EHI ($R^2=0.947$), HPI ($R^2=0.995$), ∑*n*-6 ($R^2=0.963$), UFAs ($R^2=0.784$), elongase index ($R^2=0.990$), Δ5-desaturase + Δ6-desaturase ($R^2=0.907$), and ∑*n*-3 PUFAs ($R^2=0.611$). The egg yolk fatty acid profiles significantly ($P<0.05$) negatively correlated with SFAs ($R^2=-0.804$), MUFAs ($R^2=-0.988$), ∑*n*-7 ($R^2=-0.976$), ∑*n*-9 ($R^2=-0.986$), UI ($R^2=-0.943$), AI ($R^2=-0.957$), TI ($R^2=-0.845$), DFAs ($R^2=-0.941$), UDFAs ($R^2=-0.949$), thioesterase ($R^2=-0.868$), and Δ9-desaturase (C16:1 + C:18:1) ($R^2=-0.943$). No substantial ($P>0.05$) relationships were observed between FSCM and *n*-6:*n*-3 PUFAs, linoleic acid:α-linolenic acid ratio, EPA% + DHA%, FLQ, and the activ-

Table 3. **Fatty acids composition and health-related indices of flaxseed cake.**

Fatty acid	mg/100 g as-fed basis
C3:0	0.298
C4:0	1.570
C6:0	0.308
C10:0	2.19
C12:0	5.49
C14:0	0.212
C15:0	0.286
C16:0	3.48
C17:0	3.93
C 18:0	6.36
C20:0	2.15
C21:0	1.57
Total aturatd faty acids	27.84
C16:1	1.60
C18:1	3.98
C20:1	5.35
C24:1	0.278
Totalmonounaturatd fatty acids	11.21
C18:2	2.92
C18:3-LA	5.00
C18:3*n*-4 stearidoni acid	1.68
C18:3*n*-3	0.27
C20:2*n*-6	1.58
C20:2	3.03
C20:3	2.03
C20:4	4.96
C20:5*n*3	0.307
C22:5 *n*-3 clupandnic acid (DAP)	0.004
C22:6 *n*-3 Docosahexenoic acid	0.213
Total saturated faty acids	15.84
Mono unsaturated faty acids	9.06
Poly unsaturated faty acids	15.21
Unsaturated fatty aids	24.27
Omega-6	16.01
Omega-3	11.12
Omega-6/omega-3	1.44
Atherogenic index	0.271
Thrombogenic index	3.42
Hypercholesteremic ndex	3.98
Lipid quality index	1.30
Health index	2.97

ity index. The egg yolk fatty acid profiles showed both positive and negative associations with other metrics ($P<0.05$) (Table S1).

Discussion

Phenols from plants and plant byproducts are considered vital dietary components and have considerable antioxidant activity, as well as other biological activities and health benefits[51]. The polyphenolic extract of FSCM exhibited high antioxidant activity as measured using the DPPH free radical scavenging method, which is commonly used to evaluate the free radical scavenging ability of natural products[52]. FSCM showed a strong ability to scavenge DPPH radicals, indicating that FSCM can donate substantial amounts of hydrogen to scavenge DPPH radicals. The radical scavenging activity of flaxseed extracts is comparable to that of other medicinal plants, spice extracts, and synthetic antioxidants used in food products[52]. In particular, ferulic acid is a naturally occurring phenolic antioxidant that is widely used in the food, cosmetics, pharmaceutical, and other biological industries owing to its low toxicity[53]. In agreement with the results of Akl et al.[30], phenolic compounds extracted from FSCM, such as DPPH, ascorbic acid, tannic acid, ferulic acid, and *p*-coumaric acid, showed significant biological activity.

These results indicated that FSCM is a rich source of desirable fatty acids and has health-promoting effects for laying hens, and consequently for human health. FSCM is a substantial source of omega-3 and long-chain PUFAs that promoting health index[7,17]. As shown in Table 4, dietary inclusion of 5 to 10% FSCM did not alter serum triglycerides, total cholesterol, vLDL-cholesterol, LDL-cholesterol, HDL/LDL ratio, or RHCH. These results are consistent with those of previous studies[54] with regard to serum triglyceride, total cholesterol, and the HDL-cholesterol:total cholesterol ratio. Moreover, dietary extruded flaxseed at up to 270 g/kg did not significantly affect the serum concentrations of triglycerides and LDL cholesterol[55]. However, we found that dietary 10% FSCM increased the serum concentration of HDL cholesterol compared with that in the control. Similarly, Attia et al.[3] reported that the inclusion of 12% soaked flaxseed meal increased the serum concentration of HDL cholesterol with respect to that with the control diet. Additionally, Celebi and Utlu[56] reported that the addition of 4% flaxseed oil increased the plasma HDL cholesterol concentration in ISA brown laying hens. The increase in serum HDL cholesterol content in laying hens fed 10% FSCM demonstrates the positive effects of dietary inclusion of FSCM on lipid metabolism and the elevation in healthy lipoproteins associated with increased consumption of omega-3 sources[57]. Consistent with this, several previous studies have indicated that dietary full-fat flaxseed, flaxseed meal, and flaxseed oil reduce blood plasma lipid profiles, including total plasma cholesterol, triglycerides, LDL cholesterol, and VLDL in laying hens[3,56,58].

Notably, the egg lipid profile was also not significantly affected by FSCM levels (Table 5). These results agree with those from a previous study by Abdel-Raheem and Abd-Allah[22], who reported that the inclusion of different levels of extruded flaxseed meal (10–30%) in laying hen diets did not significantly influence the total lipid and cholesterol content in the egg yolk. These results also confirmed the findings of previous studies[59,60]. However, dietary-soaked flaxseed meal reduced all lipid fractions in the egg yolk[3]. In addition, feeding extruded flaxseed

Table 4. **Effect of flaxseed cake levels on the content of lipid fractions of the blood serum in laying hens.**

Flaxseed cake, %	TRIG, mg/dL	vLDL, mg/dL	TC, mg/dL	LDL, mg/dL	HDL, mg/dL	HDL/LDL	RHCH
0	166.4	33.3	171.0	34.5	99.2[b]	2.89	0.201
5	165.0	33.0	173.1	33.5	102.4[ab]	3.00	0.204
10	165.6	33.2	173.8	34.2	103.2[a]	3.10	0.201
RMSE	1.91	0.376	2.84	1.29	2.63	0.148	0.0053
P-value	0.463	0.443	0.290	0.468	0.046	0.091	0.813

TRIG, triglycerides; vLDL, very low-density lipoprotein; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HDL/LDL, high-density lipoprotein:low-density lipoprotein ratio; RHCH, risk of hypercholesterolemia (LDL:TC); RMSE, Root mean square error.

Table 5. **Effect of flaxseed cake levels on the lipid fractions in the edible egg parts of laying hens.**

Flaxseed cake, %	TRIG, mg/g	vLDL, mg/g	TC, mg/g	LDL, mg/g	HDL, mg/g	HDL/LDL	RHCH
0	175	35.0	189	82.8	57.8	0.698	0.438
5	178	35.5	191	85.1	58.5	0.687	0.448
10	178	35.6	189	84.8	58.9	0.694	0.447
RMSE	2.24	2.24	1.32	2.27	0.913	0.017	0.009
P-value	0.290	0.291	0.302	0.178	0.153	0.515	0.177

TRIG, triglycerides; vLDL, very low-density lipoprotein; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HDL/LDL, high-density lipoprotein:low-density lipoprotein ratio; RHCH, risk of hypercholesterolemia (LDL:TC); RMSE = Root mean square error.

Table 6. **Effect of flaxseed cake levels on the blood serum antioxidants indices of laying hens.**

Flaxseed cake, %	GPX, U/mL	TAC, mmol/L	Vitamin E, µg/mL	MDA, µmol/mL	MDA/TAC
0	34.4	0.823	0.650	1.46	0.562
5	35.4	0.819	0.615	1.47	0.557
10	34.9	0.821	0.627	1.49	0.559
RMSE	1.032	0.0038	0.030	0.0059	0.0041
P-value	0.275	0.270	0.157	0.265	0.184

GPX, glutathione peroxidase; TAC, total antioxidant capacity; MDA, malondialdehyde; MDA/TAC, malondialdehyde:total antioxidant capacity ratio; RMSE= root mean square error.

Table 7. **Effect of flaxseed cake levels on antioxidative markers in the eggs of laying hens.**

Flaxseed cake, %	TAC, mmol/L	MDA, µmol/mL	TAC/MDA	Vitamin E, µg/g
0	0.768	3.80	0.202	22.1
5	0.696	3.82	0.182	20.3
10	0.732	3.80	0.193	21.1
RMSE	0.055	0.070	0.079	1.460
P-value	0.109	0.728	0.159	0.126

TAC, total antioxidant capacity; MDA, malondialdehyde; MDA/TAC, malondialdehyde/total antioxidant capacity ratio; RMSE= Root mean square error.

(18–27%) reduced the egg yolk cholesterol content[55]. Similarly, contradictory results regarding the effect of flaxseed addition on yolk HDL-cholesterol content encompass an increase[3], no change[59], or a decrease[61]. Several studies have concluded that egg yolk total cholesterol levels are not influenced by dietary omega-6:omega-3 ratios of 24.5:1, 2.7:1, 1.8:1, 1.2:1, and 1:1 *n*-6:*n*-3[15,17].

Among serum and egg antioxidant indices, dietary 5 or 10% FSCM did not significantly affect the GPX, TAC, vitamin E, MDA, or MDA/TAC ratio. Similarly, the inclusion of 6 or 12% soaked flaxseed meal did not change the serum TAC concentration[3]. Moreover, dietary incorporation of 5 or 10% flaxseed meal in laying hens diet did not significantly influence serum concentrations of GPX, superoxide dismutase, or thiobarbituric acid reactive substances[54]. It has been further postulated that dietary extruded flaxseed of up to 27% does not significantly affect serum GRX activity in laying hens[55]. The non-significant effect of the incorporation of FSCM on MDA concentration in

Table 8. **Saturated fatty acid profile (% of total fatty acids) of the egg yolk of laying hens fed different flaxseed cake levels (*n* = 4/treatment).**

Fatty acid profile	Flaxseed cake,%			RMSE	P-value
	0	5	10		
C12:0	0.00	0.00	0.04	0.052	0.414
C14:0	0.382	0.332	0.476	0.179	0.461
C15:0	ND	ND	ND	–	–
C16:0	28.1[a]	25.1[b]	24.8[b]	0.838	<0.0001
C17:0	0.298	0.132	0.296	0.132	0.119
C18:0	8.97	7.95	7.91	1.02	0.011
C21:0	0.208	0.166	0.136	0.061	0.217
∑ SFAs	38.0[a]	33.7[b]	33.6[b]	1.45	0.0007

C12:0, Lauric acid; C14:0, tetradecanoic acid; C15:0, pentadecyclic acid; C16:0, hexadecanoic acid; C17:0, heptadecanoic acid; C18:0, octadecanoic acid; C21:0, heneicosylic acid; ∑ SFAs: sum of saturated fatty acids; ND, not detectable; RMSE: standard error of the means. [a, b] Means within a row with different superscripts are significantly different (P<0.05).

Table 9. **Monounsaturated fatty acid profile of the egg yolk of laying hens fed different flaxseed cake levels (% of total fatty acids) (*n* = 4/treatment).**

Fatty acid profile	Flaxseed cake, %			RMSE	P-value
	0	5	10		
C14:1*n-5*	0.011[a]	0.001[b]	0.003[b]	0.007	<0.0001
C16:1*n-7*	4.71	4.83	4.33	0.436	0.208
C18:1*n-9*	39.6[b]	41.9[a]	38.6[b]	1.44	0.011
C18:1*n-7*	2.23	2.09	1.96	0.222	0.203
C20:1*n-7*	ND	ND	ND	-	-
C20:1*n-9*	0.227	0.178	0.320	0.161	0.354
C22:1*n-11*	0.00	0.17	0.07	0.069	0.228
C22:1*n-9*	ND	ND	ND	–	-
∑MUFAs	46.9[ab]	49.0[a]	45.3[b]	1.86	0.027

C14:1*n-5*, Myristoleic acid; C16:1*n-7*, 9-hexadecenoic acid; C18:1*n-9*, 9-octadecenoic acid; C18:1*n-7*, vaccenic acid; C20:1*n-7*, paullinic acid, *cis*-13-eicosenoic acid; C20:1*n-9*, cis-11-eicosenoic acid; C22:1*n-9*, erucic acid; ∑MUFAs, sum of monounsaturated fatty acids; RMSE, standard error of the mean; ND, not detectable. [a, b] Means within a row with different superscripts are significantly different (P<0.05).

the serum and eggs suggests that serum MDA may be maintained at normal concentrations by defensive mechanisms, such as hepatic microsomes that possess the ability to degenerate MDA[62] and prevent lipid peroxidation in the blood serum[8]. In addition, different dietary sources of *n*-3 PUFAs, including flaxseed oil and/or fish oil, did not increase serum lipid peroxidation and consequently did not induce cellular oxidative damage, but might rather provide an antioxidative effect in laying hens[5]. Alternatively, decreasing the antinutritional factor content in FSCM may reduce oxidative stress by suppressing free radical production in laying hens and prolonging the oxidative stability of eggs.

Yolk lipoprotein precursors, including vitellogenin and vLDL, are hepatically synthesized under the influence of estradiol-17β and taken up by the developing yolk through receptor-mediated endocytosis[63]. The results of the current trial showed that the dietary inclusion of 5 to 10% FSCM did not alter the plasma concentrations of vLDL or of Zn, which is indicative of circulating vitellogenin[64]. Therefore, it can be speculated that dietary FSCM did not adversely affect folliculogenesis (ovarian follicular development), which was consequently related to the egg production rate in the present study (data not shown).

In recent studies, dietary manipulation to modify the fatty acid profile of eggs has taken precedence over efforts to lower egg cholesterol content[6,65,66]. A major finding of the present study was that dietary inclusion of 5 or 10% FSCM increased ALA, EPA, DHA, and total *n*-3 PUFA concentrations in the egg yolk, whereas the *n*-6:*n*-3 PUFAs ratio decreased significantly in a dose-dependent manner. Significant (P<0.05) positive correlations were observed in the egg yolk fatty acid profile between FSCM and ∑*n*-6 and ∑*n*-3; however, no significant correlations were found between FSCM and *n*-6:*n*-3 PUFAs. The current study demonstrated successful incorporation of long-chain *n*-3 PUFAs (e.g., ALA, EPA, and DHA) into the egg yolk via dietary FSCM. These results agree with those of previous studies[55,59,67,68]. Dietary incorporation of 5 or 10% flaxseed meal in laying hen diets increased the egg yolk contents of PUFAs and *n*-3 PUFAs, whereas those of MUFAs and the *n*-3:*n*-6 ratio decreased[54]. Moreover, dietary extruded flaxseed meal (10, 20,

Table 10. **Polyunsaturated fatty acid profile of the egg yolk of laying hens fed different flaxseed cake levels (% of total fatty acids) (*n* = 4/treatment).**

Fatty acid profile	Flaxseed cake, %			RMSE	P-value
	0	5	10		
C16:3*n*-4	0.088	0.000	0.048	0.058	0.1004
C18:2*n*-6	11.39^{c}	12.59^{b}	13.70^{a}	0.321	<0.0001
C18:3*n*-6	0.126^{a}	0.00^{b}	0.00^{b}	0.003	<0.0001
C20:2*n*-6	0.132	0.000	0.438	0.433	0.3001
C20:4*n*-6 (AA)	1.82^{a}	1.04^{b}	0.510^{c}	0.341	0.0003
C22:4*n*-6	0.056	0.000	0.000	0.057	0.2451
∑ *n*-6 PUFAs	13.53	13.63	14.65	0.694	0.050
C18:3*n*-3 (ALA)	0.450^{c}	2.51^{b}	4.88^{a}	0.507	<0.0001
C 20:3*n*-3	ND	ND	ND	–	–
C20:4*n*-3	ND	ND	ND	–	–
C20:5*n*-3 (EPA)	0.00^{b}	0.00^{b}	0.142^{a}	0.008	<0.0001
C22:5*n*-3 (DPA)	0.418^{a}	0.00^{b}	0.086^{b}	0.105	<0.0001
C22:6*n*-3 (DHA)	0.622^{b}	1.08^{ab}	1.33^{a}	0.376	0.0374
∑ *n*-3 PUFAs	1.49^{c}	3.59^{b}	6.43^{a}	0.398	<0.0001
∑PUFAs	15.11^{c}	17.22^{b}	21.13^{a}	0.834	0.006
∑UFAs	61.99^{b}	66.2^{a}	66.38^{a}	1.444	<0.0001
∑ *n*-7	2.23	2.09	1.96	0.222	0.201
∑ *n*-9	39.8^{b}	42.1^{a}	38.9^{b}	1.486	0.018

C16:3*n*-4,—; C18:2*n*-6 (linoleic acid): 9,12-octadecadienoic acid (Z,Z)-; C18:3*n*-6, dihomo-gamma-linolenic acid; C20:2*n*-6: eicosadienoic acid; C20:4*n*-6 (arachidonic acid; AA), ll-cis-5,8,11,14-eicosatetraenoic acid; C22:4*n*-6, adrenic acid; ∑*n-6* PUFAs: total omega-6 polyunsaturated fatty acids; C18:3*n*-3 (α-linolenic acid; ALA), 9,12,15-octadecatrienoic acid; C20:3*n*-3: eicosatrienoic acid; C20:4*n*-3: eicosatetraenoic acid; C20:5*n*-3 (eicosapentaenoic acid; EPA), cis-5,8,11,14,17-eicosapentaenoic acid; C22:5*n*-3, docosapentaenoic acid (DPA); C22:6*n*-3 (docosahexaenoic acid; DHA), 4,7,10,13,16,19-docosahexaenoic acid; ∑*n*-3 PUFAs: total omega-3 polyunsaturated fatty acids; ∑PUFAs, total polyunsaturated fatty acids; ∑UFA, total unsaturated fatty acids; ∑*n*-7, total *n*-7 fatty acids; ∑*n*-9, total *n*-9 fatty acids; ND, not detectable; RMSE, standard error of the means. ^{a,b} Means within a row with different superscripts are significantly different (P<0.05).

and 30%) in the hen diet increased the egg yolk concentration of ALA and DPA, while reducing the arachidonic acid concentration[22]. Similarly, previous studies have reported a significant increase in the accumulation of ALA in egg yolks when laying hens were fed 10% extruded flaxseed[69] and that the dietary inclusion of flaxseed in laying hens produces omega-3-enriched eggs, particularly ALA[59,70]. Furthermore, up to 27% dietary extruded flaxseed modified the egg yolk fatty acid profile, including increased *n*-3 PUFAs and decreased *n*-6 PUFAs, SFAs, and MUFAs, consistent with the available literature[67].

In recent years, the demand for healthy foods has increased because of their relationship with improving consumer health and increasing resistance to diseases and epidemics. Thus, enhancing egg health indices represented a highly relevant finding of the present study. The AI of the 5 and 10% FSCM-derived egg yolks was significantly lower than that of the control group. TI and AI are the most widely employed nutritional indices for evaluating fatty acid content, as they clearly demonstrate important implications[71]. The TI and AI in the present study were reduced by FSCM and were comparable to those reported by Attia et al.[35]. A healthy egg is characterized by low DFAs, AI, and TI, which predicts a delay in atherosclerosis, and consequently, a reduced risk of cardiovascular disease[35,72]. These results suggest that Δ6 desaturase is used in the *n*-3 PUFAs pathway rather than the *n*-6 PUFAs pathway, which inhibits the conversion of linoleic acid into ARA[73], leading to a reduced *n*-6 PUFA concentration and *n*-6:*n*-3 PUFAs ratio in eggs produced from FSCM-fed hens.

The PUFA:SFA ratio of vegetable sources of omega-3 fatty acids is a universally used indicator for analyzing the effects of a given diet on cardiovascular health, on the assumption that all PUFAs lower LDL and total cholesterol, but that all SFAs may increase serum cholesterol. Consequently, as a direct indicator, a lower PUFA to SFA ratios indicates a greater benefit (or the opposite effect) from eating a particular egg[74]. In this case, the group fed FSCM, particularly 10% FSCM, had a significantly higher PUFA/SFA ratio (positive effect), therefore, the AI and TI were lower, and the HHR was higher in the FSCM group than in the control group. A lower ratio of *n*-6:*n*-3 PUFAs is desirable for reducing the risk of chronic diseases in both developing countries and Western societies[38]. Thus, both FSCM groups performed better than the control regarding the PUFA/SFA ratio in a dose-dependent manner, whereas on the basis of the *n*-6:*n*-3 PUFA ratio, the 10% FSCM group exhibited better performance than did the 5% FSCM group.

As linoleic acid and linolenic acid fatty acids cannot be synthesized in the human body and must be ingested through the

Table 11. **Effect of flaxseed cake levels on qualitative, nutritional, and metabolic indexing in the edible egg parts of laying hens.**

Item	Flaxseed cake, %			RMSE	P-value
	0	5	10		
Qualitative indices					
PUFAs/SFAs	0.397^{c}	0.512^{b}	0.629^{a}	0.026	<0.0001
n-6/*n*-3 PUFAs	9.39^{a}	3.80^{b}	2.29^{c}	1.672	<0.0001
Linoleic acid/α-Linolenic acid	26.2^{a}	5.04^{b}	2.88^{b}	2.884	<0.0001
EPA% + DHA%	0.623^{b}	1.08^{ab}	1.47^{a}	0.316	0.0110
Unsaturation index	85.3^{c}	92.3^{b}	99.4^{a}	1.790	<0.0001
Nutritional Indices					
Nutrition index	1.73^{b}	1.99^{a}	1.88^{ab}	0.084	0.0033
Atherogenic index	0.479^{a}	0.400^{b}	0.403^{b}	0.022	0.0004
Thrombogenic index	1.08^{a}	0.790^{b}	0.669^{c}	0.046	<0.0001
Hypocholesterolemic index	1.97^{b}	2.41^{a}	2.42^{a}	0.284	0.001
HHR	0.990^{b}	1.00^{a}	0.999^{a}	0.0213	<0.0001
Egg health index	1.81^{b}	2.07^{a}	1.96^{ab}	0.094	0.0054
Egg lipid quality index	0.623^{b}	1.08 ^{ab}	1.47^{a}	0.326	0.0110
Health-promoting index	2.09^{b}	2.51^{a}	2.49^{a}	0.124	0.0006
Fish lipid quality/Flesh lipid quality	1.64^{b}	3.20^{ab}	4.31^{a}	0.836	0.0024
Metabolic Indices					
Elongase index	31.9	31.7	31.8	3.178	0.9952
Thioesterase index	7384	7723	6477	1496	0.4962
Δ9-desaturase (C16:1 + C:18:1)	54.42^{b}	58.55^{a}	56.75^{ab}	1.710	0.0168
Δ5-desaturase + Δ6-desaturase	0.209^{a}	0.132^{b}	0.126^{b}	0.381	0.0145
Activity index	3.410^{a}	1.438^{b}	1.338^{b}	0.492	<0.0001

RMSE, standard error of the means; PUFA, poly unsaturated fatty acid; SFA, saturated fatty acid; SEM, Standard error of the mean; HHR, hypocholesterolemic/hypercholesterolemic ratio. $^{a-c}$ Means within a row within each factor not sharing similar superscripts are significantly different, P<0.05.

diet, the linoleic acid:linolenic acid ratio has been used as an evaluation method for infant feeding. These fatty acids also compete for the elongation and desaturation enzymes that enable PUFA synthesis. Because of the low conversion rate of ALA, a decrease in the LA:ALA ratio moderately improves the values of certain *n*-3 PUFAs, such as EPA and DHA[75]; consequently, the LA:ALA ratio can be considered as a first step in estimating PUFAs. Consistent with previous studies, Ryman et al.[40] reported that supplementation with FSCM increased the ALA content of egg yolks and decreased the LA:ALA ratio with increasing doses. No significant (P>0.05) associations were observed between FSCM and the LA:ALA ratio. EPA and DHA are *n*-3 PUFAs that benefit human health by preventing cardiovascular diseases and inflammation, and protecting reproductive health[40]. We found that EPA was present only in hens fed the 10% FSCM/kg diet. UI, which contains information regarding the degree of unsaturation of each FA, is computed as ΣUFAs (%) multiplied by the number of double bonds, yielding different levels of the different unsaturated types. UI was positively correlated with AI, TI, DFAs, UDFAs, ELI, and FLQ, and negatively correlated with NVI, HHR, EHI, and HPI, as well as with NVI and AI, TI, DFAs, and UDFAs. The index value was similar for the omega-3 and omega-6 series; thus, it is not very specific for dietary features, although it plays a role in determining oxidative feed stability in humans and animals and in establishing certain oxidative protective measures[41,76]. Here, the egg yolks from hens fed 5 or 10% FSCM had a higher UI value than those from control hens, indicating a higher probability of autoxidation of FAs, albeit a higher value of healthy lipids, from their egg yolks. FSCM correlated strongly and negatively with UI owing to the strong negative correlation between FSCM and both monoenoic and dienoic acids.

Nutritional indices reflect the nutritional aspects of a diet[42]. NVI, EHI, HPI, and FLQ significantly improved in the groups fed FSCM, particularly at the 5% FSCM level. FSCM strongly positively correlated with NVI, EHI, and HPI. SFAs significantly negatively correlated with NVI, HHR, EHI, and HPI. In contrast, PUFAs significantly positively correlated with HHR, EHI, and HPI.

The atherogenic index is a more specific index than the PUFA:SFA ratio for determining the atherogenicity of foods[77]. With the exception of stearic acid, which is not considered proatherogenic owing to the ability of humans to desaturate stearic acid to oleic acid, SFAs only exhibited marginally significant (P<0.05) positive correlations with AI, TI, DFAs, and UDFAs. Lauric, myristic, and palmitic SFAs promote lipocyte adherence to circulatory and immune system cells and the aggregation of

atherogenic plaques while decreasing esterified fatty acid and phospholipid content[78]. Alternatively, PUFAs significantly negatively correlated with AI, TI, DFAs, and UDFAs.

In contrast, a lower HPI value indicates that the FSCM feed has better nutritional properties. Chen et al.[79] suggested that the HPI constitutes the direct opposite of the atherogenic index. Thus, HPI showed a significantly higher nutritional value for fat and lower atherogenic and thrombogenic indices in the present study at both FSCM concentrations than the values obtained for the control. Accordingly, HPI is used to evaluate the nutritional value of fatty acids and their effects on cardiovascular disease. The thrombogenicity index is used along with the atherogenicity index to further describe the thrombogenic ability of fatty acids. Thus, the supplementation of feeds with lower thrombogenicity is valuable for public health, indirectly suggesting that FSCM has lower thrombogenicity. The HHR focuses on the relationship between dietary fatty acids and LDL in the blood in terms of DFAs (e.g., oleic acid and PUFAs) and UDFAs (e.g., lauric acid, myristic acid, and palmitic acid)[42]. In the present study, the groups receiving both 5 and 10% FSCM showed an increase in DFAs (such as the highly positive correlation between FSCM PUFAs and decreased UDFAs), which resulted in a strong correlation between FSCM and HHR.

The egg yolk fatty acid elongase and thioesterase activities were comparable across treatments, because the elongation index (C18:0:C16:0 ratio) showed that the long C18 acyl chains elongated similarly to the C16 acyl chains, and the thioesterase index (C16:0:C14:0 ratio) showed that the cleavage of C14-acyl carrier proteins (ACPs) was similar to that of C16-ACPs. FSCM correlated negatively ($P<0.05$) with thioesterase activity and positively with the elongase index. Conversely, the 5% FSCM-fed group had higher levels of Δ9-desaturase, which converts C16:0+C:18:0 saturated fatty acids to C16:1+C:18:1 monounsaturated fatty acids. This suggests that the higher levels of both fatty acids in the 5% FSCM-fed group might result from increased Δ9-desaturase activity. The FSCM negatively correlated with Δ9-desaturase and positively correlated with Δ5 desaturase plus Δ6-desaturase.

In turn, the energy consumption (β-oxidation cycle) can be inferred from the ratios of *n*-3 PUFAs and ALA[80]. In our study, compared with that of chickens fed FSCM, hens not receiving FSCM had considerably higher kinetic activity, and their yolks had a higher oxidative status than glycolytic status. This indicates that no correlation existed between the activity index and FSCM supplementation. Alternatively, positive correlations were found between the activity index and SFAs, *n*-6/*n*-3 PUFAs, linoleic acid:α-linolenic acid ratio, and TI; similarly, negative correlations were observed with UFAs and ∑*n*-3 PUFAs. Notably, the association between higher kinetic activity and more oxidative than glycolytic states in hens has been observed in other studies as well[46,81]. This indicates that the activity index accounts for the energy expended by hen locomotor activity over the course of their lifetime, considering all nutritive and qualitative effects on their yolk fatty acids.

Based on the results of the present study, it can be concluded that feeding laying hens with up to 10% FSCM does not adversely influence the blood serum lipid fractions, blood serum antioxidative properties, or antioxidative biomarkers in the plasma and eggs. Notably, the dietary inclusion of 5 or 10% FSCM increased ALA, EPA, DHA, and total *n*-3 PUFA concentrations in the egg yolk, whereas the *n*-6:*n*-3 PUFAs ratio decreased significantly in a dose-dependent manner. Moreover, egg health indices were enriched with 10% FSCM having the strongest effect, suggesting that particular application of FSCM feeding in supplementing the nutrition of laying hens may enhance egg health quality.

Acknowledgments

This work was supported by the Researchers Supporting Project (RSPD2024R581) from King Saud University, Riyadh, Saudi Arabia.

Author Contributions

Conceptualization, Y.A.A.; Methodology, Y.A.A., EL.-S.O.S.H., M.M.Q., and A.A.A.; Software, Y.A.A., F.B., V.T., and H.A.S.; Data collection, EL.-S.O.S.H. and M.M.Q.; Investigation, Y.A.A., R.A.A., M.M.Q., M.J.O., T.A.E., and EL.-S.O.S.H.; Resources, R.A.A., EL.-S.O.S.H., and H.A.S.; Writing—original draft preparation, Y.A.A., M.M.Q., and F.B.; Writing—review and editing, Y.A.A., M.M.Q, A.A.A., F.B., and V.T.; Project administration and supervising, Y.A.A.; Funding acquisition, R.A.A. and Y.A.A. All of the authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Volek Z, Ebeid TA and Uhlířová L. The impact of substituting soybean meal and sunflower meal with a mixture of white lupine seeds and rapeseed meal on rabbit doe milk yield and composition, and the growth performance and carcass traits of their litters. Anim Feed Sci Technol, **236**: 187–195. 2018. https://doi.org/10.1016/j.anifeedsci.2017.12.011

[2] Vastolo A, Calabrò S and Cutrignelli MI. A review on the use of agro-industrial CO-products in animals' diets. Ital J Anim Sci, **21**: 577–594. 2022. https://doi.org/10.1080/1828051X.2022.2039562

[3] Attia YA, Al-Harthi MA, Sagan AAA, Abdulsalam NM, Hussein EOS and Olal MJ. Egg production and quality, lipid metabolites, antioxidant status and immune response of laying hens fed diets with various levels of soaked flax seed meal. Agriculture, **12**: 1402. 2022. https://doi.org/10.3390/agriculture12091402

[4] Qota EMA, El-Ghamry AA and El-Mallah GM. Nutritional value of soaked linseed cake in broiler diets without or with pro-nutrients or formulating diets based on available amino acids value on performance of broilers. Poult Sci, **22**: 461–475. 2002.

[5] Ebeid T, Eid Y, Saleh A and Abd El-Hamid H. Ovarian follicular development, lipid peroxidation, antioxidative status and immune response in laying hens fed fish oil-supplemented diets

to produce n-3-enriched eggs. Animal, **2**: 84–91. 2008. https://doi.org/10.1017/S1751731107000882, PMID:22444966

[6] Maina AN, Lewis E and Kiarie EG. Egg production, egg quality, and fatty acids profiles in eggs and tissues in Lohmann LSL lite hens fed algal oils rich in docosahexaenoic acid (DHA). Poult Sci, **102**: 102921. 2023. https://doi.org/10.1016/j.psj.2023.102921, PMID:37499609

[7] Qaid MM, Al-Mufarrej SI, Al-Garadi MA, Al-Abdullatif AA, Alqhtani AH, Alhotan RA, Alharthi AS and BaZeyad AY. Meat fatty acids profile including metabolic, qualitative, nutritional indices, and organoleptic evaluation as affected by *Rumex nervosus* leaves meal fortified broiler diets. Ital J Anim Sci, **22**: 1050–1066. 2023. https://doi.org/10.1080/1828051X.2023.2267604

[8] Saleh AA, Ebeid TA and Eid YZ. The Effect of dietary linseed oil and organic selenium on growth performance and muscle fatty acids in growing rabbits. Pak Vet J, **33**: 450–454. 2013.

[9] Chen W, Jiang YY, Wang JP, Huang YQ and Wang ZX. Effects of dietary flaxseed meal on production performance, egg quality, and hatchability of Huoyan geese and fatty acids profile in egg yolk and thigh meat from their offspring. Livest Sci, **164**: 102–108. 2014. https://doi.org/10.1016/j.livsci.2014.03.010

[10] Xu L, Wei Z, Guo B, Bai R, Liu J, Li Y, Sun W, Jiang X, Li X and Pi Y. Flaxseed meal and its application in animal husbandry. Rev Agric (Piracicaba), **12**: 2027. 2022.

[11] Alzueta C, Ortiz LT, Rebolé A, Rodríguez ML, Centeno C and Treviño J. Effects of removal of mucilage and enzyme or sepiolite supplement on the nutrient digestibility and metabolyzable energy of a diet containing linseed in broiler chickens. Anim Feed Sci Technol, **97**: 169–181. 2002. https://doi.org/10.1016/S0377-8401(02)00030-5

[12] Rebolé A, Rodríguez ML, Ortiz LT, Alzueta C, Centeno C and Treviño J. Mucilage in linseed: effects on the intestinal viscosity and nutrient digestion in broiler chicks. J Sci Food Agric, **82**: 1171–1176. 2002. https://doi.org/10.1002/jsfa.1164

[13] Attia YA. Nutritional values of soaked linseed cake and its iinclusion in finishing diets for male broiler chicks a source of protein and n-3 fatty acids. Egyptian Poul Sci J, **23**: 739–759. 2003.

[14] Ebeid TA, Aljabeili HS, Al-Homidan IH, Volek Z and Barakat H. Volek Z andBarakat H. Ramifications of heat stress on rabbit production and role of nutraceuticals in alleviating its negative impacts: an updated review. Antioxidants, **12**: 1407. 2023. https://doi.org/10.3390/antiox12071407, PMID:37507946

[15] Ebeid T, Fayoud A, Abou El-Soud S, Eid Y and El-Habbak M. The effect of omega-3 enriched meat production on lipid peroxidation, antioxidative status, immune response and tibia bone characteristics in Japanese quail. Czech J Anim Sci, **56**: 314–324. 2011. https://doi.org/10.17221/1293-CJAS

[16] Hafez HM and Attia YA. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. Front Vet Sci, **7**: 516. 2020. https://doi.org/10.3389/fvets.2020.00516, PMID:33005639

[17] Caston L and Leeson S. Research note: dietary flax and egg composition. Poult Sci, **69**: 1617–1620. 1990. https://doi.org/10.3382/ps.0691617

[18] Scheideler SE, Jaroni D and Froning G. Strain and age effects on egg composition from hens fed diets rich in n-3 fatty acids. Poult Sci, **77**: 192–196. 1998. https://doi.org/10.1093/ps/77.2.192, PMID:9495477

[19] Richter G, Ochrimenko WI, Bargholz J, Reichardt W, Rudolph B, Leiterer M and Luebbe F. Investigations into the use of flaxe cake in broiler fattening. Wirtschaftseigene Futter (Germany). 1996.

[20] Al-Nasser AY, Al-Saffar AE, Abdullah FK, Al-Bahouh ME, Ragheb G and Mashaly MM. Effect of adding flaxseed in the diet of laying hens on both production of omega-3 enriched eggs and on production performance. Int J Poult Sci, **10**: 825–831. 2011. https://doi.org/10.3923/ijps.2011.825.831

[21] Abdel-Raheem SM, Abd-Allah SM and Hassanein KM. The effects of prebiotic, probiotic and synbiotic supplementation on intestinal microbial ecology and histomorphology of broiler chickens. Int J Agro Vet Med Sci, **6**: 277–289. 2012.

[22] Richard MJ, Portal B, Meo J, Coudray C, Hadjian A and Favier A. Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin Chem, **38**: 704–709. 1992. https://doi.org/10.1093/clinchem/38.5.704, PMID:1582024

[23] Richter G, Lüdke H, Rudolph B, Ochrimenko WI, Leiterer M, Hueller M and Schenkel H. Investigations about the use of linseed cake in laying hens. Arch Geflugelkd, **62**: 264–272. 1998.

[24] Nam KT, Lee HA, Min BS and Kang CW. Influence of dietary supplementation with linseed and vitamin E on fatty acids, α-tocopherol and lipid peroxidation in muscles of broiler chicks. Anim Feed Sci Technol, **66**: 149–158. 1997. https://doi.org/10.1016/S0377-8401(96)01108-X

[25] Attia YA, Al-Harthi MA and Abo El-Maaty HM. The effects of different oil sources on performance, digestive enzymes, carcass traits, biochemical, immunological, antioxidant, and morphometric responses of broiler chicks. Front Vet Sci, **7**: 181. 2020. https://doi.org/10.3389/fvets.2020.00181, PMID:32411732

[26] Matusiewicz M, Kosieradzka I, Zuk M and Szopa J. Effect of dose and administration period of seed cake of genetically modified and non-modified flax on selected antioxidative activities in rats. Int J Mol Sci, **16**: 14259–14275. 2015. https://doi.org/10.3390/ijms160614259, PMID:26110393

[27] Sehar S, Rabail R, Munir S, Shakeel K, Khalil AA, Tufail T, Abid M, Mukhtar K, Nabi BG, Goksen G and Aadil RM. An insight into anticancer perspectives of chickpea bioactive compounds. Food Chem Adv, **3**: 100453. 2023. https://doi.org/10.1016/j.focha.2023.100453

[28] Attia YA, Al-Sagan AA, Hussein ESOS, Olal MJ, Ebeid TA, Al-Abdullatif AA, Alhotan RA, Alyileili SR, Shehata HA and Tufarelli V. Dietary flaxseed cake influences on performance, quality, and sensory attributes of eggs, serum, and egg trace minerals of laying hens. Trop Anim Health Prod, **56**: 50. 2024. https://doi.org/10.1007/s11250-024-03897-0, PMID:38236506

[29] Table CF. Chemical composition and nutritional values of feedstuffs. Central Bureau for Livestock Feeding (CVB), Lelystad, the Netherlands. 2016.

[30] Akl EM, Mohamed SS, Hashem AI and Taha FS. Biological activities of phenolic compounds extracted from flaxseed meal. Bull Natl Res Cent, **44**: 27. 2020. https://doi.org/10.1186/s42269-020-0280-x

[31] Schanderl S. Methods in Food Analysis.701. 1970.

[32] Ragupathi Raja Kannan R, Arumugam R, Thangaradjou T

and Anantharaman P. Phytochemical constituents, antioxidant properties and p-coumaric acid analysis in some seagrasses. Food Res Int, **54**: 1229–1236. 2013. https://doi.org/10.1016/j.foodres.2013.01.027

[33] Radwan SS. Coupling of two-dimensional thin-layer chromatography with gas chromatography for the quantitative analysis of lipid classes and their constituent fatty acids. J Chromatogr Sci, **16**: 538–542. 1978. https://doi.org/10.1093/chromsci/16.11.538

[34] Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, **18**: 499–502. 1972. https://doi.org/10.1093/clinchem/18.6.499, PMID:4337382

[35] Attia YA, Al-Harthi MA, Korish MA and Shiboob MM. Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. Lipids Health Dis, **14**: 136. 2015. https://doi.org/10.1186/s12944-015-0133-z, PMID:26507616

[36] Wereńska M, Haraf G, Wołoszyn J, Goluch Z, Okruszek A and Teleszko M. Fatty acid profile and health lipid indicies of goose meat in relation to various types of heat treatment. Poult Sci, **100**: 101237. 2021. https://doi.org/10.1016/j.psj.2021.101237, PMID:34198099

[37] Simopoulos AP. The omega-6/omega-3 fatty acid ratio: health implications. Ol Corps Gras Lipides, **17**: 267–275. 2010. https://doi.org/10.1051/ocl.2010.0325

[38] Timmis A, Kazakiewicz D, Torbica A, Townsend N, Huculeci R, Aboyans V and Vardas P. Cardiovascular disease care and outcomes in West and South European countries. Lancet Reg Health Eur, **33**: 100718. 2023. https://doi.org/10.1016/j.lanepe.2023.100718, PMID:37953997

[39] Draycott SAV, Liu G, Daniel ZC, Elmes MJ, Muhlhausler BS and Langley-Evans SC. Maternal dietary ratio of linoleic acid to alpha-linolenic acid during pregnancy has sex-specific effects on placental and fetal weights in the rat. Nutr Metab (Lond), **16**: 1–12. 2019. https://doi.org/10.1186/s12986-018-0330-7, PMID:30622622

[40] Ryman VE, Packiriswamy N, Norby B, Schmidt SE, Lock AL and Sordillo LM. Supplementation of linoleic acid (C18:2n-6) or α-linolenic acid (C18:3n-3) changes microbial agonist-induced oxylipid biosynthesis. J Dairy Sci, **100**: 1870–1887. 2017. https://doi.org/10.3168/jds.2016-11599, PMID:28109600

[41] Crupi R and Cuzzocrea S. Role of EPA in inflammation: mechanisms, effects, and clinical relevance. Biomolecules, **12**: 242. 2022. https://doi.org/10.3390/biom12020242, PMID:35204743

[42] Chen Y, Qiao Y, Xiao Y, Chen H, Zhao L, Huang M and Zhou G. Differences in physicochemical and nutritional properties of breast and thigh meat from crossbred chickens, commercial broilers, and spent hens. Asian-Australas J Anim Sci, **29**: 855–864. 2016. https://doi.org/10.5713/ajas.15.0840, PMID:26954114

[43] Lo Turco V, Potortì AG, Rando R, Ravenda P, Dugo G and Di Bella G. Functional properties and fatty acids profile of different beans varieties. Nat Prod Res, **30**: 2243–2248. 2016. https://doi.org/10.1080/14786419.2016.1154056, PMID:26949141

[44] Łuczyńska J, Paszczyk B, Nowosad J and Łuczyński M. Mercury, fatty acids content and lipid quality indexes in muscles of freshwater and marine fish on the polish market. Risk assessment of fish consumption. Int J Environ Res Public Health, **14**: 1120. 2017. https://doi.org/10.3390/ijerph14101120, PMID:28946712

[45] Abrami G, Natiello F, Bronzi P, McKenzie D, Bolis L and Agradi E. A comparison of highly unsaturated fatty acid levels in wild and farmed eels (Anguilla Anguilla). Comp Biochem Physiol B, **101**: 79–81. 1992. https://doi.org/10.1016/0305-0491(92)90161-J, PMID:1499279

[46] Failla S, Buttazzoni L, Zilio DM, Contò M, Renzi G, Castellini C and Amato MG. An index to measure the activity attitude of broilers in extensive system. Poult Sci, **100**: 101279. 2021. https://doi.org/10.1016/j.psj.2021.101279, PMID:34242942

[47] Vessby B, Gustafsson I-B, Tengblad S, Boberg M and Andersson A. Desaturation and elongation of Fatty acids and insulin action. Ann N Y Acad Sci, **967**: 183–195. 2002. https://doi.org/10.1111/j.1749-6632.2002.tb04275.x, PMID:12079847

[48] Yaqoob P, Newsholme EA and Calder PC. Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. Biochim Biophys Acta Lipids Lipid Metab, **1255**: 333–340. 1995. https://doi.org/10.1016/0005-2760(94)00251-S, PMID:7734450

[49] Zhang S, Knight TJ, Stalder KJ, Goodwin RN, Lonergan SM and Beitz DC. Effects of breed, sex and halothane genotype on fatty acid composition of triacylglycerols and phospholipids in pork longissimus muscle. J Anim Breed Genet, **126**: 259–268. 2009. https://doi.org/10.1111/j.1439-0388.2008.00782.x, PMID:19630876

[50] SAS Institute. SAS/OR 9.3 User's Guide: Mathematical Programming Examples. SAS institute. 2012.

[51] Kumar N and Goel N. Phenolic acids: natural versatile molecules with promising therapeutic applications. Biotechnol Rep (Amst), **24**: e00370. 2019. https://doi.org/10.1016/j.btre.2019.e00370, PMID:31516850

[52] Pag AI, Radu DG, Draganescu D, Popa MI and Sirghie C. Flaxseed cake—A sustainable source of antioxidant and antibacterial extracts. Cellul Chem Technol, **48**: 265–273. 2014.

[53] Mikołajczak N, Pilarski W, Gęsiński K and Tańska M. Effect of Ferulic Acid and Its Derivatives on Cold-Pressed Flaxseed Oil Oxidative Stability and Bioactive Compounds Retention during Oxidation. Foods, **12**: 1088. 2023. https://doi.org/10.3390/foods12051088, PMID:36900605

[54] Shafey TM, Al-Batshan HA and Farhan AMS. The effect of dietary flaxseed meal on liver and egg yolk fatty acid profiles, immune response and antioxidant status of laying hens. Ital J Anim Sci, **14**: 3939. 2015. https://doi.org/10.4081/ijas.2015.3939

[55] Hosseini H, Esmaeili N, Sepehr A, Zare M, Rombenso A, Badierah R and Redwan EM. Does supplementing laying hen diets with a herb mixture mitigate the negative impacts of excessive inclusion of extruded flaxseed? Anim Biosci, **36**: 629–641. 2023. https://doi.org/10.5713/ab.22.0183, PMID:36397705

[56] Celebi S and Utlu N. Laying performance, serum lipoproteins, cholesterol and triglyceride of hens as influenced by dietary fat sources. J Appl Anim Res, **25**: 121–124. 2004. https://doi.org/10.1080/09712119.2004.9706488

[57] Shakoor H, Khan MI, Sahar A, Khan MKI, Faiz F and Basheer

Ahmad H. Development of omega-3 rich eggs through dietary flaxseed and bio-evaluation in metabolic syndrome. Food Sci Nutr, **8**: 2619–2626. 2020. https://doi.org/10.1002/fsn3.1522, PMID:32566179

[58] Van Elswyk ME. Comparison of *n* –3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. Br J Nutr, **78**: S61–S69. 1997. https://doi.org/10.1079/BJN19970135, PMID:9292775

[59] Venkatraman JT, Angkeow P, Satsangi N and Fernandes G. Effects of dietary n-6 and n-3 lipids on antioxidant defense system in livers of exercised rats. J Am Coll Nutr, **17**: 586–594. 1998. https://doi.org/10.1080/07315724.1998.10718807, PMID:9853538

[60] Attia YA, Abd El-Hamid AEHE, Abedalla AA, Berika MA, Al-Harthi MA, Kucuk O, Sahin K and Abou-Shehema BM. Laying performance, digestibility and plasma hormones in laying hens exposed to chronic heat stress as affected by betaine, vitamin C, and/or vitamin E supplementation. SpringerPlus, **5**: 1619. 2016. https://doi.org/10.1186/s40064-016-3304-0, PMID:27652192

[61] Ebeid TA, Eid YZ, El-Abd EA and El-Habbak MM. Effects of catecholamines on ovary morphology, blood concentrations of estradiol-17β, progesterone, zinc, triglycerides and rate of ovulation in domestic hens. Theriogenology, **69**: 870–876. 2008. https://doi.org/10.1016/j.theriogenology.2008.01.002, PMID:18316117

[62] Hayat Z, Cherian G, Pasha TN, Khattak FM and Jabbar MA. Sensory evaluation and consumer acceptance of eggs from hens fed flax seed and 2 different antioxidants. Poult Sci, **89**: 2293–2298. 2010. https://doi.org/10.3382/ps.2009-00575, PMID:20852121

[63] Cherian G. Egg quality and yolk polyunsaturated fatty acid status in relation to broiler breeder hen age and dietary n-3 oils. Poult Sci, **87**: 1131–1137. 2008. https://doi.org/10.3382/ps.2007-00333, PMID:18493002

[64] Mattioli S, Ruggeri S, Sebastiani B, Brecchia G, Dal Bosco A, Cartoni Mancinelli A and Castellini C. Performance and egg quality of laying hens fed flaxseed: highlights on n-3 fatty acids, cholesterol, lignans and isoflavones. Animal, **11**: 705–712. 2017. https://doi.org/10.1017/S175173111600207X, PMID:27819218

[65] Saleh AA, Kirrella AA, Dawood MAO and Ebeid TA. Effect of dietary inclusion of cumin seed oil on the performance, egg quality, immune response and ovarian development in laying hens under high ambient temperature. J Anim Physiol Anim Nutr (Berl), **103**: 1810–1817. 2019. https://doi.org/10.1111/jpn.13206, PMID:31518023

[66] Saleh AA, Zaki A, El- Awady A, Amber K, Badwi N, Eid Y and Ebeid TA. The effect of substituting wheat bran with cumin seed meal on laying performance, egg quality characteristics and fatty acid profile in laying hens. Vet Arh, **90**: 47–56. 2020. https://doi.org/10.24099/vet.arhiv.0500

[67] Huang S. Effects of feeding extruded flaxseed on layer performance. McGill University (Canada). 2016.

[68] Halle I and Schöne F. Influence of rapeseed cake, linseed cake and hemp seed cake on laying performance of hens and fatty acid composition of egg yolk. J Verbraucherschutz Lebensmsicherh, **8**: 185–193. 2013. https://doi.org/10.1007/s00003-013-0822-3

[69] Bean LD and Leeson S. Metabolizable energy of layer diets containing regular or heat-treated flaxseed. J Appl Poult Res, **11**: 424–429. 2002. https://doi.org/10.1093/japr/11.4.424

[70] Beynen AC. Fatty acid composition of eggs produced by hens fed diets containing groundnut, soya bean or linseed. NJAS Wagening J Life Sci, **52**: 3–10. 2004. https://doi.org/10.1016/S1573-5214(04)80026-5

[71] Chen J and Liu H. Nutritional indices for assessing fatty acids: A mini-review. Int J Mol Sci, **21**: 5695. 2020. https://doi.org/10.3390/ijms21165695, PMID:32784511

[72] Mutungi G, Ratliff J, Puglisi M, Torres-Gonzalez M, Vaishnav U, Leite JO, Quann E, Volek JS and Fernandez ML. Dietary cholesterol from eggs increases plasma HDL cholesterol in overweight men consuming a carbohydrate-restricted diet. J Nutr, **138**: 272–276. 2008. https://doi.org/10.1093/jn/138.2.272, PMID:18203890

[73] Jia W, Slominski BA, Guenter W, Humphreys A and Jones O. The effect of enzyme supplementation on egg production parameters and omega-3 fatty acid deposition in laying hens fed flaxseed and canola seed. Poult Sci, **87**: 2005–2014. 2008. https://doi.org/10.3382/ps.2007-00474, PMID:18809863

[74] Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood), **233**: 674–688. 2008. https://doi.org/10.3181/0711-MR-311, PMID:18408140

[75] Das UN. Essential fatty acids: biochemistry, physiology and pathology. Biotechnol J, **1**: 420–439. 2006. https://doi.org/10.1002/biot.200600012, PMID:16892270

[76] Untea AE, Varzaru I, Saracila M, Panaite TD, Oancea AG, Vlaicu PA and Grosu IA. Antioxidant Properties of Cranberry Leaves and Walnut Meal and Their Effect on Nutritional Quality and Oxidative Stability of Broiler Breast Meat. Antioxidants, **12**: 1084. 2023. https://doi.org/10.3390/antiox12051084, PMID:37237949

[77] Dal Bosco A, Mugnai C, Mattioli S, Rosati A, Ruggeri S, Ranucci D and Castellini C. Transfer of bioactive compounds from pasture to meat in organic free-range chickens. Poult Sci, **95**: 2464–2471. 2016. https://doi.org/10.3382/ps/pev383, PMID:26769274

[78] Omri B, Chalghoumi R, Izzo L, Ritieni A, Lucarini M, Durazzo A, Abdouli H and Santini A. Effect of dietary incorporation of linseed alone or together with tomato-red pepper mix on laying hens' egg yolk fatty acids profile and health lipid indexes. Nutrients, **11**: 813. 2019. https://doi.org/10.3390/nu11040813, PMID:30974860

[79] Chen S, Bobe G, Zimmerman S, Hammond EG, Luhman CM, Boylston TD, Freeman AE and Beitz DC. Physical and sensory properties of dairy products from cows with various milk fatty acid compositions. J Agric Food Chem, **52**: 3422–3428. 2004. https://doi.org/10.1021/jf035193z, PMID:15161209

[80] Houten SM and Wanders RJA. A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis, **33**: 469–477. 2010. https://doi.org/10.1007/s10545-010-9061-2, PMID:20195903

[81] Dal Bosco A, Mugnai C, Ruggeri S, Mattioli S and Castellini C. Fatty acid composition of meat and estimated indices of lipid metabolism in different poultry genotypes reared under organic system. Poult Sci, **91**: 2039–2045. 2012. https://doi.org/10.3382/ps.2012-02228, PMID:22802202