

NUTRITIONAL STRATEGIES TO IMPROVE EGG QUALITY: THE ROLE OF HEMP AND FLAX SEED SUPPLEMENTATION IN LAYING HENS

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ABSTRACT

This study evaluated the effects of dietary supplementation with hemp seed and flax seed on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability. A total of 240 Lohmann Brown laying hens were assigned to 4 dietary treatments in a 2 × 2 factorial experiment with two levels of non-psychoactive hemp seed (Futura variety; 0 and 40 g/kg) and two levels of non-extruded flax seed (Floral variety; 0 and 40 g/kg). The experiment lasted 12 weeks. The inclusion of flax seed significantly increased both hen-day egg production ($p < 0.001$) and egg mass ($p < 0.001$), while the addition of hemp seed resulted in a higher total egg mass ($p < 0.001$). Laying hens fed the flax seed-enriched diet exhibited a lower feed conversion ratio. The combined hemp seed and flax seed supplementation significantly increased egg weight ($p = 0.002$) and shell percentage ($p = 0.036$). The inclusion of hemp seed significantly elevated yolk γ -tocopherol concentrations ($p < 0.001$) and reduced total cholesterol ($p = 0.042$) and malondialdehyde levels in fresh yolks ($p = 0.003$), indicating improved oxidative stability. Both seed supplements and their combination increased the content of n -6 ($p = 0.022$) and n -3 ($p < 0.001$) polyunsaturated fatty acids. In particular, the levels of α -linolenic ($p < 0.001$) and eicosapentaenoic ($p < 0.001$) acids were elevated. These changes resulted in a more favorable n -6/ n -3 ratio, which was significantly affected by both hemp seed and flax seed supplementation ($p < 0.001$). In addition, all dietary treatments significantly affected the nutritional lipid quality indices, namely the index of atherogenicity and the index of thrombogenicity ($p < 0.001$).



These findings highlight the potential of combined hemp seed and flax seed supplementation as a novel nutritional strategy for producing eggs enriched with health-promoting bioactive compounds and improved nutritional quality.

Keywords: oilseeds; tocopherols; *n*-3 polyunsaturated fatty acids; nutritional indices; oxidative stability

INTRODUCTION

Eggs are an affordable and highly nutritious food, providing an excellent source of high-quality proteins and essential fats (Réhault-Godbert et al. 2019). They are also widely recognized as a valuable source of both fat-soluble and water-soluble vitamins (Adeoye et al. 2023). Owing to their rich nutritional profile, eggs are considered an ideal medium for delivering additional health-promoting bioactive compounds, the concentrations of which can be significantly influenced by the diet of laying hens. The recognized health benefits of *n*-3 polyunsaturated fatty acids (PUFAs), particularly α -linolenic acid (ALA, C18:3, *n*-3) and its long-chain derivatives eicosapentaenoic acid (EPA, C20:5, *n*-3) and docosahexaenoic acid (DHA, C22:6, *n*-3), have stimulated considerable research into increasing the *n*-3 PUFA content of eggs through dietary supplementation with *n*-3 PUFA-rich feed ingredients (Farinon et al. 2020; Usturoi et al. 2025). Seeds and their oils are among the most important dietary sources of *n*-3 PUFAs (Jing et al. 2017), including hemp seed (HS) and flax seed (FS). These oilseeds are natural sources of numerous health-promoting bioactive compounds and may exert distinct effects on laying hen performance as well as on egg quality and yolk nutritional composition. According to Bekhit et al. (2018) and Farinon et al. (2020), HS and FS contain lipids with unique and well-balanced fatty acid (FA) profiles, characterized by favorable *n*-6/*n*-3 PUFA ratios of approximately 3.5:1 and 0.3:1, respectively. Furthermore, the *n*-6/*n*-3 PUFA ratio is considered a critical indicator of dietary fat quality and is recommended to be below 4 (Simopoulos 2008). FS represents a particularly rich source of ALA (Shim et al. 2015). In addition, HS contains considerable amounts of tocopherols (Farinon et al. 2020), which are commonly used in laying hen nutrition to improve the oxidative stability of *n*-3 PUFA-enriched eggs (Ren et al. 2013). Therefore, the present study aimed to evaluate the effects of dietary supplementation with HS from the non-psychoactive variety Futura, non-extruded FS from the variety Floral, and their combination on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability.

MATERIAL AND METHODS

Experimental Design and Dietary Treatments

The experiment was performed with a total of 240 Lohmann Brown laying hens aged 50 weeks. The laying hens were housed in three-tier enriched cages within the same air-conditioned facility and randomly allocated to 4 dietary treatments, with 6 replicate cages and 10 laying hens per cage. The 2×2 factorial experiment included two levels of non-psychoactive HS (Futura variety; 0 and 40 g/kg) and two levels of non-extruded FS (Floral variety; 0 and 40 g/kg) in the diet. The first (I) control diet consisted of basal wheat–maize–soybean meal mixed feed without HS and FS. The second (II) and third (III) experimental groups of laying hens were fed diets supplemented with 40 g/kg FS and 40 g/kg HS, respectively. The laying hens from the fourth (IV) experimental group received a diet containing a combination of the above seeds, specifically 40 g/kg HS and 40 g/kg FS. Apart from the respective HS and FS supplementation, the experimental diets for laying hens were based on wheat, maize, and soybean meal as the main energy and protein sources. The diets also contained soybean oil, mineral supplements, synthetic amino acids, and a vitamin-mineral premix. Each enriched cage had an area of 7560 cm². The stocking density, cage equipment (i.e., feeders, drinkers, nests, and perches), lighting, ventilation, and vaccination complied with the requirements of the European Council Directive 1999/74/EC. The technological and microclimatic housing conditions complied with the standard procedure for the Lohmann Brown hybrid, with an average temperature of 20–22 °C and humidity between 50 and 60 %. The light cycle was 14 hours of light and 10 hours of darkness, with a light intensity of approximately 10 lx at the middle tier of the three-tier enriched cage system. Feed and fresh water complying with hygiene standards were supplied *ad libitum*. The experiment lasted for 12 weeks, including a two-week preparation period. The health status of the laying hens and the number of eggs laid were monitored daily. The hen-day egg production (%) and feed conversion ratio (FCR; kg/kg) were calculated weekly for each cage and group. Egg weights (g) were recorded three times per week for all eggs in each replicate. The eggs used for chemical analyses of the yolk were collected at the end of the experiment. Three eggs from each replicate were homogenized to form one sample ($n = 6$).

Physical Analysis of Eggs

For the analysis of physical parameters, eggs from a whole day of production were collected from laying hens at 61 weeks of age. A total of 219 eggs were analyzed. The values were averaged per cage ($n = 6$). The color of the yolk was determined using a DSM yolk color fan (DSM Nutritional Products, Basel, Switzerland) with a scale ranging from 1 to 15. The percentage of shell was determined on the basis of the individual weight of each egg (g) and the corresponding weight of its shell (g). All eggs and their shells were weighed using a digital scale (KERN & SOHN GmbH, Balingen, Germany) with an accuracy of 0.1 g.

Chemical Analyses of Egg Yolks

The concentrations of α -tocopherol, γ -tocopherol, and β -carotene (mg/kg dry matter; DM) in the lyophilized egg yolks ($n = 6$) were determined using a Shimadzu high-performance liquid chromatography system (VP series; Shimadzu, Kyoto, Japan) equipped with a diode array detector (HPLC-DAD), according to the European Standards EN 12822-1 and EN 12823-2. The FA profiles (mg/100 g fresh weight) of the egg yolks ($n = 6$) were determined following chloroform-methanol extraction of total lipids according to Folch et al. (1957). The FAs were converted to methyl esters (FAMES) by alkaline trans-methylation. The resulting FAMES were analyzed by gas chromatography using an HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionization detector (GC-FID). The cholesterol concentration (g/kg egg yolk) in the egg yolks ($n = 6$) was determined following lipid saponification and extraction of the unsaponifiable fraction with diethyl ether according to ISO 3596:2011. The cholesterol was derivatized to silyl derivatives using trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) silylation reagents (Sigma-Aldrich, Prague, Czech Republic) and subsequently analyzed by GC-FID (Agilent Technologies, Inc., Santa Clara, CA, USA). Lipid peroxidation in the egg yolks ($n = 6$) from fresh eggs and eggs stored for 28 days at 18 °C and 50–55% relative humidity on paper trays was determined using a modified method based on Czauderna et al. (2011). The oxidative stability of the egg yolk lipids in both fresh and stored eggs was expressed as malondialdehyde (MDA) concentration (mg/kg fresh egg yolk), as determined by HPLC-DAD analysis (VP series; Shimadzu, Kyoto, Japan).

Calculations of Nutritional Indices

On the basis of the FA profiles determined, nutritional indices (i.e., index of atherogenicity – IA and index of thrombogenicity – IT) related to the nutritional value of egg yolk lipids were calculated with the formulas provided below.

The formula for calculating the index of atherogenicity (IA) was as follows:

$$IA = [C12:0 + (4 \times C14:0) + C16:0] / \Sigma \text{UFAs}$$

where lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) represent the main classes of saturated fatty acids (SFAs), and Σ UFAs denotes the sum of unsaturated fatty acids (UFAs), including monounsaturated fatty acids (MUFAs) and PUFAs.

The formula for calculating the index of thrombogenicity (IT) was as follows:

$$IT = (C14:0 + C16:0 + C18:0) / (0.5 \times \Sigma \text{MUFAs}) + (0.5 \times \Sigma n-6 \text{ PUFAs}) \\ + (3 \times \Sigma n-3 \text{ PUFAs}) + (n-3 \text{ PUFAs} / n-6 \text{ PUFAs})$$

where myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) represent the main classes of SFAs; Σ MUFAs represents the sum of MUFAs; and Σ $n-6$ PUFAs and Σ $n-3$ PUFAs denote the sums of $n-6$ PUFAs and $n-3$ PUFAs, respectively.

Statistical Analysis

The experimental data obtained were analyzed with a two-way analysis of variance (ANOVA) with the general linear model (GLM) procedure in the Statistical Analysis System (SAS 2013). The main effects were the level of HS in the diet, the level of FS in the diet, and the interaction effect between these factors (i.e., HS*FS). Differences between the groups were tested with Duncan's multiple range test. The experimental unit was a cage ($n = 6$). The results in Table 1 are presented as the means (a, b, c, d), the standard error of the mean (SEM), and the probability (p) values. All differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effects of dietary HS and FS supplementation on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability are summarized in Table 1.

Performance Characteristics of Laying Hens

The inclusion of FS in the experimental diets (II and IV) of laying hens significantly increased both hen-day egg production ($p < 0.001$) and egg mass ($p < 0.001$). In contrast, HS supplementation had no significant effect on overall hen-day egg production. Similarly, Neijat et al. (2014) reported no significant differences in the overall performance of laying hens when graded levels of either HS (up to 300 g/kg) or HS oil (up to 90 g/kg) were included in the diet compared with laying hens fed the control diet. However, in the present study, the addition of HS to the diets (III and IV) resulted in a higher total egg mass ($p < 0.001$). In addition, laying hens fed the FS-enriched diet exhibited a lower FCR (II; 2.09 kg/kg) compared with those fed the combination of HS and FS (IV; 2.14 kg/kg) or the control diet (I; 2.17 kg/kg). FS is rich in lipids and represents a valuable source of n -3 PUFAs, particularly ALA (Shim et al. 2015), thereby increasing the energy density of the feed. This may reduce feed intake while maintaining production performance, consequently improving FCR. However, published findings regarding the effects of FS supplementation on the performance characteristics of laying hens remain inconsistent. Such inconsistency has also been reported by Imran et al. (2015).

Physical Egg Quality Characteristics

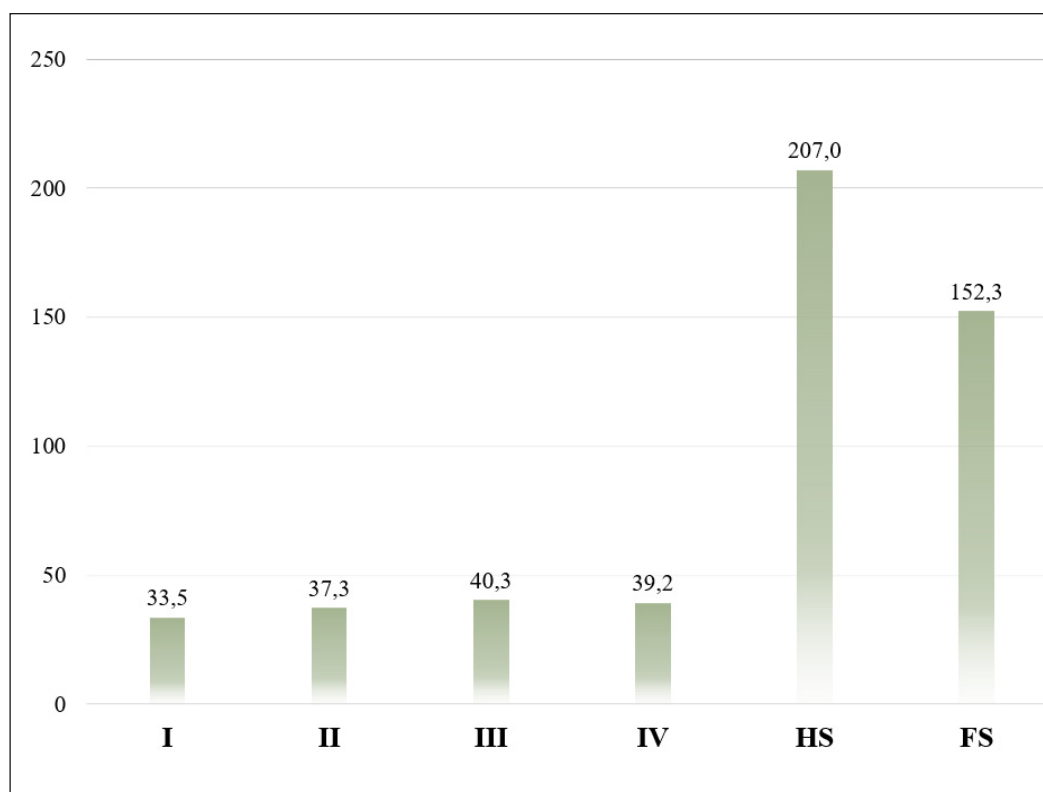
The mixed feed containing both HS and FS (IV) significantly increased egg weight (HS*FS; $p = 0.002$) by almost 2% compared with the control group (I). The highest egg weight was observed in laying hens fed the diet with HS supplementation alone (III; 62.4 g), followed by the combination of HS and FS (IV; 62.3 g) and FS supplementation alone (II; 61.7 g), while the lowest value was recorded in the control group (I; 60.5 g). Similarly, previous studies have reported a positive effect of HS supplementation on egg mass or egg weight (Halle and Schöne 2013; Skřivan et al. 2019). Neither HS nor FS supplementation significantly affected yolk color. A significant HS*FS interaction ($p = 0.014$) was observed. Numerically,

slightly higher yolk color values were found in all seed-supplemented groups (II, III, and IV) compared with the control group (I). The yolk color intensity is primarily determined by the presence of carotenoids, which represent one of the most widespread groups of natural lipophilic pigments with a wide range of bioactive and health-promoting properties (Szmek et al. 2026). Because laying hens are incapable of synthesizing these pigments *in vivo*, they must be entirely supplied through the diet. There are various natural dietary sources of carotenoids. For example, Halle and Schöne (2013) reported that FS meal increased yolk color more markedly than rapeseed meal or HS meal did. Furthermore, in the present study, the combined HS and FS supplementation in the diet (IV) resulted in a significantly higher shell percentage (HS*FS; $p = 0.036$) than that in group III, which received HS alone.

Tocopherol and β -carotene Concentrations in Egg Yolks

While egg yolks are a valuable source of α -tocopherol, the content of γ -tocopherol is negligible. In the present study, the inclusion of HS in the experimental diets (III and IV) of laying hens resulted in elevated levels of γ -tocopherol in egg yolks ($p < 0.001$), with the highest value (62.1 mg/kg DM) observed in group IV, which received the combined HS and FS supplementation. The addition of HS alone in the experimental diet (III) resulted in a γ -tocopherol content of 61.5 mg/kg DM in egg yolks. In contrast, Skřivan et al. (2019) reported that a considerably higher dietary inclusion level of HS (90 g/kg) resulted in a lower γ -tocopherol content in egg yolks (43.3 mg/kg DM). According to Wang et al. (2010), the main factors influencing yolk tocopherol content include nutrition, age, and laying hen genotype. Although rapeseed oil was used in the study by Skřivan et al. (2019), soybean oil served as the primary dietary fat source in our experimental diets. Although soybean oil naturally contains γ -tocopherol and therefore contributed to its overall concentration in the feed, our results suggest that the majority of this compound originated directly from the supplemented HS. According to our HPLC-DAD analysis (Graph 1), HS contained a 1.4-fold higher concentration of γ -tocopherol than FS did (207.0 mg/kg fresh weight vs. 152.3 mg/kg fresh weight, respectively). According to Kriese et al. (2004), γ -tocopherol is the predominant tocopherol isomer in HS. Furthermore, Skřivan et al. (2019) observed that increasing the HS concentration in the diets of laying hens led to elevated concentrations of both α -tocopherol and γ -tocopherol in egg yolks. In the present study, no significant effects of dietary treatments were observed on α -tocopherol concentration. Tocopherols are well known for their strong antioxidant properties, which may contribute to increasing the antioxidant potential and oxidative stability of eggs. Among all tocopherol isomers, α -tocopherol exhibits the highest antioxidant activity and represents the most bioavailable form (Es-Sai et al. 2025) with vitamin E activity in both animals and humans. Unlike other tocopherol isomers, γ -tocopherol effectively neutralizes reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby providing robust cellular protection against oxidative

damage and lipid peroxidation (Es-Sai et al. 2025). In addition to its strong antioxidant properties, particularly in lipophilic matrices (Porto et al. 2014), γ -tocopherol is also characterized by anti-inflammatory activity through the modulation of pathways involving cyclooxygenase-2 (COX-2) and tumor necrosis factor- α (TNF- α), thereby reducing chronic inflammation and associated health risks (Es-Sai et al. 2025). Finally, a significant HS*FS interaction ($p = 0.029$) was detected for the β -carotene content of egg yolks. The control group (I) showed the highest β -carotene level (0.387 mg/kg DM), whereas the lowest value (0.312 mg/kg DM) was recorded in group III, which received HS alone. The elevated γ -tocopherol concentration observed in egg yolks from laying hens fed the HS-enriched diets (III and IV) may be associated with the antioxidant properties of carotenoids, which can protect this lipophilic compound against oxidative degradation in the upper gastrointestinal tract (Mortensen et al. 2001). This may explain the lowest β -carotene concentration recorded in group III, suggesting that a greater proportion of this carotenoid was utilized for antioxidant protection.



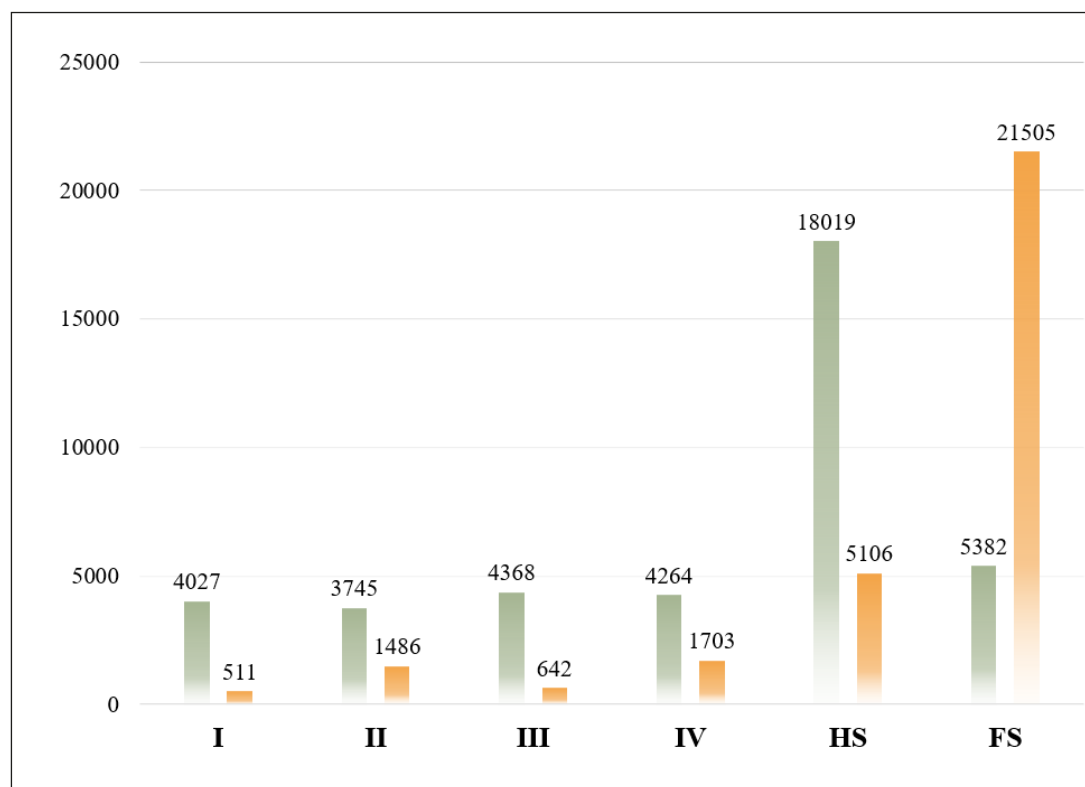
Graph 1: Concentrations of γ -tocopherol (mg/kg fresh weight) in the experimental diets (I, II, III, and IV), HS, and FS

Fatty Acid Composition and Cholesterol Concentrations in Egg Yolks

From a nutritional perspective, pronounced beneficial changes were observed in the lipid profile of egg yolks. Both the individual seed supplements (HS and FS) and, in particular, their combination (HS*FS) increased the content of n -6 ($p = 0.022$) and n -3 ($p < 0.001$) PUFAs. The highest total PUFA content in group IV, receiving combined HS and FS supplementation, was mainly driven by a marked

increase in ALA, with the highest level observed in group IV (866 mg/100 g), reflecting the strong *n*-3 PUFA enrichment effect of FS ($p < 0.001$). According to our GC-FID analysis (Graph 2), FS contained a 4.2-fold higher concentration of *n*-3 PUFAs than HS did (21505 mg/100 g fresh weight vs. 5106 mg/100 g fresh weight, respectively). ALA, the principal *n*-3 PUFA, is an essential nutrient for both birds and mammals, as it cannot be synthesized *de novo* and must be supplied through the diet. Through desaturation and elongation pathways, ALA serves as a precursor for long-chain FAs such as EPA and DHA, which are important for multiple physiological processes and overall health in both animals and humans (Jing et al. 2017; Farinon et al. 2020). The enrichment of eggs with *n*-3 PUFAs through dietary FS and HS supplementation has been widely documented in poultry. FS (Cherian and Quezada 2016) and HS (Rbah et al. 2025) have both been shown to increase ALA deposition in egg yolks. Moreover, Omri et al. (2019) demonstrated that dietary inclusion of FS at 45 g/kg altered yolk FA composition and increased EPA and DHA levels. In agreement with these findings, the present experiment showed that combined HS and FS supplementation in the diet (IV) of laying hens markedly enhanced the deposition of *n*-3 PUFAs in egg yolks, with ALA, EPA, and DHA levels being 2.2-, 1.8-, and 1.6-fold higher than in the control group (I), respectively. The conversion of ALA to EPA and DHA in both animals and humans depends on a balanced *n*-6/*n*-3 PUFA ratio; otherwise, metabolism is shifted toward arachidonic acid (AA, C20:4, *n*-6) synthesis from linolenic acid (LA, C18:2, *n*-6). In general, lipid mediators derived from *n*-3 PUFAs tend to be less pro-inflammatory and more pro-resolving, whereas those originating from *n*-6 PUFAs are typically more pro-inflammatory (de Batlle et al. 2012). Therefore, beyond absolute PUFA levels, the *n*-6/*n*-3 PUFA ratio is a key nutritional indicator associated with the prevention of chronic degenerative diseases (Simopoulos 2008). In the present study, the *n*-6/*n*-3 PUFA ratio in egg yolks decreased from 8.31 (I) to 5.44 (IV). This result was significantly affected by both HS ($p < 0.001$) and FS ($p < 0.001$) supplementation. In previous studies, similar reductions have been reported for both HS- and FS-based dietary interventions. For example, Taaifi et al. (2023) reported a decrease in the *n*-6/*n*-3 PUFA ratio from 8.19 in the control group to 4.89 in laying hens fed 300 g/kg HS by day 84. Consistently, numerous studies have shown that FS and HS supplementation improve yolk lipid profiles and enhance *n*-3 PUFA enrichment in line with current nutritional recommendations.

All dietary treatments also affected nutritional lipid quality indices, namely the IA and IT ($p < 0.001$). These indices are widely used indicators for evaluating the nutritional quality of lipids, including egg yolk lipids, in relation to human health. The IA reflects the atherogenic potential of FAs, indicating their capacity to promote the formation of atherosclerotic plaques in arteries (Ulbricht and Southgate 1991). In contrast, the IT characterizes the thrombogenic potential of FAs, reflecting the tendency to promote clot formation in blood vessels and the balance between pro-thrombogenic FAs (mainly SFAs) and anti-thrombogenic FAs (mainly MUFAs and PUFAs; Ulbricht and Southgate 1991). Consumption of foods



Graph 2: Concentrations of *n*-6 and *n*-3 PUFAs (mg/100 g fresh weight) in the experimental diets (I, II, III, and IV), HS, and FS

with lower IA and IT values has been associated with reduced total and low-density lipoprotein (LDL) cholesterol concentrations in human blood plasma, thereby contributing to improved cardiovascular health (Chen and Liu 2020). Although the IA and IT values in all experimental groups remained below the recommended thresholds (0.5 and 1.0, respectively), their further reduction ($p < 0.001$) in the FS-supplemented groups (II and IV) indicates improved nutritional quality of egg yolk lipids. The lowest IA (0.321) and IT (0.632) values observed in group IV may suggest a synergistic effect of combined HS and FS supplementation (HS*FS; $p < 0.001$). In agreement with our findings, Omri et al. (2019) reported that FS-supplemented diets significantly reduced the IT value. Similarly, Mierliță (2019) observed that dietary inclusion of HS and HS meal decreased the IT value in egg yolks, although no significant effect on IA was detected.

Finally, HS supplementation significantly reduced the total cholesterol content in egg yolks ($p = 0.042$). This finding is consistent with the study by Skřivan et al. (2019), who reported a reduction in egg yolk cholesterol from 12.2 g/kg in the control group to 10.6 g/kg in laying hens fed a diet containing 90 g/kg HS. According to Rbah et al. (2025), the hypocholesterolemic effect of HS is likely associated with several components of its chemical composition, particularly phytosterols (e.g., β -sitosterol), proteins, and insoluble fiber.

Oxidative Stability of Egg Yolks

Lipid peroxidation, a process compromising the nutritional and sensory quality of eggs, was evaluated by determining MDA concentrations in egg yolks. MDA, one of the major oxidation products of PUFAs, is widely recognized as a reliable marker of lipid peroxidation in food products. In fresh eggs, HS supplementation significantly reduced MDA levels in egg yolks ($p = 0.003$), indicating improved oxidative stability of yolk lipids. A similar effect was reported by Mierliță (2019), who observed the lowest MDA concentrations in egg yolks from laying hens fed HS (0.22 mg/kg in fresh eggs and 0.35 mg/kg in eggs after 30 days of storage). According to the author, the unexpectedly low degree of lipid peroxidation despite the elevated n -3 PUFA content may be associated with the relatively high concentration of tocopherols, particularly α -tocopherol, in egg yolks. A similar explanation may also apply to our findings, although HS supplementation in the present study specifically resulted in a marked increase in γ -tocopherol concentration in egg yolks. This tocopherol isomer, the predominant form of vitamin E in HS (Kriese et al. 2004), is considered a highly potent antioxidant in lipophilic matrices (Porto et al. 2014). It may effectively protect n -3 PUFAs against peroxidation, thereby contributing to enhanced oxidative stability of the produced eggs (Es-Sai et al. 2025).

Tab. 1: Effects of dietary HS and FS supplementation on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability (n = 6)

Group (diet)	I	II	III	IV	SEM	Probability		
Hemp seed (%)	0	0	4	4		HS	FS	HS*FS
Flax seed (%)	0	4	0	4				
Hen-day egg production (%)	94.3	95.7	94.0	96.2	0.24	NS	<0.001	NS
Feed conversion ratio (kg/kg)	2.17 ^a	2.09 ^c	2.12 ^{bc}	2.14 ^{ab}	0.008	NS	NS	<0.001
Egg mass (g/hen/day)	57.1	59.1	58.7	59.9	0.18	<0.001	<0.001	NS
Egg weight (g)	60.5 ^c	61.7 ^b	62.4 ^a	62.3 ^{ab}	0.12	<0.001	0.017	0.002
DSM yolk color fan	13.5 ^b	13.7 ^a	13.6 ^a	13.6 ^{ab}	0.02	NS	NS	0.014
Shell percentage (%)	10.2 ^{ab}	10.2 ^{ab}	10.1 ^b	10.3 ^a	0.03	NS	NS	0.036
α-tocopherol (mg/kg DM)	181	171	183	184	2.7	NS	NS	NS
γ-tocopherol (mg/kg DM)	54.0	49.0	61.5	62.1	1.45	<0.001	NS	NS
β-carotene (mg/kg DM)	0.387 ^a	0.332 ^{ab}	0.312 ^b	0.348 ^{ab}	0.0107	NS	NS	0.029
LA (mg/100 g)	5901 ^c	6435 ^b	6420 ^b	7313 ^a	97.8	<0.001	<0.001	0.023
ALA (mg/100 g)	394 ^d	635 ^b	463 ^c	866 ^a	33.2	<0.001	<0.001	<0.001
EPA (mg/100 g)	6.09 ^c	7.45 ^b	7.61 ^b	11.24 ^a	0.360	<0.001	<0.001	<0.001
DHA (mg/100 g)	330	403	399	516	13.1	<0.001	<0.001	NS
n-6 PUFAs (mg/100 g)	6495 ^c	7004 ^b	7082 ^b	7991 ^a	104.4	<0.001	<0.001	0.022
n-3 PUFAs (mg/100 g)	782 ^d	1114 ^b	943 ^c	1472 ^a	46.8	<0.001	<0.001	<0.001
n-6/n-3 PUFA ratio	8.31	6.29	7.51	5.44	0.201	<0.001	<0.001	NS
Index of atherogenicity	0.362 ^a	0.350 ^b	0.362 ^a	0.321 ^c	0.0033	<0.001	<0.001	<0.001
Index of thrombogenicity	0.792 ^a	0.712 ^b	0.778 ^a	0.632 ^c	0.0117	<0.001	<0.001	<0.001
Cholesterol (g/kg)	12.0	11.2	11.1	11.0	0.15	0.042	NS	NS
MDA – fresh eggs (mg/kg)	0.564	0.512	0.429	0.403	0.0508	0.003	NS	NS
MDA – stored eggs (mg/kg) ¹	0.603	0.530	0.509	0.528	0.0237	NS	NS	NS

SEM = standard error of the mean; HS = hemp seed; FS = flax seed; HS*FS = interaction between hemp seed and flax seed; NS = not significant; ^{a,b,c,d} in the same row indicate significant differences; DM = dry matter; LA = linoleic acid (C18:2, n-6); ALA = α-linolenic acid (C18:3, n-3); EPA = eicosapentaenoic acid (C20:5, n-3); DHA = docosahexaenoic acid (C22:6, n-3); PUFAs = polyunsaturated fatty acids; MDA = malondialdehyde; ¹ indicates the oxidative stability of egg yolks from eggs stored for 28 days.

CONCLUSION

In conclusion, the present study demonstrated that dietary supplementation with HS and FS significantly enhanced the nutritional profile and oxidative stability of eggs. While FS primarily served as a rich source of *n*-3 PUFAs, particularly ALA, and improved laying hen performance, HS supplementation effectively elevated γ -tocopherol concentrations in egg yolks, reduced yolk cholesterol content, and decreased lipid peroxidation. Importantly, the combined supplementation with HS and FS exerted a synergistic effect, characterized by a higher shell percentage and increased deposition of *n*-3 PUFAs, especially ALA and EPA, in egg yolks, resulting in the most favorable *n*-6/*n*-3 PUFA ratio and improved nutritional lipid quality indices. These findings highlight the potential of combined HS and FS supplementation as a novel nutritional strategy for producing eggs enriched with health-promoting bioactive compounds and improved lipid quality, thereby supporting the development of value-added poultry products.

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