

Modulation of Egg Yolk Lipid Composition in Laying Hens under Dietary Administration of High-Protein Sunflower Concentrate

Mykhailo Sychov, Ihor Ilchuk*, Yulia Osadcha, Vladyslav Pitera, Roman Vozniuk

National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources,
P.D. Pshenychnyi Department of Animal Nutrition and Feed Technology, Kyiv, Ukraine

Article Details: Received: 2025-09-25 | Accepted: 2026-03-06 | Available online: 2026-09-30

<https://doi.org/10.15414/afz.2026.29.03.270-279>



Licensed under a Creative Commons Attribution 4.0 International License



Polyunsaturated fatty acids (PUFAs) are critical biomolecules that underpin a wide range of physiological and metabolic functions in both human and animal systems. Among them, linoleic acid and α -linolenic acid are recognized as essential for humans due to the absence of endogenous biosynthetic pathways, thereby necessitating dietary intake. A practical approach to ensuring sufficient PUFA consumption involves the inclusion of functional foods enriched with these compounds, such as eggs. With increasing scientific and consumer awareness regarding the health-promoting properties of omega-3 fatty acids, the market demand for eggs fortified with omega-3 and omega-6 fatty acids is expected to expand further, presenting both new opportunities and technological challenges for the poultry industry. The present study was designed to evaluate the influence of dietary supplementation with high-protein sunflower concentrate, as a partial replacement for soybean meal, on the lipid composition and cholesterol content of egg yolk in laying hens. Results demonstrated that incorporation of sunflower concentrate into the diet altered the lipidomic profile of egg yolk. Specifically, feeding trials revealed increases in oleic acid (+5.29%), linolenic acid (+3.35%), and linoleic acid (+0.09%), coupled with a significant reduction in palmitic acid levels (-7.91%). Conversely, the concentrations of myristic, palmitoleic, stearic, vaccenic, arachidonic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids remained unaffected. Egg yolks derived from hens provided with a diet enriched in high-protein sunflower concentrate demonstrated an 11.6% decrease in total cholesterol, accompanied by rises of 21.8% in vitamin E and 9.9% in calcium content.

Keywords: protein concentrate; hens; lipid profile

1 Introduction

Polyunsaturated fatty acids (PUFAs) constitute a heterogeneous group of lipids that exert indispensable physiological functions in both human and animal organisms. Of particular importance are linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3), which are recognized as essential in adult human nutrition due to the absence of endogenous biosynthetic pathways. Consistent evidence from epidemiological and clinical studies indicates that dietary substitution of saturated fatty acids with polyunsaturated counterparts confers cardioprotective effects and reduces the risk of metabolic disorders such as obesity, insulin resistance, and dyslipidemia (Huether et al., 2025; Nilsen et al., 2025). Within this nutritional framework, omega-3 and omega-6 fatty acids are integral to maintaining cellular membrane

structure and functionality, with omega-3 derivatives such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibiting well-documented benefits for cardiovascular health, neurocognitive performance, and inflammatory modulation. Conversely, omega-6 fatty acids play a central role in intracellular signaling pathways and immunological regulation, contributing significantly to anti-inflammatory responses (Zamani et al., 2024).

Given the growing demand for functional foods enriched with bioactive lipids, research has increasingly focused on feed strategies capable of modulating the fatty acid composition of animal-derived products. Sunflower meal concentrates, a co-product of industrial sunflower oil extraction, has emerged as a promising feed ingredient in this context, primarily due to its substantial content

***Corresponding Author:** Ihor Ilchuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, P.D. Pshenychnyi Department of Animal Nutrition and Feed Technology, Horikhuvatskyi shliakh 19 str., 03041 Kyiv, Ukraine
e-mail: ilchukigor@nubip.edu.ua ORCID <https://orcid.org/0000-0003-0961-6613>

of bound phosphates and sulfates and its dual role as a protein and dietary fiber source (Lira-Ricárdez et al., 2024). Its incorporation into poultry diets has been associated not only with reduced feed costs but also with lower emissions of ammonia (NH_3) and hydrogen sulfide (H_2S) from manure, thereby conferring both economic and environmental advantages (Vidosavljević et al., 2025). Experimental studies have demonstrated that inclusion levels ranging from 100 to 150 $\text{g}\cdot\text{kg}^{-1}$ exert no adverse effects on digestive physiology, growth performance, or egg quality (Ruan et al., 2022). Furthermore, in laying hens, dietary supplementation with sunflower concentrate has been shown to enhance egg quality through yolk enrichment with polyunsaturated fatty acids (PUFAs) and concurrent reductions in yolk cholesterol content (Saleh et al., 2021). Consequently, sunflower meal concentrate not only represents a sustainable alternative protein source but also holds considerable potential in the development of nutritionally enhanced poultry products tailored to meet contemporary human health needs.

Current international dietary frameworks stipulate that a daily intake of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) should not fall below 250 mg for the general population, an amount conventionally attained through the consumption of approximately two servings of oily marine fish (Mozaffarian & Wu, 2011). Augmentation of dietary PUFA availability can be achieved via two principal strategies:

- a) direct consumption of naturally occurring lipid-rich matrices, including marine-derived fish oils and plant-based sources such as flaxseed (Balić et al., 2020);
- b) incorporation of functional food products biofortified with omega-3 and omega-6 fatty acids.

Among the latter, hen's eggs represent an optimal delivery vehicle due to their widespread consumption, high bioavailability of lipids, and metabolic amenability to nutritional modulation.

Eggs enriched with omega-3 fatty acids have been extensively documented to exert hypocholesterolemic effects via modulation of hepatic cholesterol biosynthesis and lipoprotein metabolism, as well as systemic anti-inflammatory actions mediated through attenuation of pro-inflammatory eicosanoid synthesis. Such modifications translate into measurable benefits for cardiovascular homeostasis and neurocognitive performance, thereby classifying omega-3-enriched eggs as a nutraceutical intervention of clinical relevance (Usturoi et al., 2025). Empirical evidence indicates that dietary incorporation of flaxseed into layer rations can substantially increase yolk concentrations of alpha-linolenic acid ($\approx 200 \text{ mg}\cdot\text{egg}^{-1}$) and docosahexaenoic acid

($\approx 90 \text{ mg}\cdot\text{egg}^{-1}$), thereby enhancing both the nutritional density and functional value of eggs (Lemahieu et al., 2015). Equally critical to the nutraceutical efficacy of such biofortification is the regulation of the dietary omega-6/omega-3 ratio, a determinant of the equilibrium between pro-inflammatory and anti-inflammatory lipid mediators. Optimizing this ratio in accordance with contemporary nutritional guidelines is essential for aligning the biochemical profile of enriched eggs with current recommendations for cardiovascular and systemic metabolic health (Attia et al., 2024).

Fortification of table eggs with long-chain omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) is achieved through targeted nutritional modulation of laying hens' diets by incorporating lipid substrates enriched in these essential fatty acids (Djuricic & Calder, 2021). This biofortification strategy has garnered significant attention within both scientific and commercial domains owing to the escalating consumer demand for functional foods that provide physiologically active health benefits beyond conventional nutritional value. Empirical investigations have demonstrated that omega-3-enriched eggs exhibit markedly elevated concentrations of n-3 fatty acids compared with conventional eggs, with some studies documenting up to a twelve-fold increase in yolk omega-3 content (Ahmed & Abdelati, 2009). With the growing recognition of the cardioprotective, neurocognitive, and anti-inflammatory properties of omega-3 fatty acids, the consumer market for PUFA-enriched eggs is anticipated to expand further, creating both novel opportunities and technological challenges for producers in an increasingly dynamic agri-food sector (Usturoi et al., 2025).

The enrichment process predominantly relies on the incorporation of omega-3-rich feedstuffs such as flaxseed and canola oils, marine-derived fish oils, or algal biomass, all of which have been shown to significantly elevate yolk concentrations of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Filipiak-Florkiewicz et al., 2024). In addition, alternative oilseed substrates, including sunflower seeds and their derivatives, have been successfully utilized to remodel yolk lipidomic profiles, thereby modulating the ratio of saturated to unsaturated fatty acids and enhancing the overall nutraceutical value of eggs (Aguillón-Páez et al., 2020; Teneva et al., 2025).

Sunflower concentrate is also characterized by a substantial content of chlorogenic acid, approximately 26 $\text{g}\cdot\text{kg}^{-1}$, which accounts for nearly 700 $\text{g}\cdot\text{kg}^{-1}$ of the total phenolic fraction (Ruan et al., 2022). As a principal bioactive constituent, chlorogenic acid exhibits a broad spectrum of biological activities, including pronounced antioxidant, anti-inflammatory, antibacterial, antidiabetic,

and nutraceutical properties (Nguyen et al., 2024). *In vitro* assays have further demonstrated its superior radical-scavenging efficacy compared to α -tocopherol (vitamin E) (Liu et al., 2023), while *in vivo* applications have been associated with enhanced antioxidant status and improved qualitative parameters of poultry-derived products, including egg yolk and muscle tissue (Bi et al., 2024). Moreover, chlorogenic acid has been implicated in the modulation of key transcriptional regulators and enzymatic pathways governing lipid metabolism, thereby suggesting a mechanistic link between its dietary inclusion and metabolic remodeling at the systemic level (Nguyen et al., 2024).

Previous studies have evaluated the efficacy of high-protein sunflower concentrate in poultry nutrition. Shkarban and Sychov (2025) demonstrated that the complete and partial replacement of soybean meal with sunflower concentrate in broiler diets significantly improved growth performance, carcass characteristics, and meat quality parameters. In subsequent research,

Sychov et al. (2025) showed that the inclusion of this ingredient in laying hen diets optimised productive performance and improved egg quality traits.

Nevertheless, despite growing scientific interest, the influence of sunflower concentrate supplementation on the clinical biochemical indices of laying hen serum serving as biomarkers of systemic physiological status as well as its capacity to modulate the lipidomic architecture of egg yolk, remains insufficiently elucidated. This knowledge gap provided the rationale and primary objective of the present investigation.

2 Material and Methods

The biological material for this investigation comprised commercial egg-laying hens (*Gallus gallus domesticus*) of the industrial cross Lohmann Brown. The experimental protocol was developed and conducted in full accordance with the ethical principles outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and was consistent with prevailing institutional and national regulatory frameworks governing animal research.

During the trial, hens were maintained under controlled environmental conditions with unrestricted access (*ad libitum*) to potable water and a nutritionally balanced compound diet formulated to meet or exceed species-specific requirements. Housing, stocking density, microclimatic parameters, and general management practices adhered strictly to the standards specified (Lohmann Brown Management Guide, 2024).

Comprehensive descriptions of husbandry conditions, environmental parameters, and the detailed proximate and nutrient composition of the experimental rations are presented in Tables 1–3.

Table 1 Experimental housing and management conditions of laying hens

Characteristic	Group of chickens	
	1 (control)	2
Number of laying hens	120	120
Cage dimensions:		
– length (cm)	60	
– depth (cm)	62	
– area (cm ²)	3,720	
Chickens are placed in 1 cage, number	10	
Area availability (cm ² ·hen ⁻¹)	372.0	
Nipples in the cage (pcs.)	1	
Feeding front (cm)	7.1	

Table 2 Ingredient composition of experimental diets for laying hens (% , as-fed basis)

Ingredient	Pre-laying		Laying 1		Laying 2		Laying 3	
	1	2	1	2	1	2	1	2
Corn	50.380	52.793	50.540	51.102	52.506	52.718	55.208	55.263
Sunflower concentrate CP44	–	25.764	–	23.553	–	21.757	–	18.971
Soybean meal CP 45	17.817	–	23.700	–	21.822	–	18.295	–
Wheat	15.000	15.000	15.000	15.000	15.000	15.000	15.000	15.000
Limestone flour Ca 36%	1.332	1.443	5.160	5.345	5.338	5.525	5.586	5.766
Sunflower meal CP35	10.471	–	–	–	–	–	0.911	–
Soybean premix	5.000	–	5.000	–	5.000	–	5.000	–
Premix concentrate	–	5.000	–	5.000	–	5.000	–	5.000
Soybean oil	–	–	0.600	–	0.334	–	–	–
Total	100	100	100	100	100	100	100	100

Table 3 Chemical and nutritional profile of experimental diets for laying hens

Indicator	Pre-laying		Laying 1		Laying 2		Laying 3	
	1	2	1	2	1	2	1	2
Energy (kcal)	2,700	3,014	2,725	2,902	2,725	2,903	2,725	2,911
Crude protein (%)	17.5	17.5	16.5	16.5	15.8	15.8	14.8	14.8
Crude fat (%)	2.52	2.49	2.99	2.4	2.78	2.44	2.51	2.49
Crude fibre (%)	4.03	3.55	2.39	3.33	2.35	3.23	2.44	3.06
Calcium (%)	2.2	2.2	3.57	3.57	3.67	3.67	3.75	3.75
Phosphorus (%)	0.4	0.4	0.37	0.37	0.34	0.34	0.32	0.32
Sodium (%)	0.17	0.17	0.16	0.17	0.15	0.17	0.15	0.17
Chlorine (%)	0.25	0.32	0.217	0.333	0.2	0.32	0.21	0.32
Digestible lysine (%)	0.7	0.7	0.71	0.71	0.67	0.67	0.64	0.64
Digestible methionine and cystine (%)	0.63	0.63	0.64	0.64	0.6	0.6	0.58	0.58
Digestible threonine (%)	0.533	0.5	0.5	0.5	0.48	0.47	0.45	0.45
Choline (mg·kg ⁻¹)	600	600	600	600	600	600	600	600

The high-protein sunflower concentrate employed in the present study was derived from sunflower (*Helianthus annuus* L.) cultivated in Ukraine. In the experimental group, soybean meal was completely replaced (100%) with high-protein sunflower concentrate throughout all productive periods of laying hens, whereas the control group received conventional soybean meal-based diets. The high-protein sunflower concentrate used in the present study is characterized by a reduced crude fibre content (not exceeding 8%) and an elevated crude protein content (not less than 47% on a dry matter basis). The proximate chemical composition of the concentrate was analyzed using the classical Weende system of feed analysis, and the results are presented in Table 4.

The experimental trial was conducted over a period of 44 weeks, spanning from the onset of lay (18 weeks of age) to 62 weeks of age in Lohmann Brown hens (Table 5). At the termination of the feeding trial (62 weeks of age), six individual whole blood samples (1.0–1.5 mL, obtained from the axillary vein) were collected from randomly selected hens in each treatment group.

Blood samples were processed for serum separation, and a comprehensive panel of biochemical and enzymatic indicators was determined using an automated biochemical analyser (BioChem FC-360, Hightechnology Inc.) at the accredited diagnostic laboratory “Bald” (Certificate No. LB/02/2016).

The following biochemical constituents and enzyme activities were analyzed: total protein, albumin, glucose, creatinine, urea, bilirubin, cholesterol, inorganic phosphorus, calcium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH). Quantification of serum analyses was performed with commercial diagnostic kits (High Technology Inc., USA), applying the following standardized methodologies: glucose – HT-G242 (oxidase method, endpoint, $\lambda = 500$ nm); total protein – HT-T251 (biuret method, endpoint, $\lambda = 540$ nm); albumin –

Table 4 Chemical and proximate composition of high-protein sunflower concentrate (g·kg⁻¹)

Indicator	Dry matter content
Crude protein	474.5 ± 0.72
Crude fat	10.5 ± 0.06
Crude fibre	79.8 ± 0.52
Raw ash	73.7 ± 0.31
NFE	361.5 ± 1.11
NDF	241.6 ± 0.97
ADF	84.9 ± 0.26
Lignin	11.3 ± 0.09

Table 5 Blood serum biochemical indices of laying hens ($n = 6/\text{group}$)

Indicator	Group of laying hens	
	1 (control)	2
Total protein (g·l ⁻¹)	56.2 ± 1.13 ^a	59.8 ± 1.32 ^b
Albumin (g·l ⁻¹)	18.7 ± 0.56 ^a	18.9 ± 0.73 ^a
Glucose (mmol·l ⁻¹)	19.7 ± 0.67 ^a	20.1 ± 0.59 ^a
Creatinine (μmol·l ⁻¹)	27.1 ± 1.04 ^a	26.7 ± 0.96 ^a
Uric acid (mmol·l ⁻¹)	228.7 ± 6.44 ^a	248.2 ± 7.1 ^b
Bilirubin (μmol·l ⁻¹)	1.93 ± 0.045 ^a	1.97 ± 0.052 ^a
Cholesterol (mmol·l ⁻¹)	4.4 ± 0.04 ^a	3.6 ± 0.03 ^b
Phosphorus (mmol·l ⁻¹)	2.47 ± 0.052 ^a	2.43 ± 0.068 ^a
Calcium (mmol·l ⁻¹)	4.54 ± 0.078 ^a	4.56 ± 0.064 ^a

a, b – differences are significant ($p < 0.05$) within the indicator

HT-A203 (bromocresol green method, endpoint, $\lambda = 630$ nm); creatinine – HT-C225 (Jaffe reaction, kinetic, $\lambda = 510$ nm); urea – HT-U254 (Trinder/uricase method, endpoint, $\lambda = 520$ nm); cholesterol – HT-C218 (enzymatic method, endpoint, $\lambda = 500$ nm); phosphorus – HT-P244 (ammonium molybdate method, endpoint, $\lambda = 340$ nm); calcium – HT-C216 (OKF method, endpoint, $\lambda = 570$ nm); alanine aminotransferase – HT-A206 (IFCC, kinetic, $\lambda = 340$ nm); aspartate aminotransferase – HT-A109 (IFCC, kinetic, $\lambda = 340$ nm); alkaline phosphatase – HT-A205 (kinetic, $\lambda = 405$ nm); lactate dehydrogenase – HT-L236 (modified Wacker/Tris method, kinetic, $\lambda = 340$ nm); gamma-glutamyltransferase – HTI-G7571-120 (modified Szasz method, kinetic, $\lambda = 405$ nm). Concentrations of biochemical components were calculated in accordance with the manufacturer's instructions.

At the end of the laying period, six representative eggs from each experimental group were collected for lipidomic evaluation of yolk composition. The following fatty acids were quantified: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), vaccenic (C18:1 trans-11), linoleic (C18:2 n-6), α -linolenic (C18:3 n-3), arachidonic (C20:4 n-6), eicosapentaenoic (EPA, C20:5 n-3), docosapentaenoic (DPA, C22:5 n-3), and docosahexaenoic acids (DHA, C22:6 n-3), in addition to total yolk fat and cholesterol concentrations. Fatty acid methyl esters (FAMES) were prepared according to standard protocols and subsequently analysed by gas chromatography (Shimadzu GC-4 CM, PFE) equipped with a flame ionization detector (FID).

For quantification of vitamin E and calcium, pooled yolk samples were homogenized in 0.054 mol/L sodium phosphate dibasic buffer (adjusted to pH 7.0 with HCl). Following extraction with absolute ethanol and hexane, the upper α -tocopherol phase was evaporated under nitrogen and reconstituted in ethanol prior to analysis by high-performance liquid chromatography (HPLC) with UV detection ($\lambda = 290$ nm). Total cholesterol content of yolk was determined via lipid extraction with a chloroform-methanol mixture (2 : 1, v/v), followed by spectrophotometric quantification in accordance with established protocols.

All data were analysed using a general linear model (GLM) framework to evaluate the effects of dietary treatment on measured parameters. One-way analysis of variance (ANOVA) was employed to test for overall group differences, with the Tukey-Kramer procedure applied as a post-hoc multiple comparison method to adjust for pairwise contrasts. In addition, effect sizes (η^2) were calculated to assess the magnitude of treatment effects.

The assumptions of parametric testing were verified prior to analysis. Normality of residual distributions was assessed

using the Kolmogorov-Smirnov test, and homogeneity of variances was evaluated with Levene's test. In instances where the data deviated significantly from normal distribution, or variance homogeneity was not satisfied, the non-parametric Mann-Whitney U test was employed as a robust alternative.

Where appropriate (e.g., for repeated measurements across laying periods), data were analysed using repeated-measures ANOVA within the GLM framework to account for within-subject variability. All results are expressed as mean $\pm$ standard error of the mean (SEM). Differences between groups were considered statistically significant at $p < 0.05$.

All statistical analyses were performed using SPSS Statistics v.29.0 (IBM Corp., Armonk, NY, USA) and validated by confirmatory runs in R software (version 4.3.2, R Core Team, Vienna, Austria). Graphical visualizations were generated using GraphPad Prism 10.0 (GraphPad Software, San Diego, CA, USA).

3 Results and Discussion

The biochemical parameters of blood serum, considered as markers of the physiological status of laying hens, exhibited measurable alterations in response to dietary supplementation with high-protein sunflower concentrate (Table 5). The observed increase in serum total protein (59.8 vs. 56.2 g·L⁻¹; $p < 0.05$) indicates an intensification of protein metabolism and likely reflects an improved amino acid supply associated with the inclusion of high-protein sunflower concentrate in the diet. Concurrently, a significant elevation in serum uric acid concentration was recorded (248.2 vs. 228.7 μ mol·L⁻¹; $p < 0.05$). Given that uric acid represents the principal end-product of nitrogen metabolism in avian species, its increase may reflect not only enhanced amino acid turnover but also an intensification of nitrogen catabolism. Importantly, the measured values remained within the physiological reference range for laying hens and were not accompanied by alterations in hepatic enzyme activity, suggesting the absence of pathological impairment. Therefore, the observed changes may be interpreted as an adaptive metabolic response to dietary modification; however, under long-term feeding conditions, the potential for increased nitrogen load should be taken into consideration.

The significant reduction in serum cholesterol (-18.2%) is consistent with previously described hypocholesterolemic effects of polyunsaturated fatty acids and phenolic compounds. High-protein sunflower concentrate is particularly rich in linoleic acid and chlorogenic acid, both of which have been implicated in the regulation of lipid metabolism. Linoleic acid

Table 6 Serum enzymatic activity in laying hens ($n = 6/\text{group}$)

Indicator (units·l ⁻¹)	Group of laying hens	
	1 (control)	2
ALT	3.1 ± 0.05 ^a	3.2 ± 0.04 ^a
AST	211.6 ± 2.13 ^a	214.4 ± 2.34 ^a
GGT	29.2 ± 1.17 ^a	29.9 ± 0.97 ^a
ALP	916.4 ± 21.36 ^a	922.7 ± 22.15 ^a
LDH	2136.4 ± 27.89 ^a	2095.6 ± 21.38 ^a

a, b – differences are significant ($p < 0.05$) within the indicator

serves as a ligand for peroxisome proliferator-activated receptors (PPAR α/γ), which stimulate β -oxidation of fatty acids and downregulate hepatic cholesterol synthesis. Concurrently, chlorogenic acid has been shown to inhibit the activity of hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase), the rate-limiting enzyme of cholesterol biosynthesis, while also modulating sterol regulatory element-binding proteins (SREBP-1c, SREBP-2).

Collectively, these biochemical shifts suggest that inclusion of sunflower concentrate in layer diets exerts a dual effect:

- modulation of protein and nitrogen metabolism through improved protein supply and utilization;
- remodeling of lipid metabolism toward a more favorable profile, mediated via both fatty acids signaling pathways and bioactive phytochemicals.

Such metabolic remodeling may contribute to improved systemic homeostasis, enhanced productivity, and the production of eggs with a healthier lipid profile.

No differences in the activity of serum enzymes were observed in laying hens in response to the administration of a high-protein sunflower concentrate (Table 6). The absence of significant changes in serum enzyme activities (ALT, AST, GGT, ALP, LDH) indicates that dietary inclusion of high-protein sunflower concentrate did not adversely affect hepatic or renal function in laying hens. The stability of enzymatic markers suggests that the observed metabolic responses, including alterations in lipid and protein metabolism, reflected adaptive and beneficial processes rather than pathological changes. Furthermore, the antioxidant potential of sunflower-derived bioactive compounds, such as chlorogenic acid and α -tocopherol, may have contributed to maintaining cellular integrity and preventing oxidative stress-induced enzyme leakage into the bloodstream.

Polyunsaturated fatty acids (PUFAs) represent a heterogeneous group of fatty acids that exert essential physiological functions, among which linoleic acid (C18:2 n-6) and α -linolenic acid (C18:3 n-3) are considered indispensable for human health due to the lack of de novo biosynthetic capacity (Aguillón-Páez et al., 2020). In avian species, and particularly in laying hens, the fatty acid composition of egg yolk is determined by the complex interplay between hepatic de novo lipogenesis, intestinal absorption of dietary

Table 7 Egg yolk lipid profile of laying hens (% , $n = 6/\text{group}$)

Indicator	Group of laying hens	
	1 (control)	2
Myristic acid (C14:0)	0.23 ± 0.028 ^a	0.22 ± 0.026 ^a
Palmitic acid (C16:0)	27.73 ± 1.143 ^a	19.82 ± 1.095 ^b
Palmitoleic acid (C16:1)	2.86 ± 0.432 ^a	2.62 ± 0.511 ^a
Stearic acid (C18:0)	8.31 ± 0.476 ^a	7.86 ± 0.383 ^a
Oleic acid (C18:1 n-9c)	41.67 ± 1.362 ^a	46.96 ± 1.499 ^b
Vaccenic acid (C18:1 n-7)	1.94 ± 0.121 ^a	1.90 ± 0.098 ^a
Linoleic acid (C18:2 n-6)	13.96 ± 0.984 ^a	17.31 ± 1.123 ^b
Linolenic acid (C18:3 n-3)	0.43 ± 0.011 ^a	0.52 ± 0.013 ^b
Arachidonic acid (C20:4 n-6)	1.83 ± 0.113 ^a	1.79 ± 0.098 ^a
Eicosapentaenoic acid (C20:5 n-3)	0.026 ± 0.001 ^a	0.025 ± 0.001 ^a
Docosapentaenoic acid (C22:5n-3)	0.115 ± 0.009 ^a	0.112 ± 0.011 ^a
Docosahexaenoic acid (C22:6n-3)	0.899 ± 0.016 ^a	0.863 ± 0.017 ^a
Total fat (g·100 g yolk ⁻¹)	29.34 ± 0.98 ^a	29.08 ± 1.14 ^a
Total cholesterol (mg·100 g yolk ⁻¹)	136.96 ± 2.87 ^a	121.11 ± 3.11 ^b
Vitamin E (mg·100 g ⁻¹)	4.86 ± 0.010 ^a	5.92 ± 0.012 ^b
Calcium (mg·100 g ⁻¹)	0.724 ± 0.004 ^a	0.796 ± 0.005 ^b

a, b – differences are significant ($p < 0.05$) within the indicator

lipids, and the subsequent hepatic re-esterification and transport of lipoproteins to the developing oocyte (Hu et al., 2024). Consequently, dietary modulation represents a powerful tool to alter yolk lipid composition and to enrich eggs with polyunsaturated fatty acids, thereby enhancing their functional food value (Attia et al., 2024).

The findings of the present investigation (Table 7) demonstrated that partial replacement of soybean meal with high-protein sunflower concentrate significantly modified the yolk fatty acid profile. Specifically, dietary inclusion of sunflower concentrate resulted in a statistically significant ($p < 0.05$) increase in oleic acid content by 5.29%, α -linolenic acid by 3.35%, and linoleic acid by 0.09%, while simultaneously reducing palmitic acid concentration by 7.91% compared with yolks derived from hens fed conventional soybean meal-based diets. These shifts in fatty acid proportions indicate that the substitution of soybean meal with sunflower concentrate promotes enrichment of yolk lipids in monounsaturated and polyunsaturated fatty acids while reducing the proportion of saturated fatty acids. From a nutritional perspective, such changes are considered favorable, as they align yolk lipid composition more closely with contemporary recommendations for human dietary fat intake and may contribute to improved cardiovascular health outcomes in consumers.

The enrichment of egg yolk with oleic acid (+5.29%) observed in the present study can be attributed, at least in part, to the high intrinsic content of oleic acid in sunflower-derived feed ingredients. Moreover, sunflower bioactives, particularly chlorogenic acid, may influence hepatic lipid metabolism through modulation of sterol regulatory element-binding proteins (SREBP-1c) and peroxisome proliferator-activated receptors (PPAR γ). Upregulation of $\Delta 9$ -desaturase activity in hepatocytes, which catalyzes the conversion of stearic acid (C18:0) into oleic acid (C18:1 n-9), represents a plausible biochemical mechanism contributing to the increased yolk oleic acid deposition.

The elevated α -linolenic acid content (+3.35%) in egg yolk can be explained by the presence of precursor fatty acids in the sunflower concentrate as well as by enhanced hepatic absorption and subsequent re-esterification into yolk triacylglycerols and phospholipids. The slight but significant increase in linoleic acid (+0.09%) indicates that even modest contributions of dietary n-6 PUFA can be effectively transferred into egg yolk lipids.

Conversely, the marked reduction in palmitic acid (-7.91%) likely reflects the downregulation of hepatic de novo lipogenesis. Palmitic acid is the primary product of fatty acid synthase (FASN), and its decreased proportion

in yolk lipids may be linked to both substrate dilution by unsaturated fatty acids and the inhibitory action of chlorogenic acid on lipogenic transcription factors, including SREBP-1c. Such an effect is consistent with reports of reduced lipogenesis and improved lipid quality in poultry diets supplemented with polyphenol-rich feedstuffs.

From a nutraceutical perspective, the remodeling of yolk lipid composition toward higher proportions of oleic and α -linolenic acids and lower saturated fatty acids represents a health-promoting shift. Oleic acid has been associated with favorable effects on plasma lipid profiles, while α -linolenic acid is a precursor for long-chain n-3 fatty acids (EPA, DHA), known for their anti-inflammatory and cardioprotective roles. Thus, dietary incorporation of high-protein sunflower concentrate not only modifies yolk lipid composition through direct nutrient transfer but also through modulation of hepatic metabolic pathways governing lipid synthesis, desaturation, and partitioning.

The dietary inclusion of high-protein sunflower concentrate did not exert a significant influence on the concentrations of myristic, palmitoleic, stearic, vaccenic, arachidonic, eicosapentaenoic (EPA), docosapentaenoic (DPA), or docosahexaenoic acids (DHA) in egg yolk, indicating that these fatty acid classes are less responsive to dietary modulation under the conditions of the present study. Notably, although total yolk lipid content remained unchanged, substitution of soybean meal with sunflower concentrate significantly altered cholesterol metabolism. Specifically, yolk cholesterol concentration decreased by 15.85 mg·100 g⁻¹ (-11.6%; $p < 0.05$) in the experimental group relative to controls. These findings are in agreement with previous reports (Saleh et al., 2021), which similarly documented hypocholesterolemic effects of sunflower meal substitution. From a nutritional standpoint, this outcome is of considerable consumer relevance, as hypercholesterolemia is a well-established risk factor for cardiovascular morbidity (Mohseni et al., 2023).

The mechanisms underlying the cholesterol-lowering effect of sunflower-derived feed ingredients appear multifactorial. One potential pathway involves attenuation of hepatic de novo lipogenesis, leading to reduced cholesterol biosynthesis (Saleh et al., 2021). Additionally, sunflower seeds are rich in phytosterols, compounds structurally analogous to cholesterol, which competitively inhibit intestinal cholesterol absorption and thereby reduce systemic and yolk cholesterol levels (Liu et al., 2010). Dietary fibre, also present in sunflower concentrate, may further potentiate hypocholesterolemic effects through increased fecal bile acid excretion. Given that cholic and deoxycholic acids are synthesized in

the liver from cholesterol and conjugated with glycine and taurine (Fleishman & Kumar, 2024), enhanced bile acid turnover would increase cholesterol utilization and thereby lower yolk deposition.

Beyond lipid remodeling, high-protein sunflower concentrate contributed to the enrichment of egg yolk with key micronutrients. Sunflower seeds contain exceptionally high levels of α -tocopherol ($\approx 608 \text{ mg}\cdot\text{kg}^{-1}$), the most biologically active isoform of vitamin E (Saleh et al., 2021). Unlike γ - and δ -tocopherols, which predominate in soybean and cottonseed oils and exhibit higher thermal stability (Egea et al., 2023), α -tocopherol is more heat-sensitive but physiologically superior as an antioxidant. In the present study, hens fed sunflower concentrate-based diets produced eggs with a significantly higher yolk vitamin E content ($+1.06 \text{ mg}\cdot 100 \text{ g}^{-1}$, $+21.8\%$; $p < 0.05$), consistent with the antioxidant potential of sunflower bioactives.

Furthermore, sunflower meal is recognized as a nutritionally rich source of calcium, phosphorus, and B-group vitamins (Petraru et al., 2021; Puraikalan & Scott, 2023). Our results confirmed this nutritional advantage, demonstrating a significant increase in yolk calcium content by $0.072 \text{ mg}\cdot 100 \text{ g}^{-1}$ ($+9.9\%$; $p < 0.05$) in hens receiving high-protein sunflower concentrate. Collectively, these findings highlight the potential of sunflower-derived feed ingredients not only to improve the lipid quality of eggs but also to enhance their micronutrient density, thereby strengthening their role as functional foods of elevated nutritional and health-promoting value.

4 Conclusions

The present study demonstrates that replacing soybean meal with high-protein sunflower concentrate in the diets of laying hens significantly affects egg yolk composition and serum biochemical markers, thereby enhancing the nutritional and functional value of eggs. Dietary inclusion of sunflower concentrate resulted in a significant increase in the deposition of oleic acid ($+5.29\%$), α -linolenic acid ($+3.35\%$), and linoleic acid ($+0.09\%$), while reducing palmitic acid levels by 7.91% , leading to a more favorable fatty acid profile consistent with contemporary dietary recommendations for human health. Importantly, total yolk cholesterol content was reduced by 11.6% , reflecting a hypocholesterolemic effect likely mediated through the combined actions of polyunsaturated fatty acids, chlorogenic acid, plant sterols, and dietary fibre.

In parallel, hens receiving sunflower concentrate-based diets exhibited increased serum total protein and uric acid concentrations, as well as enhanced deposition

of vitamin E ($+21.8\%$) and calcium ($+9.9\%$) in egg yolk. These findings indicate not only improved egg quality but also enrichment of eggs with micronutrients of public health relevance. Notably, other fatty acids, including myristic, palmitoleic, stearic, vaccenic, arachidonic, eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids, remained unaffected by dietary treatment.

Overall, replacing soybean meal with high-protein sunflower concentrate represents a promising nutritional strategy for producing value-added eggs with reduced cholesterol content, improved fatty acid profile, and enhanced antioxidant and mineral levels, offering tangible benefits to both consumers and the poultry industry.

Acknowledgments

The research was carried out within the framework of the state scientific research project 04493706 No. 0124U000924.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

Mykhailo Sychoy and Ihor Ilchuk developed the research scheme and methodology, organized the experiment. Yulia Osadcha performed biometric data processing and wrote the manuscript. Vladyslav Pitera and Roman Voznyuk conducted the experiment, collected and processed the raw data. All authors read and agreed with the published version of the manuscript.

AI and AI-assisted technologies use declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

References

- Ahmed, M.E., & Abdelati, K.A. (2009). Effect of Dietary Graded Levels of *Leucaena leucocephala* Seeds on Layers Performance, Egg Quality and Blood Parameters. *International Journal of Poultry Science*, 8, 475–479. <https://doi.org/10.3923/ijps.2009.475.479>
- Aguillón-Páez, Y.J., Romero, L.A., & Diaz, G.J. (2020). Effect of full-fat sunflower or flaxseed seeds dietary inclusion on performance, egg yolk fatty acid profile and egg quality in laying hens. *Animal Nutrition*, 6(2), 179–184. <https://doi.org/10.1016/j.aninu.2019.12.005>
- Attia, Y., Al Sagan, A.A., Hussein, E.-S.O.S., Olal, M.J., Ebeid, T.A., Alabdullatif, A.A., Alhotan, R.A., Qaid, M.M., Tufarelli, V., Pugliese, G., Asiry, K.A., Nagadi, S.A., & Shehta, H.A. (2024). Enhancing the nutritional values of egg yolks of laying hens

by different dietary sources of omega-3 fatty acids, vitamin E and trace elements. *Livestock Science*, 289, article number 105573. <https://doi.org/10.1016/j.livsci.2024.105573>

Balić, A., Vlašić, D., Žužul, K., Marinović, B., & Bukvić Mokos, Z. (2020). Omega-3 versus omega-6 polyunsaturated fatty acids in the prevention and treatment of inflammatory skin diseases. *International journal of molecular sciences*, 21(3), 741. <https://doi.org/10.3390/ijms21030741>

Bi, R., Yang, M., Liu, X., Guo, F., Hu, Z., Huang, J., Abbas, W., Xu, T., Liu, W., & Wang, Z. (2024). Effects of chlorogenic acid on productive and reproductive performances, egg quality, antioxidant functions, and intestinal microenvironment in aged breeder laying hens. *Poultry Science*, 103(9), article number 104060. <https://doi.org/10.1016/j.psj.2024.104060>

Djuricic, I., & Calder, P.C. (2021). Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. *Nutrients*, 13, article number 2421. <https://doi.org/10.3390/nu13072421>

Egea, M.B., de Oliveira Filho, J.G., Bertolo, M.R.V., de Araújo, J.C., Gautério, G.V., & Lemes, A.C. (2023). Bioactive Phytochemicals from Sunflower (*Helianthus annuus* L.) Oil Processing By-products. In Ramadan Hassanien, M.F. (ed.), *Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products. Reference Series in Phytochemistry* (pp. 49–64). Springer. https://doi.org/10.1007/978-3-030-91381-6_4

Filipiak-Florkiewicz, A., Dymińska-Czyż, M., Szymczyk, B., Franczyk-Żarów, M., Kostogryś, R., Florkiewicz, A., & Lukasiewicz, M. (2024). Design of Physicochemical Properties of Eggs as a Result of Modification of the Fat Fraction of Laying Feed. *Molecules*, 29(6), article number 1242. <https://doi.org/10.3390/molecules29061242>

Fleishman, J.S., & Kumar, S. (2024). Bile acid metabolism and signaling in health and disease: molecular mechanisms and therapeutic targets. *Signal Transduction and Targeted Therapy*, 9, article number 97. <https://doi.org/10.1038/s41392-024-01811-6>

Huether, K.M., Lamb, J.M., Skelly, J., Brigham, E., McCormack, M.C., Bose, S., Garrow, O.J., & Dixon, A.E. (2025). Omega-3 fatty acid intake potentiates bronchodilator response in patients with obesity and poorly controlled asthma. *Respiratory Medicine*, 243, article number 108131. <https://doi.org/10.1016/j.rmed.2025.108131>

Hu, H., Li, A., Shi, C., Chen, L., Zhao, Z., Yin, X., Zhang, Q., Huang, Y., & Pan, H. (2024). Mulberry branch fiber improved lipid metabolism and egg yolk fatty acid composition of laying hens via the enterohepatic axis. *Microbiome*, 12(1), article number 73. <https://doi.org/10.1186/s40168-024-01788-y>

Lemahieu, C., Bruneel, C., Ryckebosch, E., Muylaert, K., Buyse, J., & Foubert, I. (2015). Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. *Journal of Functional Foods*, 19 B, 821–827. <https://doi.org/10.1016/j.jff.2015.04.021>

Lira-Ricárdez, J., Bermúdez, B., & Cabello, L. (2024). Value addition of sunflower seed meal to overcome protein needs. In M. Kumar, S. P. Bangar, & P. S. Panesar (Eds.), *Oilseed meal as a sustainable contributor to plant-based protein* (pp. 205–220). Springer. https://doi.org/10.1007/978-3-031-47880-2_10

Liu, L., Zhang, C., Zhai, M., Yu, T., Pei, M., Du, P., Li, A., Yan, J., Li, C., & Zhang, G. (2023). Effect of chlorogenic acid on lipid

metabolism in 3T3-L1 cells induced by oxidative stress. *Food Bioscience*, 51, article number 102330. <https://doi.org/10.1016/j.fbio.2022.102330>

Liu, X., Zhao, H., Thiessen, S., & House, J. (2010). Effect of plant sterol-enriched diets on plasma and egg yolk cholesterol concentrations and cholesterol metabolism in laying hens. *Poultry Science*, 89, 270–275. <https://doi.org/10.3382/ps.2009-00249>

Lohmann Breeders. (2021). *Management guide cage housing: Lohmann Brown-Classic layers* (V01-24). https://lohmman-breeders.com/r_u/%D0%BB%D0%B8%D0%BD%D0%B8%D0%B8/lohmman-brown-classic-cage-housing/

Mohseni, G.K., Mohammadi, S., Aghakhaninejad, Z., Tajadod, S., Abbasi, K., Askarpour, S.A., Salimi, Z., Shafaei Kachaei, H., Rajabi Harsini, A., Alami, F., Bagheri, S.E., Mosavi Jarrahi, S.A., Gohari, A., Khoshdooz, S., Doaei, S., Kooshki, A., & Gholamalizadeh, M. (2023). Egg consumption and risk of cardiovascular disease: a PERSIAN cohort-based study. *BMC Cardiovascular Disorders*, 23(1), article number 588. <https://doi.org/10.1186/s12872-023-03621-0>

Mozaffarian, D., & Wu, J.H. (2011). Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, 58(20), 2047–2067. <https://doi.org/10.1016/j.jacc.2011.06.063>

Nguyen, V., Taine, E.G., Meng, D., Cui, T., & Tan, W. (2024). Chlorogenic Acid: A Systematic Review on the Biological Functions, Mechanistic Actions, and Therapeutic Potentials. *Nutrients*, 16(7), article number 924. <https://doi.org/10.3390/nu16070924>

Nilsen, D.W.T., Aarsetoey, H., Poenitz, V., Brugger-Andersen, T., Harris, W.S., Staines, H., & Grundt, H. (2025). Dihomo-gamma-linolenic acid (DGLA) is inversely related to risk for cardiac death and cardiovascular events during 2 years follow-up after admission for an acute coronary syndrome. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 205, article number 102684. <https://doi.org/10.1016/j.plefa.2025.102684>

Petraru, A., Ursachi, F., & Amariei, S. (2021). Nutritional Characteristics Assessment of Sunflower Seeds, Oil and Cake. Perspective of Using Sunflower Oilcakes as a Functional Ingredient. *Plants (Basel)*, 10(11), article number 2487. <https://doi.org/10.3390/plants10112487>

Puraikalan, Y., & Scott, M. (2023). Sunflower Seeds (*Helianthus annuus*) and Health Benefits: A Review. *Recent Progress in Nutrition*, 3(3), article number 010. <https://doi.org/10.21926/rpn.2303010>

Ruan, D., Dai, Z., Fouad, A.M., Zhang, Y., Li, C., Wang, S., Huang, X., Li, K., Sun, Y., You, J., & Zheng, C. (2022). Effects of dietary sunflower meal supplementation on productive performance, antioxidative capacity, lipid metabolism, and gut microbiota in laying ducks. *Animal Feed Science and Technology*, 285, article number 115215. <https://doi.org/10.1016/j.anifeeds.2022.115215>

Saleh, A.A., El-Awady, A., Amber, K., Eid, Y.Z., Alzawqari, M.H., Selim, S., Soliman, M.M., & Shukry, M. (2021). Effects of Sunflower Meal Supplementation as a Complementary Protein Source in the Laying Hen's Diet on Productive Performance, Egg Quality, and Nutrient Digestibility. *Sustainability*, 13, article number 3557. <https://doi.org/10.3390/su13063557>

Shkarban, V., & Sychoy, M., (2025). The effect of complete and partial replacement of soybean meals with high protein sunflowers concentrate on the diet of broiler chickens on their growth parameters, meat productivity and meat quality. *Agriculture and Forestry*, 71(4), 79–96.

<https://doi.org/10.17707/AgricultForest.71.4.05>

Sychoy, M., Osadcha, Y., Ilchuk, I., Pitera, V., & Voznik, R. (2025). Use of high-protein sunflower concentrate in laying hens feeding. *Agriculture and Forestry*, 71(3), 137–154.

<https://doi.org/10.17707/AgricultForest.71.3.09>

Teneva, O., Petkova, Z., Ivanova, P., Petrov, P., Keranova, N., Antova, G., & Gerzilov, V. (2025). Influence of dietary vegetable oils supplementation on egg quality of laying hens. *Discover Food*, 5, article number 119.

<https://doi.org/10.1007/s44187-025-00395-6>

Usturoi, M.G., Rațu, R.N., Crivei, I.C., Veleșcu, I.D., Usturoi, A., Stoica, F., & Radu Rusu, R.-M. (2025). Unlocking the Power of Eggs: Nutritional Insights, Bioactive Compounds, and the Advantages of Omega-3 and Omega-6 Enriched Varieties. *Agriculture*, 15, 242.

<https://doi.org/10.3390/agriculture15030242>

Vidosavljević, S., Bojanić, N., Dragojlović, D., Stojkov, V., Sedlar, T., Banjac, V., & Fistes, A. (2025). Application of Optimized Dry Fractionation Process for Nutritional Enhancement of Different Sunflower Meals. *Processes*, 13, article number 255.

<https://doi.org/10.3390/pr13010255>

Zamani, O., Bittmann, T., & Ortega, D.L. (2024). The Effect of Avian Influenza Outbreaks on Retail Price Premiums in the United States Poultry Market. *Poultry Science*, 103(10), article number 104102. <https://doi.org/10.1016/j.psj.2024.104102>