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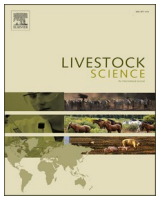

Enhancing the nutritional values of egg yolks of laying hens by different dietary sources of omega-3 fatty acids, vitamin e and trace elements

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HIGHLIGHT

- Dietary whole flaxseed, fish oil and vitamin E with organic form of Se, Zn, and Fe had a positive effect on antioxidative properties in blood and egg.
- Dietary whole flaxseed, fish oil and vitamin E with nano form of Se, Zn, and Fe improved the egg yolk lipids, fatty acids profile, and health indices.

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ABSTRACT

The present study aims to investigate the effect of whole flaxseed (WFS), fish oil (FO), and different sources of Se, Zn, and Fe on lipid metabolites, antioxidant status, immunity, egg yolks' minerals (Se, Zn and Fe) and fatty acids and eggs' lipid and health index in laying hens. A total of 144 hens were divided into 6 groups with 6 replicated of 4 hens each. The laying hens were fed diets containing 0, 7.5 % whole flaxseed (WFS)+1.5 % fish oil (FO), 7.5 %WFS+1.5 % FO+175 mg kg^{-1} vitamin E (VE), 7.5 %WFS+1.5 % FO+ 175 mg kg^{-1} VE +inorganic sources of Se, Zn, and Fe (ISeZnFe), 7.5 %WFS+1.5 % FO+175 mg kg^{-1} VE+ organic sources of Se, Zn, and Fe (OSeZnFe) and 7.5 %WFS+1.5 % FO+ 175 mg kg^{-1} VE +nano-source of Se, Zn, and Fe (NSeZnFe) from 40 to 50 weeks of age. Results clarified that incorporation of 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased serum concentration of HDL-cholesterol and HDL/LDL ratio, while reduced LDL-cholesterol and the hypercholesteremia index (RHCH) in comparison with control. Dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased concentrations of total antioxidants capacity and vitamin E and reduced concentrations of malonaldehyde (MDA) in blood serum and egg contents. Interestingly, dietary inclusion of 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and total n-3 PUFAs concentrations in egg yolk, while reduced arachidonic acid (ARA) in the egg yolks, whereas n-6/n-3 PUFAs ratio was decreased significantly. Moreover, incorporation of 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 %FO+VE+NSeZnFe improved egg health indices. It might be concluded that, inclusion of 7.5 % WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 %FO+VE+NSeZnFe in hens' diets had a positive effect on

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antioxidative properties in blood serum and eggs yolks contents; at the same time, it improved the egg yolk lipids, fatty acids and its related health and nutritional indices.

1. Introduction

Recently, traditional protein feed resources, like soybean meal, have been suffering from a long-term imbalance between supply and demand, leading to an increase in feed cost and a decrease in the maintainability of poultry and livestock production. Also, in countries that do not grow soybeans, there is a trend to substitute it with alternative sources of vegetable protein and crop co-products in animal production (Volek et al., 2018). Therefore, there are a growing interest in finding alternatives to soybean meal such as other plant protein feeds. Besides, large amounts of grain and feed by-product are produced each year. Thus, another purpose of using crop co-products is reducing the feed cost and environmental pollution. Moreover, the co-products could be an important source of bioactive molecules with significant advantages for the environment, human, and animal health (Vastolo et al., 2022).

Hen eggs are a staple in many cuisines around the world, known for their high nutrient content and adaptability. Hen eggs play an important role in human nutrition and have the ability to improve their nutritional content, including: Eggs are a superb source of high-quality protein, as they contain all nine essential amino acids in the proper amounts. This makes them ideal for muscle healing and overall body health (Ebeid et al., 2008). Eggs contain several essential vitamins, including vitamin A, which is important for vision, immune function, and skin health; vitamin D, which aids calcium absorption; and cobalamin (vitamin B12) and folic acid (vitamin B9), which work together to help make red blood cells, help iron work properly in the body, and promote healthy cell growth and function. In addition, eggs contain vital minerals including iron, phosphorus, and selenium. Iron is essential for oxygen delivery in the blood, phosphorus promotes bone health, and selenium functions as an antioxidant. Eggs contain beneficial fats, such as omega-3 fatty acids, especially those from hens on a high-fat diet. Omega-3 fatty acids promote heart health and cognitive function (Maina et al., 2023). Eggs include antioxidants such as lutein and zeaxanthin, which are good for the eyes and may lower the risk of age-related macular degeneration. Despite its various advantages, there is opportunity to improve the nutritional profile of eggs to better match modern dietary needs (Qota et al., 2002), such as: The omega-3 content of eggs can be raised by giving hens an omega-3 fatty acid-rich diet (for example, flaxseed and fish oil). This is helpful to both heart health and cognitive function. Enhancing the mineral content of eggs, such as raising iron and calcium levels, may correct deficits in these vital elements in some populations. Thus, hen eggs are a very nutritious food with numerous benefits, but there is room for further nutritional enhancement through dietary and management breakthroughs.

Marine products (including fish oil; FO) and plant products that can enhance the nutritive value of eggs and meat are the omega-3 fatty acids sources (Ebeid et al., 2008; Maina et al., 2023; Qota et al., 2002). Flaxseed (*Linum usitatissimum* L.) is a critical crop worldwide as a food and feed-stuff due to its oil and fiber components. Worldwide, the production of flax/linseed reached up to 8.7 million tons in 2016, which was priced about 70.2 $ (https://www.statista.com/). Recently, there are a growing interest in using flaxseeds as a source of nutrients for humans and animals since they are a good source of high-quality proteins (20–25 %), oil (35–45 %), ME value (3800–3960 kcal/kg), fibers (20–25 % including 10 % soluble fibers), and ash (3–4 %) (Maina et al., 2023; Saleh et al., 2013). Whole flaxseed (WFS) generally provides 35–45 % oil, including 9–10 % of saturated fatty acids (mainly palmitic and stearic acids), around 20 % monounsaturated fatty acids (mainly oleic acid), and >70 % α-linolenic acid (Al-Madhagy et al., 2023). However, WFS contain non-starch polysaccharides, such as mucilage and numerous anti-nutritional factors such cyanogenic glycosides, tannins, phytic acid, trypsin inhibitors, and anti-vitamin B6 (Xu et al., 2022). These anti-nutritional factors impaired digestion and absorption as well as increased viscosity of ingesta, which ultimately limited the using of WFS in poultry production (Alzueta et al., 2002). Therefore, several methods are used to destroy such anti-nutritional factors in WFS, including physicochemical method (boiling, soaking in warm water, solvent, extrusion, roasting, autoclaving, and microwave) and biological processes (enzymatic and microbial fermentation) (Qota et al., 2002; Alzueta et al., 2002).

Several studies documented that WFS and its products might be used in poultry production to enhance the quality of eggs and meat (Al-Nasser et al., 2011; Attia et al., 2022). Flaxseed, flaxseed meal, or flaxseed oil is a primary source of α-linolenic acid (ALA, 18:3, n-3) for broiler chickens (Qota et al., 2002), laying hens (Attia et al., 2022), and Japanese quails (Ebeid et al., 2011). It is well documented that omega-3-enriched eggs and meat is a successful strategy to reduce the incidence of such lifestyle diseases and enhance the human health index (Hafez and Attia, 2020). It was reported that dietary incorporation of 10–20 % WFS in laying hens' diets elevated ALA concentration in egg yolk by 10-fold or 20-fold, respectively (Caston and Leeson, 1990). Similarly, Scheideler, Jaroni (Scheideler et al., 1998) observed that dietary15 % WFS increased yolk ALA content to be 7.07 g/100 g of fatty acids compared with the control diet (0.26 g/100 g of fatty acids). However, there is a discrepancy in the recommended level of WFS in poultry rations, including 2–15 % for broiler chickens (Qota et al., 2002; Attia, 2004; Richter et al., 1996) and up to10 % for laying hens (Al-Nasser et al., 2011; Leeson et al., 2000). Leeson et al. (2000) reported that dietary 20 % ground flaxseeds to laying hens diets had a negative impact on laying performance, compared to zero and 10 % treatments. Nam et al. (1997) that the inclusion of 10 % WFS had no noteworthy impact on body weight gain, feed intake and feed conversion ratio (FCR) in broilers. Gonzalez-Esquerra and Leeson (2000) concluded that single comb White Leghorn roosters could tolerate up to 10 % WFS in comparison with broiler chicks which is significantly lower tolerance manifesting as diarrhea. Similarly, it was reported that dietary 10 % WFS increased omega-3 fatty acids and had no negative impacts on laying performance (Al-Nasser et al., 2011).

Trace minerals like Se, Zn, and Fe are involved in biochemical-physiological functions, including growth, reproduction, lipid metabolism, antioxidative status, and immune response (Ebeid et al., 2023). Optimal mineral concentration are essential for laying hens due to their role in egg production, eggshell formation, egg quality, immune response and antioxidative properties (Saleh et al., 2020). Trace minerals are provided in form of inorganic, organic, or nano-particles. It was documented that organic sources are higher bioavailability and stability more than inorganic ones, allowing for the addition of feed containing lower amounts (Kannan et al., 2022). Currently, there is a growing interest on using the elemental nanoparticles due to low toxicity, high bioavailability, and great catalytic efficiency in broilers (El-Deep et al., 2016), laying hens (Kannan et al., 2022) and rabbits (Abdelsalam et al., 2019).

Therefore, the objective of the present study is to investigate the role of omega-3 fatty acids derived from marine (fish oil) and plant sources (whole flaxseed) as well as different sources of trace minerals (Se, Zn, Fe) on lipid metabolites, antioxidant status, immune status, egg yolks minerals (Se, Zn and Fe), egg yolk lipids, and overall health, qualitative nutritional, and metabolic indicators of egg yolk fatty acids in laying hens.

2. Materials and methods

2.1. Ethical statement

This work was done with general humane treatment of animals that does not cause distress, suffering, pain, or harm, as reported by Royal Decree number M59 in 14/9/1431H and institutional approval code ACUC-22–1–2.

2.2. Whole flaxseed

A whole flaxseed (brown variety) was obtained from a commercial supplier and used in the current experiment.

2.3. Experimental design

A total of 144 forty -week-old Hisex white shell eggs laying hens with 1703±28.7 g were used in the present experiment. The trail was lasted for 10 weeks, from 40 to 50 weeks of age. The initial 2 weeks, known as the preliminary trial period, served as a period of adaptation and was

Table 1
Composition and chemical analysis of the experimental diets fed to laying hens (40–50 weeks of age) according to Attia et al. (2024).

Ingredients	Diets					
	Control	7.5 %+WFS+1.5 %FO	7.5 %WFS+1.5 % FO+ VE	7.5 %WFS+1.5 %FO +VE+ISEZNFE [1]	7.5 %WFS+1.5 %FO +VE+OSEZNFE [2]	7.5 %WFS+1.5 %FO +VE+NSEZNFE[3]
Yellow corn	64.25	52.515	52.515	52.515	52.515	52.515
Soybean meal, 48 % CP	21.50	20.10	20.10	20.10	20.10	20.10
Corn gluten meal	1.50	1.40	1.40	1.40	1.40	1.40
Whole Flaxseed	0.0	7.5	7.5	7.5	7.5	7.5
What bran	0.0	0.65	0.65	0.65	0.65	0.65
Dicalcium phosphate	1.55	1.51	1.51	1.51	1.51	1.51
Calcium carbonate	9.00	9.00	9.00	9.00	9.00	9.00
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vit.-Min. premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10
L-Lysine	0.20	0.175	0.175	0.175	0.175	0.175
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10	0.10
Choline chloride 50 %	0.05	0.05	0.05	0.05	0.05	0.05
Sardine oil	0.0	1.5	1.5	1.5	1.5	1.5
Building sand	0.95	4.60	4.60	4.60	4.60	4.60
Vit. E (α-Toc., 50 %) mg kg^{-1}	0.0	0.0	350	350	350	350
Se, mg kg^{-1}	0.0	0.0	0.0	4.4	100	0.20
Zn, mg kg^{-1}	0.0	0.0	0.0	76.4	220	55
Fe, mg kg^{-1}	0.0	0.0	0.0	1102.5	2450	441
Determined analysis						
Dry matter %	90.87	90.63	90.57	91.43	91.13	90.71
Crude protein %	16.54	16.63	16.34	16.44	16.44	16.58
Crude fat %	2.71	6.21	6.48	6.51	6.55	6.63
Crude fiber %	3.27	3.63	4.13	4.13	4.13	4.13
Ash	16.31	18.93	15.67	15.81	15.76	15.68
Se, mg kg^{-1}	0.341	0.344	0.351	0.583	0.591	0.571
Zn, mg kg^{-1}	91.4	95.3	97.6	140.5	141.2	148.6
Fe, mg kg^{-1}	30.3	36.1	35.7	450.5	461.8	455.7
Vit. E, mg kg^{-1}	21.4	19.75	186.3	191.3	187.4	195.7
Calculated analysis						
ME, kcal /kg	2700	2701	2701	2701	2701	2701
Calcium %	4.04	4.04	4.04	4.04	4.04	4.04
Phosphorus av. %	0.346	0.340	0.340	0.340	0.340	0.340
Se, ppm	0.367	0.378	0.378	0.578	0.578	0.578
Zn, ppm	93.4	94.4	94.4	149.4	149.4	149.4
Fe, ppm	33.3	40.4	40.4	481.4	481.4	481.4
Methionine %	0.507	0.507	0.507	0.507	0.507	0.507
Lysine,%	0.901	0.893	0.893	0.893	0.893	0.893
C18–1, %	0.595	1.166	1.166	1.166	1.166	1.166
C18–2 PUFAs, %	1.37	1.53	1.53	1.53	1.53	1.53
C18–3 PUFAs, %	0.046	1.382	1.382	1.382	1.382	1.382
C18:2/C18:3 ratio	29.7	1.11	1.11	1.11	1.11	1.11
Vit. E	25.5	25.5	200.5	200.5	200.5	200.5

[1] The 7.5 %WFS+1.5 %FO+VE+ISEZNFE-diets containing 7.5 % flaxseed + 1.5 % fish oil + 175 mg kg^{-1} Vit *E* + 0.2 mg kg^{-1} inorganic Seas sodium selenite 4.5 % Se +55 mg kg^{-1} inorganic Zn from Zn oxide 72 % Zn + 441 mg kg^{-1} inorganic Fe from Fe carbonate 40 % Fe.

[2] The 7.5 %WFS+1.5 %FO+VE+OSEZNFE-diets containing 7.5 % flaxseed + 1.5 % fish oil + 175 mg kg^{-1} Vit *E* + 0.2 mg kg^{-1} organic Se from Bio-Sel, Se yeast (*Saccharomyces cerevisiae*, 0.2 % with seleno-methionine, min 70 %) + 55 mg kg^{-1} organic Zn from Zn chelate of glycine hydrate + 441 mg kg^{-1} organic Fe from Fe chelate of glycine. Organic Se was added as Bio-Sel 2000 Se yeast (0.2 % Se, Seleno -methionine 70 %), IBEX international. The organic sources of Zn and Fe are products of Wspleramy nature, Arkop SP, z.oo., ul. Kolejowa, Poland, as Glystatr-25 % Zn and Glystar®-Fe-17 %, respectively.

[3] The 7.5 %WFS+1.5 %FO+VE+OSEZNFE-diets containing 7.5 %flax seed + 1.5 % fish oil +175 mg kg^{-1} Vit *E* + 0.2 mg kg^{-1} Nano Se + 55 mg kg^{-1} Nano-Zn + 441 mg kg^{-1} Nano Fe respectively from Nano source containing 100 % concentration of each element. [4] Provided per kg diet: Vit. A 1,200,000 IU/kg, Vit D3 3000.000 IU/kg, VE 25.5 mg kg^{-1}, VC 30 mg kg^{-1}, Vit K3 (MNB) 2.05 mg kg^{-1}, Vit. B1 2.00 mg kg^{-1}, Vit. B2 6.00 mg kg^{-1}, Vit. B6 3.00 mg kg^{-1}, Vit. B12 0.03 mg kg^{-1}, niacin (Niacin) 30.00 mg kg^{-1}, folic acid 1.00 mg kg^{-1}, pantothenic acid 10.00 mg kg^{-1}, biotin 0.10 mg kg^{-1}, cobalt 0.40 mg kg^{-1}, copper 10 mg kg^{-1}, iodine 1.50 mg kg^{-1}, iron 9 mg kg^{-1}, manganese 80 mg kg^{-1}, selenium 0.30 mg kg^{-1}, zinc 80 mg kg^{-1}, methionine 0.15 %. [2]Se, Zn and Fe were added replacing the same amount of sand.

discarded. Throughout the study, laying hens were housed in floor pens of 1.5 × 0.6 × 2.0 m, with 4 hens per pen and 6 replicates per treatment. Each pen was equipped with a laying feeder and a manual drinker, ensuring that food and water were available *ad libitum*. All pens were furnished was wood shaving and hens kept in an environmentally controlled room at 22−24 °C with a 16/8 light/dark daily cycle. The dose level for whole flaxseed, fish oil, vitamin E, and various sources of Se, Zn, and Fe was decided based on prior research by Attia et al. (2024) and others authors (Kannan et al., 2022; Kannan et al., 2023; Arbabi--Motlagh et al., 2022). The experimental design had 6 experimental treatments, each containing 6 replicates (4 hens/each) housed in floor pens. Laying hens were supplied diets comprising 0, 7.5 % whole flaxseed (WFS)+1.5 % fish oil (FO), 7.5 %WFS+1.5 % FO+175 mg kg^{-1} vitamin E (VE), 7.5 %WFS+1.5 % FO+ 175 mg kg^{-1} VE +inorganic sources of Se, Zn, and Fe (ISeZnFe), 7.5 %WFS+1.5 % FO+175 mg kg^{-1} VE+ organic sources of Se, Zn, and Fe (OSeZnFe) and 7.5 %WFS+1.5 % FO+ 175 mg kg^{-1} VE +nano-source of Se, Zn, and Fe (NSeZnFe) from 40 to 50 weeks of age. The diets were supplemented with 0.20 mg kg^{-1} inorganic Se + 55 mg kg^{-1} inorganic Zn from Zn oxide 72 % Zn + 441 mg kg^{-1} inorganic Fe from ferrous carbonate 40 % ferrous (WFSFOVE-Inorganic-SeZnFe), 7.5 %WFS+1.5 %FO+ 0.20 mg kg^{-1} organic Se from Bio-Sel, Se yeast (*Saccharomyces cerevisiae*, 0.2 % with seleno-methionine, min 70 %) + 55 mg kg^{-1} organic Zn from Zn chelate of glycine hydrate + 441 mg kg^{-1} organic Fe from Ferrous chelate of glycine hydrate (WFSFOVE-Organic-SeZnFe), or 7.5 %flaxseed+1.5 % fish oil + 0.2 mg kg^{-1} Nano Se + 55 mg kg^{-1} Nano-Zn + 441 mg kg^{-1} Nano Fe (WFSFOVE-Nano-SeZnFe), respectively from Nano source containing 100 % concentration of each element. Organic Se was added as Bio-Sel 2000 Se yeast (0.2 % Se, Seleno -methionine 70 %), IBEX international. The organic sources of Zn and Fe are products of Wspleramy nature, Arkop SP, z. o.o., ul. Kolejowa, Poland, as Glystatr-25 % Zn and Glystar®-Fe-17 %, respectively. The nano compounds were prepared by mechanical grinding (Ball mill) by interaction with ascorbic acid (the number of acid equivalents is equal to the number of salt equivalents and therefore equal to the number of prepared nano equivalents), which performs the bio-reduction of ions and converts them into charge less nanoparticles with mechanical grinding that reduces the size of nanoparticles to reach Average size from 1 to 100 nanometers. They are chemically stable compounds that are not affected by long-term storage. And since the number of equivalents is equal in the preparation, there is no increase in acid or salt during the reaction, and therefore the concentration of the prepared nano is 100 %, and since the compound is stable, it can be added to the feed mixture throughout the production cycle, but for safety, if the production cycle is 45 days, it can be added every 15 days. The feedstuffs and the composition of the experimental diets are displayed in Table 1. The experimental diets' nutritional makeup and fatty acids contents were computed based on the analytical values of feedstuffs (Table-CVB-Feed 2016) and the abovementioned chemical analyses of WFS. The supplemented minerals sources were added to the diets replacing the same amount of building sand. Table 2 displayed the fatty acid composition of the oil used to formulate the laying hens' experimental meals.

2.4. Antioxidants of whole flaxseed

The antioxidant scavenging activity of 1,1-diphenyl-2-picryl-hydrazil (DPPH%) free radicals was measured as described in Akl et al. (2020). The tannin content in whole flaxseed was determined with the Folin–Denis reagent based on Schanderl (1970) method. Quantitative ferulic acids and *p*-coumaric were analyzed by HPTLC in Kannan et al. (2023). Vitamin C concentration was measured as described by Kannan et al. (2023).

Table 2
Fatty acids profile of flaxseed used in diets for laying hens according to Attia et al. (2024).

Fatty acid	mg/100 g flaxseed as feed basis
C3:0	0.371
C4:0	1.46
C6:0	0.341
C10:0	1.78
C12:0	0.241
C14:0	0.099
C15:0	0.381
C16:0	0.281
C17:0	0.331
C 18:0	0.821
C20:0	0.191
C21:0	1.52
Total SFA	7.82
C16:1	0.091
C18:1	0.712
C20:1	0.261
C24:1	0.258
Total MUFA	1.32
C18:2	0.110
C18:3-ALA	0.263
C18:3 n-4 Stearidonic acid	1.11
C18:3 n3	0.286
C20:2 n-6	1.56
C20:2	0.397
C20:3	0.237
C20:4	4.13
C20:5 n-3	0.271
C22:5 n-3 Clupandnioc acid (DAP)	0.007
C22:6 n-3 Docosahexaenoic acid	0.083
Total PUFA	8.45
UFAs	9.78
N6	7.56
N3	0.886
N6/N3	8.53
AI	0.101
TI	1.30
Hypocholestermic index	15.34
Lipid quality index	2.81
Health index	5.45

2.5. Assessment of efficacy

2.5.1. Yolk lipid profile, fatty acids profile, and chemical composition

Six yolk samples were randomly collected per treatment (1 egg yolk/ replicate) and used to determine yolk chemical composition. The yolks were chosen for analyses because of its nutritional value of eggs and practically fats. Yolk lipids were extracted to determine the total yolk lipids, triglycerides, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), according to Attia et al. (2022). The hypercholesteremia index (RHCH) was calculated as the LDL and total cholesterol ratio.

The fatty acids profiles such as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) of egg yolks were measured using 4 eggs per treatment (four individual samples/ treatment; each sample run in duplicate =8 biological samples/ treatment). After lipid excretion, the Methylation of lipid was done using 50 mg of the excreted lipid by adding 5 ml of methanolic sulphuric acid (1 ml concentrated sulphuric acid, and 100 ml methanol) and 2 ml of benzene in the tube. Then the tube was closed well and placed in a water bath at 90 °C for an hour and a half. Then the tube was cooled, and 5 ml petroleum ether and 8 ml of distilled water were added; the tube was shaken strongly, and the ethereal layer separated into a dry tube. Evaporate to dryness. Then the fatty acids profiles were determined according to Radwan (1978) using the coupling of two-dimensional thin-layer chromatography with GC, for the quantitative analysis of lipid classes and their constituents of fatty acids using HP (Hewlett Packard) 6890 GC with Flame Ionization

Detector (FID detector). The detector temperature: 250 °C, the injector temperature: 220oC, and the injection volume 2 µl, split fewer modes. The column was HP-5 (5 % diphenyl, 95 % dimethyl polysiloxane), 30 m, 0.32 mm ID, and 0.25 µm film thickness. The carrier gas was Nitrogen with a gas flow: 1 ml/mi. The oven program was initially temp. 150 °C for 2 min.

Eggs yolk, Se (Prohaska et al., 2000), Zn (De Blas et al., 1994), Fe (Ng et al., 1983), and Cu (McMillin et al., 2009) were determined (n = 6/treatment) using a Varian ICP-Optical Emission Spectrometer (Varian 720-ES, ICP-OEM, Leuven, Belgium).

2.5.2. Blood biochemical constituents and antioxidative status

Six blood samples were randomly collected from each treatment to represent all treatment replicates for the hens at 50 weeks of age. The blood serum was separated after centrifugation of the blood at 1500 g × for 10 min. Then, serum lipid profiles were determined, including the total lipids, triglycerides, total serum cholesterol, serum LDL-cholesterol, and serum HDL-cholesterol according to Attia et al. (2022). Additionally, the serum very-low-density lipoprotein (VLDL) was calculated by dividing the triglycerides by 5. Digenetic kits manufactured by Diamond Diagnostics (23 EL-Montazah St. Heliopolis, Cairo, Egypt, http://www.diamonddiagnostics.com) were used for the determination of lipid profile. The HDL/LDL ratio was measured by dividing HDL/LDL. Moreover, the risk of hypercholesterolemia and very-low-density lipoprotein (VLDL cholesterol) was measured as (mg/dL) according to Friedewald et al. (1972) equation: VLDL = triglycerides/5.

The risk of hypercholesterolemia was computed using the following equation according to Attia et al. (2015):

Risk of hypercholesterolemia= LDL cholesterol /total cholesterol.

The serum total antioxidant capacity (TAC) and malondialdehyde (MAD) were determined according to Richard et al. (1992). Then, the antioxidant balance was calculated as the ratio between TAC and MDA (Attia et al., 2020).

2.5.3. Qualitative, nutritional, and metabolic indices of fatty acids profile analysis

The yolk health lipid indices such as qualitative, nutritional, and metabolic indices were estimated (n = 4/treatment) as described in the study by Qaid et al. (2023) and by Wereńska et al. (2021).

2.5.3.1. Qualitative indexes. Polyunsaturated fatty acid/Saturated fatty acid (PUFAs/SFAs) = (ΣPUFAs)/(ΣSFAs) (Wereńska et al., 2021; Simopoulos, 2008).

Σn-6PUFAs/Σn-3PUFAs ratio = (C18:2n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6)/(C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n3) (Wereńska et al., 2021; Simopoulos, 2008).

Linoleic acid/α-linolenic acid (LA/ALA) ratio = (C18:2n-6)/(C18:3n-3) (Ryman et al., 2017).

Sum of eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA%) = %C20:5n-3 + %C22:6n-3 (Crupi and Cuzzocrea, 2022; Untea et al., 2023).

Unsaturation index (UI) = (% monoenoic) + (2 × % dienoic) + (3 × % trienoic) + (4 × % tetraenoic) + (5 × % pentaenoic) + (6 × % hesaenoic) (Qaid et al., 2023).

2.5.3.2. Nutritional indexes. Nutrition value index = (C18:0 + C18:1n9)/(C16:0) (Qaid et al., 2023).

Atherogenicity index (AI) = [C12:0 + (4 × C14:0) + C16:0]/(ΣUFAs);

Thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5 × MUFAs) + (0.5 × Σn-6 PUFAs) + (3 × Σn-3 PUFAs) + (Σn-3 PUFAs/Σn-6 PUFAs)].

Platelet aggregation and thrombus formation can occur when AI and TI levels are elevated. As a result, lower values benefit human health (Lo Turco et al., 2016).

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

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

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

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

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

The lipid quality parameter expresses the percentage correlation between the main n-3 PUFAs [eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)] and total lipids. Higher values of this index are synonymous with a higher quality of the dietary lipid source (Łuczyńska et al., 2017). Therefore, the expression used to calculate lipid quality was:

Lipid quality = 100 x [EPA + DHA]/ [% of total fatty acids].

Fish lipid quality/Flesh lipid quality (FLQ) = 100 × (C20:5n-3 + C22:6n-3)/(ΣSFAs) (Qaid et al., 2023).

2.5.3.3. Metabolic indexes. Elongase index = (C18:0/C16:0) × 100, whereas

Thioesterase index = (C16:0/C14:0) × 100 (Wereńska et al., 2021).

Estimated Δ9-, Δ5- and Δ6-desaturase activities can be used as surrogates of the measure of true desaturase activity in the laboratory (Qaid et al., 2023).

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

Δ5-desaturase + Δ6-desaturase = (C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3

+ C22:6n-3)/(C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:4n-6 + C20:5n-3 + C22:5n-3

+ C22:6n-3)

Activity index of n-3 β-oxidation in the muscles = (Σ n-3PUFAs)/(C18:3n-3) (Failla et al., 2021).

2.6. Statistical analysis

The differences among treatments were statistically analyzed by one-way ANOVA Statistical Analysis Software computer program (SAS-Institute 2012). The replicate was the experimental unit. Tukey's post hoc compared the significant differences among means of treatments at significance level 0.05 %. All percentages were transformed to $\log^{10}$ to normalize the data distribution before running the statistical analysis.

3. Results

3.1. Antioxidants levels of whole flaxseed

An antioxidant levels of whole flaxseed were showed in Table 3. whole flaxseed was a rich source of antioxidants compounds. DPPH %, ferulic acid (mg g^{-1}), ascorbic acid %, tannic acid %, and p-coumaric acid (mg g^{-1}) were 33.20±2.13 %, 1.64±0.34 %, 1.19±0.34 %, 0.096±0.19 mg g^{-1}, and 0.096±0.19 mg g^{-1}.

Table 3
Level of antioxidants in whole flaxseed (as mean of nine replicates; mean ± SD).

Item	
1,1-diphenyl-2-picryl-hydrazil (DPPH%)	33.20 ± 2.13
Ascorbic acid %	1.19 ± 0.34
Tannic acid %	0.096 ± 0.19
Ferulic Acid (mg g^{-1})	1.64 ± 0.34
p-Coumaric Acid (mg g^{-1})	0.95 ± 0.14

3.1. Effect of dietary treatments on blood serum lipid profile

The influence of dietary WFS, FO, and different sources of Se, Zn, and Fe on blood serum lipid fractions of Hisex laying hens is presented in Table 4. Dietary treatments had a significant effect on serum concentrations of total lipids, triglycerides, total cholesterol, VLDL-cholesterol, total cholesterol, LDL-cholesterol, HDL/LDL ratio, and RHCH. Dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 % FO+VE+NSeZnFe increased serum concentration of HDL-cholesterol and HDL/LDL ratio, while reduced LDL-cholesterol and RHCH in comparison with control.

3.2. Effect of dietary treatments on antioxidant indices and trace minerals in blood serum and eggs

Regarding the effect of WFS, FO, and different sources of Se, Zn, and Fe on serum antioxidative status indices of Hisex laying hens, Table 5 displays that dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 % WFS+1.5 %FO+VE+NSeZnFe increased blood serum content of TAC and vitamin E, while, blood serum concentrations of glutathione peroxidase (GPX), lipid peroxidase and MDA were reduced significantly.

Similar trend was observed on antioxidant markers in the egg yolks. As shown in Table 6, dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased egg yolks content of TAC and vitamin E, while, egg concentrations of MDA and TAC/MDA ratio were minimized.

Table 7 shows that the dietary inclusion of 7.5 %WFS+1.5 % FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased egg content of Se, Zn, and Fe, while, blood serum contents of Se and Zn did not be affected.

3.3. Effect of dietary treatments on egg yolk lipids and fatty acids profiles

Table 8 presents the results of egg yolk lipids profiles of laying hens fed WFS, FO, and different sources of Se, Zn, and Fe. All egg lipid profiles were significantly affected by dietary treatments. Compared with the control diet, dietary 7.5 %WFS+1.5 %FO+VE+ISeZnFe, 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %+1.5 %FO+VE+NSeZnFe reduced egg contents of total lipids, triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol, and RH, while, egg contents of HDL-cholesterol and HDL/LDL ratio were significantly increased. Results concerning the effect of dietary inclusion of WFS, FO, and different sources of Se, Zn, and Fe on egg yolks fatty acids profile, including SFAs, MUFAs, and PUFAs are displayed in Tables 9, 10, and 11; respectively. Compared with the control diet, dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 % WFS+1.5 %FO+VE+NSeZnFe had no significant effect on total SFAs (Table 9) and total MUFAs (Table 9), with a concomitant increase in total PUFAs and total n-3 PUFAs, however, total n-6 PUFAs and n6/n3 PUFAs ratio were decreased (Table 10). As shown in Table 11, dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 % FO+VE+NSeZnFe increased concentrations of ALA, EPA, docosapentaenoic acid (DPA), and DHA, while reduced ARA concentration in the egg yolks.

3.4. Effect of dietary treatments on egg yolks qualitative, nutritional and metabolic indices

Based on the fatty acids profile of the egg yolk, the qualitative, nutritive, and metabolic indices of the eggs were significantly affected by the dietary treatments, with the exception of elongase (Table 12). Compared with the control diet, the dietary treatments significantly improved the qualitative indices by decreasing the ratio of n6/n3 PUFAs and linoleic acid/α-linolenic acid and improving EPA% + DHA%, PUFAs/SFAs, and the unsaturation index. In terms of nutritional indices, the 7.5 % WFS +1.5 %FO +VE+ ISeZnFe group outperformed the other groups by having the highest nutritional index, egg health index, hypocholesterolemic index, health-promoting index, egg health index, and the lowest atherogenic index and thrombogenic index. Compared to the control diet, the egg fat quality index was highest, especially in the group treated with 5 % WFS plus 1.5 %FO. The quality of fish/meat lipids was best in all groups compared to the control diet, which can be attributed to the 1.5 % FO diet in these groups, which increased the EPA and DHA content. The control group had the highest thioesterase index and activity index compared to the other groups, and the 7.5 % WFS +1.5 %FO +VE+ ISeZnFe group had the highest Δ9-desaturase (C16:1 + C:18:1), and the 7.5 %WFS +1.5 %FO +VE group had the highest Δ5-desaturase + Δ6-desaturase.

3.5. Correlation between dietary treatments and egg health indices

The correlation between the dietary treatments and the fatty acid profile and their health indices is shown in Table 13. Omega-3 meal (7.5 % flaxseed +1.5 % fish oil) correlated strongly negatively ($p < 0.05$) with n6/n3-PUFAs (R^2=−0.980), linoleic acid/α-linolenic acid (R^2=−0.983), thrombogenic index (R^2=−0.909), activity index (R^2=−0.924). In the fatty acid profile of hens' egg yolk, there were strong positive significant ($p < 0.05$) correlations between omega-3 meal (7.5 %WFS +1.5 %FO) with C16N4 (R^2=0.709), with C18:3N3 (R^2=0.928), C22:6N3 (R^2=0.699), N3 (R^2=0.917), PUFAs (R^2=0.765), H:H ratio (R^2=0.700), PUFAs:SFAs (R^2=0.727), unsaturation index (R^2=0.861), lipid quality index for eggs (R^2=0.739), and for fish/meat lipid quality (R^2=0.775).

Dietary vitamin E was weakly negatively correlated ($p < 0.05$) with n6/n3 PUFAs (R^2=−0.633), linoleic acid/α-linolenic acid (R^2=−0.631), thrombogenic index (R^2=−0.501), activity index (R^2=−0.624). There were weak positive significant ($p < 0.05$) correlations between dietary vitamin E and C14 (R^2=0.535), with C18:3N3 (R^2=0.595), and with mega-3 (R2=−0.517) in the diet. The diet with the metal source SeZnFe correlated weakly negatively ($p < 0.05$) with the metabolic indices of the fatty acid profile of the eggs.

Table 4
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe fed on lipid fractions of blood serum (n = 6/treatment) of laying hens.

Dietary treatments	Total lipids, mg/dL	Triglycerides, mg/dL	VLDL, mg/dL	Cholesterol, mg/dL	LDL, mg/dL	HDL, mg/dL	HDL/LDL	RHCH
Control	572.6^{e}	156.2^{ab}	31.2^{ab}	172.2^{a}	33.9^{b}	104.7^{c}	3.08^{d}	0.197^{ab}
7.5 %WFS+1.5 %FO	590.9^{d}	147.1^{c}	29.4^{c}	165.5^{b}	33.4^{b}	108.5^{b}	3.26^{c}	0.202^{a}
7.5 %WFS+1.5 %FO+VE	613.7^{c}	147.4^{c}	29.5^{c}	166.7^{b}	31.8^{c}	111.0^{b}	3.47^{b}	0.191^{b}
7.5 % WFS +1.5 %FO+VE+ISeZnFe	631.4^{b}	153.7^{b}	30.7^{b}	170.8^{ab}	35.3^{a}	117.6^{a}	3.34^{bc}	0.207^{a}
7.5 %WFS +1.5 %FO+VE+OSeZnFe	641.1^{a}	155.2^{b}	31.0^{b}	173.2^{a}	28.3^{d}	120.4^{a}	4.25^{a}	0.164^{c}
7.5 %WFS +1.5 %FO+VE+NSeZnFe	641.7^{a}	160.2^{a}	32.1^{a}	174.5^{a}	28.3^{d}	119.8^{a}	4.23^{a}	0.162^{c}
RMSE	4.99	3.39	0.679	3.89	0.969	2.34	0.135	0.007
p value	<0.0001	<0.0001	<0.0001	.001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a–c}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE = Root mean square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); LDL = Low-density lipoprotein; HDL = High-density lipoprotein; HDL/LDL = High-density lipoprotein/low-density lipoprotein ratio; RHCH = Risk of hypercholesteremia = LDL/TC.

Table 5
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe on the serum antioxidants indices (n = 6/treatment) of laying hens.

Dietary treatments	GPX, mu/ml	TAC, mg/dL	Vit E, mg/100 ml	Lipid peroxidase, nmol/ml	MAD, mmol/dl	MDA/TAC
Control	24.7^{a}	0.853^{b}	0.664^{c}	1.88^{a}	1.52^{b}	0.561^{a}
7.5 %WFS+1.5 %FO	23.9^{a}	0.850^{c}	0.670^{bc}	1.87^{ab}	1.52^{a}	0.558$^{e.}$
7.5 %WFS+1.5 %FO+VE	23.9^{a}	0.856^{ab}	0.672^{bc}	1.87^{ab}	1.52^{a}	0.563^{d}
7.5 % WFS +1.5 %FO+VE+ISeZnFe	23.8^{a}	0.857^{a}	0.677^{b}	1.86^{b}	1.51^{a}	0.565^{c}
7.5 %WFS +1.5 %FO+VE+OSeZnFe	19.3^{b}	0.858^{a}	0.691^{a}	1.86^{b}	1.50^{b}	0.570^{b}
7.5 %WFS +1.5 %FO+VE+NSeZnFe	20.3^{b}	0.858^{a}	0.689^{a}	1.86^{b}	1.49^{c}	0.574^{a}
RMSE	1.89	0.002	0.006	0.005	0.006	0.002
p value	<0.0001	<0.0001	<0.0001	0.007	<0.0001	<0.0001

$^{a\text{-}c}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE = Root mean square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; GPX=Glutathione peroxidase, TAC= Total antioxidant capacity; MDA= Malondialdehyde.

Table 6
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe on antioxidant markers in egg yolks (n = 6/treatment) of laying hens.

Flaxseed Meal,%	TAC, mmol/L	MDA, uM/ml	TAC/ MDA	Vitamin E, ug/g
Control	0.551^{e}	3.99^{a}	7.23^{a}	19.1^{f}
7.5 %WFS+1.5 %FO	0.691^{d}	3.89^{ab}	5.64^{b}	23.3^{e}
7.5 %WFS+1.5 %FO+VE	0.831^{c}	3.86^{ab}	4.85^{c}	25.8^{d}
7.5 % WFS +1.5 % FO+VE+ISeZnFe	0.961^{b}	3.77^{bc}	394^{d}	28.5^{c}
7.5 %WFS +1.5 % FO+VE+OSeZnFe	1.02^{b}	3.69^{c}	3.58^{d}	31.2^{b}
7.5 %WFS +1.5 % FO+VE+NSeZnFe	1.15^{a}	2.92^{d}	2.55^{e}	33.1^{a}
RMSE	0.041	0.096	0.217	0.521
p value	<0.0001	<0.0001	<0.0001	<0.0001

$^{a\text{-}e}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE= Root means square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; TAC= total antioxidant capacity; MDA= malondialdehyde.

3.6. Correlation between dietary treatments and trace minerals in egg and serum

The correlation between omega-3 fatty acids (flaxseed, fish oil), vitamin E, and different metal sources with selenium, zinc and furious supplementation of Hisex laying hens with selenium, zinc and furious in egg yolks and in serum is shown in Table 14. Dietary metal source was weakly positively correlated with dietary vitamin E (p<.0001; R^2=0.707). Se content in egg yolks was negatively correlated (p < 0.05) with diet plus omega-3 (R^2=−0.844), diet plus vitamin E (R^2=−0.642), and diet plus metal source (R^2=−0.523). Serum Fe content correlated weakly negatively with serum Zn content (p = 0.002; R^2=−0.553), and weakly positively with egg yolks Zn content (p<.0001; R^2=0.671).

4. Discussion

The laying performance as well as interior and exterior egg characteristics of laying hen are studied recently (Attia et al., 2024). Phenols from plants and their derivatives are considered essential dietary components with high antioxidant activity, as well as other biological activities and health benefits (Kumar and Goel, 2019). In this study, the antioxidant activity of the polyphenols extracted from flaxseed is considerably high when measured by the DPPH free radical scavenging method, which is commonly used to evaluate the free radical scavenging activity of natural plants (Pag et al., 2014). The exceptional performance of flaxseed in scavenging DPPH radicals is equivalent to that of other natural and synthetic antioxidants, suggesting that flaxseed or its by-products can provide more hydrogen to scavenge DPPH radicals (Pag et al., 2014). Ferulic acid, a natural phenolic antioxidant, is used in a variety of biological applications due to its low toxicity (Mikołajczak et al., 2023). In agreement with Akl et al. (2020), phenolic chemicals isolated from whole flaxseeds, such as DPPH, ferulic acid, ascorbic acid, tannic acid, and p-coumaric acid, showed significant biological activity.

4.1. Effect dietary treatments on blood serum and lipid profile

Results showed that dietary inclusion of 7.5 %WFS+1.5 % FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased serum concentration of HDL-cholesterol and HDL/LDL ratio, while reduced LDL-cholesterol and RHCH in comparison with control. Several previous studies indicated that dietary full fat flaxseed, flaxseed meal, and flaxseed oil reduced blood serum lipid profile, including LDL-cholesterol, VLDL, total cholesterol, and triglycerides in laying hens (Attia et al., 2022; Celebi and Utlu, 2004; Van Elswyk, 1997). Likewise,

Table 7
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe on the trace minerals (n = 6/treatment) in blood serum and egg yolks of laying hens.

Dietary treatments	Serum trace minerals			Egg yolks trace minerals		
	Se, ug/ml	Zn, ppm	Fe, ug/dl	Se, ppm	Zn, ppm	Fe, ppm
Control	0.186	5.57	9.33^{ab}	5.97^{e}	69.4^{e}	117.0^{c}
7.5 %WFS+1.5 %FO	0.148	5.98	10.33^{a}	6.75^{d}	71.5^{d}	118.6^{b}
7.5 %WFS+1.5 %FO+VE	0.158	5.13	8.72^{ab}	7.46^{c}	74.8^{c}	118.8^{b}
7.5 % WFS +1.5 %FO+VE+ISeZnFe	0.209	5.03	9.27^{ab}	8.27^{b}	76.9^{b}	118.9^{b}
7.5 %WFS +1.5 %FO+VE+OSeZnFe	0.236	6.16	10.19^{a}	8.73^{ab}	78.5^{a}	119.8^{a}
7.5 %WFS +1.5 %FO+VE+NSeZnFe	0.171	4.71	7.50^{b}	9.06^{a}	78.8^{a}	119.1^{a}
RMSE	0.076	0.961	1.40	0.321	0.291	0.390
p value	0.171	0.158	0.043	<0.0001	<0.0001	0.0062

$^{a\text{–}e}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE = Root mean square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); NSeZnFe= Nano-source of Se, Zn and Fe.

Table 8
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe on lipid fractions in egg yolks (n = 6/treatment) of laying hens.

Dietary treatments	TL, mg g^{-1}	TRIG, mg g^{-1}	TC, mg g^{-1}	LDL, mg g^{-1}	HDL, mg g^{-1}	HDL/LDL	VLDL, mg g^{-1}	RH
Control	351.3^{a}	184.5^{a}	193.1^{a}	96.9^{a}	59.8^{e}	0.618^{f}	36.9^{a}	0.502^{a}
7.5 %WFS+1.5 %FO	335.2^{a}	179.6^{b}	190.9^{a}	92.5^{b}	59.8^{e}	0.646^{e}	35.9^{b}	0.484^{b}
7.5 %WFS+1.5 %FO+VE	311.2^{b}	161.8^{c}	185.4^{b}	84.2^{c}	60.7^{d}	0.721^{d}	32.4^{c}	0.454^{c}
7.5 % WFS +1.5 %FO+VE+ISeZnFe	270.8^{b}	155.7^{d}	175.8^{c}	79.9^{d}	62.4^{c}	0.781^{c}	31.1^{d}	0.455^{c}
7.5 %WFS +1.5 %FO+VE+OSeZnFe	241.1^{d}	147.8^{e}	165.7^{d}	74.3^{e}	63.9^{b}	0.859^{b}	29.6^{e}	0.449^{c}
7.5 %WFS +1.5 %FO+VE+NSeZnFe	231.9^{d}	139.8^{f}	158.7^{e}	69.8^{f}	66.6^{a}	0.953^{a}	28.0^{f}	0.440^{d}
RMSE	10.9	2.32	1.54	0.384	0.391	0.007	0.464	0.004
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a–f}$ Means within a column within each factor not sharing similar superscripts are significantly different, P< .05; RMSE = Root mean square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of selenium (0.20 mg kg^{-1}), zinc (55 mg kg^{-1}), and ferrous (441 mg kg^{-1}); OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe (441 mg kg^{-1}); NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; TL = total lipids; TRIG = triglycerides; TC = total cholesterol; LDL = low-density lipoprotein; HDL = high-density lipoprotein; HDL/LDL = high-density lipoprotein/low-density lipoprotein ratio; vLDL =: very-low-density lipoprotein; RHCH= risk of hypercholesteremia = LDL/TC.

Table 9
Total saturated fatty acid profile of in egg yolks of laying hens fed whole flaxseed, fish oil and different sources of Se, Zn and Fe (n = 4/treatment).

Fatty acids	Dietary treatments						RMSE	*p-value*
	0 % control	7.5 % WFS+1.5 %FO	7.5 %WFS +1.5 %FO+VE	7.5 %WFS +1.5 % FO+VE+ISeZnFe	7.5 %WFS +1.5 % FO+VE+OSeZnFe	7.5 %WFS +1.5 % FO+VE+NSeZnFe		
C12:0	0.00	0.00	0.100	0.100	0.075	0.000	0.093	0.350
C14:0	0.352	0.382	0.423	0.453	0.483	0.540	0.083	0.054
C15:0	0.00^{b}	0.075^{a}	0.00^{b}	0.00^{b}	0.075^{a}	0.00^{b}	0.029	0.001
C16:0	27.1^{a}	25.1^{b}	25.3^{b}	24.7^{b}	26.8^{a}	26.4^{a}	0.487	<0.0001
C17:0	0.162^{c}	0.352^{ab}	0.273^{bc}	0.258^{bc}	0.468^{a}	0.370^{ab}	0.081	0.001
C18:0	8.73	9.22	9.50	7.74	9.37	8.32	0.869	0.073
C21:0	0.170^{ab}	0.173^{ab}	0.188^{a}	0.130^{b}	0.173^{ab}	0.153^{ab}	0.024	0.055
ΣSFAs	36.5^{a}	35.3^{a}	35.7^{a}	33.3^{b}	37.3^{a}	35.7^{a}	1.01	0.0008

$^{a-c}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE= Root means square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; C12:0: Lauric acid, C14:0: Tetradecanoic acid, C15:0: Pentadecylic acid, C16:0: Hexadecanoic acid, C17:0: Heptadecanoic acid, C18:0: Octadecanoic acid, C21:0, ΣSFAs: Sum saturated fatty acid. ND: Not detectable.

Table 10
Monounsaturated fatty acid profile of egg yolks of laying hens fed whole flaxseed, fish oil and different sources of Se, Zn and Fe (n = 4/treatment).

Fatty acids	Dietary treatments						RMSE	*p-value*
	Control	7.5 % WFS+1.5 %FO	7.5 %WFS +1.5 %FO+VE	7.5 %WFS +1.5 % FO+VE+ISeZnFe	7.5 %WFS +1.5 % FO+VE+OSeZnFe	7.5 %WFS +1.5 % FO+VE+NSeZnFe		
C14:1 *n-5*	0.00^{b}	0.075^{a}	0.00^{b}	0.00^{b}	0.100^{a}	0.00^{b}	0.021	<0.0001
C16:1 *n – 7*	4.43^{a}	3.64^{b}	3.89^{ab}	3.94^{ab}	3.90^{ab}	4.50^{a}	0.332	0.012
C18:1 *n – 7*	2.17	2.03	2.05	1.88	2.01	2.12	0.158	0.218
C18:1 *n – 9*	41.56	39.19	40.56	41.45	38.60	38.9	1.46	0.031
C20:1 *n – 7*	ND	ND	ND	ND	ND	ND	ND	ND
C20:1 *n – 9*	0.220	0.218	0.270	0.217	0.383	0.290	0.122	0.386
C22:1 *n – 11*	0.00	0.00	0.00	0.00	0.00	0.050	0.029	0.132
C22:1 *n – 9*	ND	ND	ND	ND	ND	ND	ND	ND
ΣMUFAs	48.4	45.1	46.8	47.5	45.0	45.9	1.69	0.068

$^{a-b}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE= Root means square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; 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, ΣMUFAs: Sum monounsaturated fatty acid. SEM: Standard error of the means, ND: Not detectable.

Cherian and Hayat Cherian and Hayat (2009) revealed that long-term feeding of 10 % WFS to laying hens minimized hepatic and plasma fat content, hepatic triglycerides, and total number of fat vacuoles in ISA Brown Leghorn hens. Moreover, Attia et al. (2022) reported that the inclusion of 12 % of soaked flaxseed meal increased plasma concentration of HDL-cholesterol with respect to the control diet. Additionally, Celebi and Utlu (2004) reported that adding 4 % flaxseed oil increased plasma HDL-cholesterol concentration in ISA brown laying hens. The raise of serum HDL-cholesterol content in laying hens fed 7.5 %WFS demonstrates the positive effects of dietary inclusion of WFS on lipid metabolism and the elevation in healthy lipoproteins connected with increasing the consumption of omega-3 sources (Shakoor et al., 2020).

Table 11
Polyunsaturated fatty acid profile of egg yolks of laying hens fed whole flaxseed, fish oil and different sources of Se, Zn and Fe (n = 4/treatment).

Fatty acids	Dietary treatments						RMSE	*p-value*
	0 % control	7.5 % WFS+1.5 % FO	7.5 %WFS +1.5 % FO+VE	7.5 %WFS +1.5 % FO+VE+ISeZnFe	7.5 %WFS +1.5 % FO+VE+OSeZnFe	7.5 %WFS +1.5 % FO+VE+NSeZnFe		
C16:3 *n* − *4*	0.130^{c}	0.203^{ab}	0.190^{ab}	0.198^{ab}	0.168^{b}	0.228^{a}	0.021	<0.0001
C18:2 *n* − *6*	11.97^{c}	13.19^{b}	11.65^{c}	14.42^{a}	11.70^{c}	12.46^{c}	0.533	<0.0001
C18:3 *n* − *6*	0.090^{a}	0.032^{b}	0.000^{b}	0.000^{b}	0.110^{a}	0.000^{b}	0.032	0.0002
C20:2 *n* − *6*	0.038	0.083	0.000	0.000	0.038	0.040	0.046	0.173
C20:4 *n* − *6* (ARA)	1.49^{a}	1.31^{ab}	1.21^{ab}	0.758^{b}	1.29^{ab}	0.923^{ab}	0.266	0.012
C22:4 *n* − *6*	ND	ND	ND	ND	ND	ND	ND	ND
Σ*n-6* PUFAs	13.6^{b}	14.8^{a}	12.9^{b}	15.2^{a}	13.1^{b}	13.4^{b}	0.581	0.0001
C18:3 *n* − *3* (ALA)	0.380^{c}	2.35^{ab}	2.07^{b}	2.28^{ab}	2.43^{ab}	2.72^{a}	0.267	<0.0001
C 20:3 *n* − *3*	0.190^{a}	0.000^{b}	0.000^{b}	0.000^{b}	0.048^{b}	0.188^{a}	0.038	<0.0001
C20:4 *n* − *3*	ND	ND	ND	ND	ND	ND	ND	ND
C20:5 *n* − *3* (EPA)	0.000 ^{c}	0.038 ^{b}	0.120 ^{a}	0.113 ^{a}	0.033 ^{b}	0.110 ^{a}	0.057	0.028
C22:5 *n* − *3* (DPA)	0.293^{a}	0.000^{c}	0.230^{a}	0.213^{ab}	0.100^{b}	0.193^{ab}	0.064	<0.0001
C22:6 *n* − *3* (DHA)	0.498^{c}	2.14^{a}	1.98^{a}	1.08^{b}	1.65^{ab}	1.49^{ab}	0.342	<0.0001
Σ*n-3* PUFAs	1.36^{c}	4.53^{a}	4.40^{ab}	3.68^{b}	4.26^{ab}	4.70^{a}	0.421	<0.0001
ΣPUFAs	15.1^{c}	19.6^{a}	17.5^{b}	19.1^{a}	17.6^{b}	18.3^{ab}	0.871	<0.0001
ΣUFAs	63.4^{bc}	64.5^{b}	64.2^{bc}	66.5^{a}	62.6^{c}	64.2^{bc}	0.982	0.001
Σ*n*−7	2.17	2.03	2.04	1.87	2.00	2.11	0.162	0.225
Σ*n*−9	41.8	39.4	40.8	41.7	40.0	39.2	1.473	0.041

$^{a-c}$ Means within a column within each factor not sharing similar superscripts are significantly different, P < 0.05; RMSE= Root means square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; C18:2*n*−6: (Linoleic acid: 9,12-Octadecadienoic acid (Z,Z); C20:2*n*−6: Eicosadienoic acid; C18:3*n*-6: Dihomo-gamma-linolenic acid; C20:4*n*−6: Arachidonic acid: all-cis-5,8,11,14-eicosatetraenoic acid; C22:4*n*−6: Adrenic acid; Σ*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: Docosapentaenoic acid: cis-5,8,11,14,17-Eicosapentaenoic acid (EPA), C22:5*n*−3: Docosapentaenoic acid (DPA), C22:6 *n* − 3: (Docosahexaenoic acid (DHA): 4,7,10,13,16,19-docosahexaenoic acid, Σ*n*-3 PUFAs: Total omega−3 polyunsaturated fatty acids, C16:3*n*−4, ΣPUFAs: Total polyunsaturated fatty acid, ΣUFA: Total unsaturated fatty acid. ND: Not detectable.

Furthermore, dietary Zn proteinate reduced plasma concentrations of triglycerides and LDL-cholesterol in laying hens (Kannan et al., 2022). Organic and inorganic Fe supplementation reduced serum total cholesterol and triglycerides levels (Kannan et al., 2023). Fe nano-oxide supplementation reduced serum lipid concentrations (triglycerides, total cholesterol, and LDL- cholesterol) of Bovens layer hens while increasing serum HDL concentration (Javadifar et al., 2020).

4.2. *Effect dietary treatments on antioxidant indices and trace minerals in blood serum and eggs*

One of the major interested results of the present study is that dietary 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 % FO+VE+NSeZnFe increased concentrations of TAC and vitamin E and reduced concentrations of MDA in blood serum and egg contents. These results are in correspondence with previous studies (Moghadam et al., 2020; Buckiuniene et al., 2018; Panaite et al., 2019). Mattioli et al. (2017) stated that dietary 10 % extruded flaxseed enhanced plasma TAC and reduced yolk lipid peroxidation (TBARS) in comparison with control group. Similarly, Moghadam et al. (2020) observed that dietary 15 % of heated flaxseed + 0.1 % enzyme mixture enhanced oxidative stability of eggs (decreasing of TBARS concentration in egg yolk) which might be connected with improving egg sensory attributes. Also, Panaite et al. (2019) proved that dietary 5 % WFS + dried tomato waste (2.5 %, 5.0 %, and 10.0 %) reduced yolk concentration of TBARS and peroxide value. Buckiuniene et al. (2018) reported that dietary rapeseed oil+ organic or inorganic Se + vitamin E decreased MDA content in egg yolk. Ghasemi et al. (2022) pointed out that dietary inclusion of chelated trace minerals (Fe, Zn, Cu. Cr, Mn, Se, and I) enhanced serum concentration of glutathione peroxidase, superoxide dismutase, TAC and minimized serum MDA concentration in laying hens. Also, dietary trace minerals had a positive effect on yolk lipid oxidative stability (Arbabi-Motlagh et al., 2022; Buckiuniene et al., 2016). Dietary 7.5 %WFS+1.5 % FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased blood serum content of TAC and vitamin E, while, blood serum concentrations of GPX, lipid peroxidase and MDA (a lipid peroxidation marker) decreased, indicating that those treatments protected cells against lipid peroxidation and thus kept antioxidant enzymes at normal levels. The concentration of Vit. E increased in treatments 7.5 % WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe compared to the respective Vit. E-supplemented treatments, implying that VE with trace mineral in organic and nano form exerts high antioxidant activity and contributes to lipid peroxidation reduction (low mmol/dl concentration of MAD). Arbabi-Motlagh et al. (2022) concluded that inclusion of organic Zn (20 mg kg^{-1}) plus organic Se (0.2 mg kg^{-1}) in the oxidized fat diets enhanced oxidative stability (in form of reducing yolk MDA concentration). Buckiuniene et al. (2016) reported that different dietary Fe sources reduced yolk concentration of MDA in fresh egg in comparison with the control group. Similarly, dietary supplementation of Zn proteinate (Kannan et al., 2022), organic Fe (Fe-Lys-Glu), Cao et al. (2022) enhanced serum TAC and reduced serum MDA concentration. Likewise, MDA concentration in serum and egg yolk was reduced due to the dietary inclusion of chelated trace minerals in laying hens (Ghasemi et al., 2022).

Results showed that the dietary inclusion of 7.5 %WFS+1.5 % FO+VE+OSeZnFe or 7.5 %WFS+1.5 %FO+VE+NSeZnFe increased egg content of Se, Zn, and Fe. These results confirmed the previous studies (Kannan et al., 2022; Kannan et al., 2023; Ghasemi et al., 2022) which indicated that dietary trace minerals increased their concentrations in egg yolk. Increasing Zn proteinate levels in the diet (up to 80 mg kg^{-1}) improved egg Zn content (Kannan et al., 2022). Increased organic Fe (75 mg kg^{-1} Fe-Lys-Glu) levels resulted in increasing the Fe concentrations in

Table 12
Effect of whole flaxseed, fish oil and different sources of Se, Zn and Fe fed on qualitative nutritive, and metabolic indices of egg yolks of laying hens ($n = 4$/treatment).

Items	Control	7.5 %WFS+ 1.5 %FO	7.5 %WFS +1.5 %FO +VE	7.5 % WFS +1.5 %FO +VE+ ISeZnFe	7.5 %WFS +1.5 %FO +VE+ OSeZnFe	7.5 %WFS +1.5 %FO +VE +NSeZnFe	RMSE	*p*-value
Qualitative indices								
n6/n3 PUFAs	10.0^{a}	3.37^{c}	2.92^{c}	4.16^{b}	3.08^{c}	2.86^{c}	0.302	<0.0001
PUFAs/SFAs	0.413^{d}	0.553^{a}	0.489^{bc}	0.573^{a}	0.471^{c}	0.514^{b}	0.018	<0.0001
Linoleic acid/α-Linolenic acid	32.0^{a}	5.74^{b}	5.66^{b}	6.41^{b}	4.85^{b}	4.65^{b}	2.00	<0.0001
EPA% + DHA%	0.497^{c}	2.18^{a}	2.10^{a}	1.19^{b}	1.69^{ab}	1.60^{ab}	0.325	<0.0001
Unsaturation index	85.2^{c}	98.1^{a}	95.4^{ab}	94.9^{ab}	92.5^{b}	94.4^{b}	1.61	<0.0001
Nutritive indices								
Nutrition value index	1.86^{ab}	1.93^{ab}	1.98^{a}	1.99^{a}	1.79^{b}	1.79^{b}	0.067	0.001
Atherogenic index	0.450^{ab}	0.413^{c}	0.423^{bc}	0.401^{c}	0.462^{a}	0.445^{ab}	0.013	<0.0001
Thrombogenic index	1.03^{a}	0.790^{c}	0.813^{bc}	0.773^{c}	0.870^{b}	0.800^{c}	0.026	<0.0001
Hypocholesterolemic index	2.12^{d}	2.37^{ab}	2.32^{bc}	2.46^{a}	2.12^{d}	2.19^{dc}	0.059	<0.0001
Hypercholesterolemic index	2.14^{d}	2.37^{ab}	2.33^{bc}	2.47^{a}	2.13^{d}	2.20^{dc}	0.059	<0.0001
H/H	0.993^{b}	1.00^{a}	0.996^{ab}	0.996^{ab}	0.996^{ab}	0.997^{ab}	0.003	0.009
Egg health index	1.94^{ab}	2.00^{ab}	2.06^{a}	2.07^{a}	1.87^{b}	1.87^{b}	0.070	0.002
Egg lipid quality index	0.497^{c}	2.18^{a}	2.11^{a}	1.20^{bc}	1.69^{ab}	1.60^{ab}	0.317	<0.0001
Health-promoting index	2.23^{bc}	2.43^{a}	2.37^{ab}	2.50^{a}	2.18^{c}	2.25^{bc}	0.069	<0.0001
Fish lipid quality/Flesh lipid quality	1.36^{c}	6.10^{a}	5.89^{a}	3.59^{b}	4.50^{ab}	4.48^{ab}	0.751	<0.0001
Metabolic indices								
Elongase index	32.2	36.7	37.5	31.4	35.0	31.6	3.29	0.053
Thioesterase index	7683^{a}	6566^{ab}	6079^{ab}	5517^{b}	5690^{b}	5161^{b}	865	0.009
Δ9-desaturase (C16:1 + C:18:1)	56.2^{ab}	55.4^{ab}	56.1^{ab}	58.3^{a}	54.0^{b}	55.6^{ab}	1.58	0.035
Δ5-desaturase + Δ6-desaturase	0.163^{bc}	0.207^{ab}	0.232^{a}	0.122^{c}	0.199^{ab}	0.169^{abc}	0.030	0.001
Activity index	3.63^{a}	1.92^{b}	2.13^{b}	1.62^{b}	1.76^{b}	1.75^{b}	0.262	<0.0001

$^{a\text{-}c}$ Means within a row within each factor not sharing similar superscripts are significantly different, $P < 0.05$; RMSE= Root means square error; WFS=Whole flaxseed; FO= Fish oil; VE= Vitamin E (175 mg kg^{-1}); ISeZnFe= Inorganic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; OSeZnFe= Organic source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; NSeZnFe= Nano-source of Se (0.20 mg kg^{-1}), Zn (55 mg kg^{-1}), and Fe; n6/n3 PUFAs = Omega 6 fatty acids/omega 3 polyunsaturated fatty acids. Atherogenic index=,C12:0 + 4 × C14:0 + C16:0/∑UFA; TI: Thrombogenic index =,C14:0 + C16:0 + C18:0/[,0.5 × ΣMUFAs +,0.5 × ∑PUFAs $n-6$ +,3 × ∑PUFAs $n-3$ +,∑PUFAs $n-3$/∑ $n-6$]. Hypocholesterolemic index=,C18:0 + ∑MUFAs + ∑PUFAs. Hypercholesterolemic index: UFAs,C14:0 + C16:0. Lipid quality = 100 x [EPA + DHA]/ [% of total fatty acids. Egg health index=,C180+C181/C16:0 (Wereńska et al., 2021).

egg yolk with the highest content at 75 mg Fe/kg (Cao et al., 2022). Hens fed 45 mg Fe/kg organic Fe (Fe-Lys-Glu) had higher yolk Fe contents than those provided with the exact dosage of inorganic Fe (FeSO4) (Cao et al., 2022). Inorganic Fe (ferrous sulfate) supplementation increased egg Fe content by 20.4 to 35.3 %, and organic Fe (ferrous proteinate) supplementation increased egg Fe content by 32.87 to 42.44 % in White Leghorn layers (Kannan et al., 2022). Also, Fe nano-oxide supplementation increased serum Fe and Zn concentrations (Javadifar et al., 2020). Regarding yolk Zn and Se contents, advanced chelate compound-based trace minerals produced the best results of laying hens (Ghasemi et al., 2022). It well knows that trace minerals like Se, Zn, and Fe are involved in several physiological and metabolic processes, including growth, reproduction, lipid metabolism, antioxidative status, and immune response (Ebeid et al., 2023). Therefore, it is noteworthy to indicate that dietary combination of WFS+FO+VE+SeZnFe enhanced the antioxidative properties and minimized lipid peroxidation in blood serum and egg yolk, which probably translates into enhancing immunity and disease resistance in laying hens as well as improving the oxidative stability and consequently prolonging the shelf life of egg.

Yolk lipoprotein precursors, including vitellogenin and VLDL are hepatically synthesized under the influence of estradiol-17β and taken up by developing yolk through receptor-mediated endocytosis (Attia et al., 2016). Plasma Zn concentration indicates circulating vitellogenin (Ebeid et al., 2008). Results of the current trail showed that dietary inclusion of 7.5 %WFS+1.5 %FO+VE+OSeZnFe or 7.5 %WFS+1.5 % FO+VE+NSeZnFe did not alter serum concentrations of VLDL and Zn compared with control group. Therefore, it might be speculated that dietary WFS in combination with FO, VE and SeZnFe had no adverse effect on folliculogenesis (ovarian follicular development) which consequently related to the egg production rate in the present study (Data not shown).

4.3. Effect dietary treatments on egg yolk lipids and fatty acids profiles

Similarly, to blood serum lipid profile, dietary 7.5 %WFS+1.5 % FO+VE+ISeZnFe, 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %+1.5 % FO+VE+NSeZnFe reduced egg contents of total lipids, triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol, and RH, while, egg contents of HDL-cholesterol and HDL/LDL ratio were significantly increased compared with the control diet. These results agree with Hosseini et al. (2023) who postulated that dietary extruded flaxseed (18 – 27 %) reduced egg yolk cholesterol content. Also, Attia et al. (2022) concluded that dietary-soaked flaxseed meals reduced all lipid fractions in egg yolk. However, Imran et al. (2015) reported that the inclusion of different levels of extruded flaxseed meal (10–30 %) in laying hens' diet had no significant effect on total lipids and cholesterol content in egg yolk. Also, Moghadam et al. (2020) observed that dietary 15 % of heated flaxseed had no significant effect on egg total lipids content. Indeed, there are contradictory results of the effect of flaxseed addition on yolk HDL-cholesterol content in the form of an increase (Attia et al., 2022), a decrease (Mattioli et al., 2017), or no modify (Hayat et al., 2010). Several investigations found that dietary omega 6/omega 3 ratios of 24.5:1, 2.7:1, 1.8:1, 1.2:1, and 1:1 had no effect on egg yolk total cholesterol levels (Ebeid et al., 2011; Caston and Leeson, 1990).

Dietary changes to alter the fatty acid composition of eggs take precedence over efforts to minimize the cholesterol content of eggs (Maina et al., 2023; Saleh et al., 2020). In this study, strong positive ($p < .05$) correlations were found between omega-3 meals and the mega-3 content of egg yolk, and strong negative correlations were found between omega-3 meals and the n-3:n-6 ratio; however, no significant ($p > .05$) correlations were found between omega-3 meals and mega-6. In addition, dietary vitamin E and dietary metal sources were not significantly correlated with total n-3 PUFAs, total n-6 PUFAs, and the n-6/n-3 ratio. Interestingly, the intake of 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or

Table 13
Pearson's correlation coefficients between dietary omega 3, vitamin E, and different trace minerals sources with fatty acid profiles and its related indices in eggs yolks of laying hens.

Pearson's Correlation Coefficients							
Fatty acid profiles in egg yolks	**Diet+ omega 3**	**Diet + Vit E**	**Die + metal source**	**Egg yolks' health indices**	**Diet + Omega 3**	**Diet + Vit E**	**Diet + metal source**
C12:0	0.225	0.355	0.137	n-6	0.111	−0.263	0.078
	0.291	0.088	0.523		0.606	0.215	0.717
C14:0	0.410	0.535	0.562	n-3	0.917	0.517	0.327
	0.047	0.007	0.004		<0.0001	0.010	0.119
C14:1 *n*-5	0.287	−0.130	0.092	SFAs	−0.262	−0.134	−0.136
	0.174	0.546	0.670		0.217	0.532	0.528
C15:0	0.258	−0.204	0.000	MUFAs	−0.462	−0.120	−0.171
	0.223	0.339	1.000		0.023	0.577	0.423
C16:0	−0.553	−0.165	0.043	PUFAs	0.765	0.233	0.298
	0.005	0.441	0.843		<0.0001	0.273	0.157
C16:1 *n*-7	−0.403	0.023	0.149	UFAs	0.256	0.104	0.110
	0.051	0.915	0.488		0.228	0.629	0.609
C17:0	0.572	0.336	0.433	n-6:n-3	−0.980	−0.633	−0.404
	0.004	0.108	0.034	Ratio	<0.0001	0.001	0.050
C16:3 *n*-4	0.709	0.393	0.331	Hypo CHO	0.459	0.094	−0.045
	0.0001	0.057	0.114		0.024	0.664	0.834
C18:0	0.039	−0.120	−0.350	Hyper CHO	0.449	0.103	−0.036
	0.858	0.577	0.094		0.028	0.632	0.866
C18:1 n-9	−0.393	−0.134	−0.227	HH Ratio	0.700	0.395	0.224
	0.058	0.532	0.286		0.0001	0.056	0.294
C18:1 n-7	−0.343	−0.245	−0.242	PUFAs:SFAs	0.727	0.243	0.298
	0.101	0.248	0.254		<0.0001	0.253	0.158
C18:2 n-6	0.254	−0.053	0.236	Linoleic acid/α-	−0.983	−0.631	−0.455
	0.230	0.806	0.266	Linolenic acid	<0.0001	0.001	0.026
C18:3 n-6	−0.430	−0.299	−0.039	Unsaturation index	0.861	0.291	0.122
	0.036	0.156	0.856		<0.0001	0.168	0.570
C18: 3 n-3	0.928	0.595	0.547	Nutrition value	0.155	−0.018	−0.314
α-linolenic acid	<0.0001	0.002	0.006	index	0.469	0.935	0.136
C20:1 n-9	0.173	0.281	0.255	Health-promoting	0.346	−0.007	−0.118
	0.418	0.183	0.229	index	0.098	0.975	0.583
C21:0	−0.095	−0.182	−0.454	Atherogenic index	−0.334	−0.012	0.122
	0.660	0.395	0.026		0.111	0.955	0.571
C20:2 n-6	−0.043	−0.398	−0.147	Thrombogenic	−0.909	−0.501	−0.350
	0.843	0.054	0.492	index	<0.0001	0.013	0.094
C20:3 n-3	−0.587	−0.188	0.083	Egg health index	0.143	−0.026	−0.319
	0.003	0.378	0.701		0.506	0.904	0.128
C20:4 n-6	−0.438	−0.501	−0.522	Egg lipid quality	0.739	0.232	−0.076
	0.033	0.013	0.009	index	<0.0001	0.276	0.723
C22:1 n-11	0.120	0.189	0.267	Fish/Flesh lipid	0.775	0.244	−0.076
	0.578	0.377	0.207	quality (FLQ)	<0.0001	0.251	0.723
C22:5 n-3	−0.493	0.161	−0.027	Elongase index	0.217	−0.076	−0.377
	0.014	0.453	0.902		0.309	0.725	0.069
C22:6 n-3	0.699	0.176	−0.105	Thioesterase index	−0.628	−0.639	−0.591
	0.0001	0.411	0.624		0.001	0.001	0.002
C20:5 n-3	0.453	0.521	0.240	Δ9-desaturase	−0.060	0.047	0.020
	0.026	0.009	0.260	(C16:1 + C:18:1)	0.779	0.826	0.928
n-7	−0.356	−0.273	−0.260	Δ5- + Δ6-desaturase	0.203	−0.040	−0.417
	0.088	0.196	0.220		0.341	0.852	0.043
n-9	−0.385	−0.116	−0.211	Activity index	−0.924	−0.624	−0.587
	0.063	0.591	0.322		<0.0001	0.001	0.003

7.5 %WFS+1.5 %FO+VE+NSeZnFe increased the concentrations of ALA, EPA, DPA, DHA, and total n-3 PUFAs in egg yolk, whereas the ARA concentration in egg yolk decreased, while the ratio of n-6/n-3 PUFAs was significantly decreased. The data from the current study show that the intake of long-chain n-3 PUFAs (ALA, EPA, and DHA) in egg yolk via a 7.5 % WFS diet was successful. These results are in line with several previous studies (Panaite et al., 2019; Mattioli et al., 2017; Westbrook and Cherian, 2019). In addition, Buckiuniene et al. (2018) showed that the addition of a diet containing sunflower oil + organic or inorganic Se + vitamin E increased PUFAs 0.35 percent in egg yolk. In addition, Westbrook and Cherian (2019) showed that the addition of a 0.05- 0.1 % enzyme mixture to a 10 % WFS diet increased the concentration of ALA, DHA, and total n–3 PUFAs in egg yolk and decreased the ARA concentration. Mattioli et al. (2017) found that a diet containing 10 % extruded flaxseed altered the fatty acid profile in terms of an increase in ALA, EPA, DHA, and total n-3 PUFA, while decreasing the yolk concentration of MUSFs, total n-6 PUFAs, and the n-6/n-3 ratio. Panaite et al. (2019) demonstrated that a 5 % WFS diet with different amounts of dried tomato waste (2.5 %, 5.0 %, and 10.0 %) increased the content of total PUFAs and n-3 PUFAs in egg yolk, while n-3 PUFAs and n-6/n-3 were reduced. In addition, a dietary n-3:n-6 PUFAs ratio of 1:3 + 200 ppm organic Zn gave the best n-3:n-6 ratio by decreasing n-6 PUFAs and increasing n-3 PUFAs in duck egg yolk (Alifian et al., 2023). Eggs enriched with n-3 PUFAs (vegetable oils, flaxseed and/or rapeseed), Se, VE and lutein reduced n-6 PUFAs compared to conventional eggs (Kralik et al., 2023). It could be hypothesized that Δ6-desaturase is utilized for the n-3 PUFAs pathway rather than the n-6 PUFAs pathway, which inhibits the conversion of linoleic acid to ARA (Jia et al., 2008), leading to a decrease in n-6 PUFA concentration and n6/n3 PUFA ratio in eggs from FSC-fed hens.

Table 14
Correlation between dietary omega 3, Vitamin E, and different trace mineral sources in egg yolks and serum of laying hens.

Pearson's Correlation Coefficients									
Prob > |r| under H0: Rho = 0									
	Diet + Omega 3	**Diet + Vitamin E**	**Diet + metal source**	**Fe egg**	**Se egg**	**Zn egg**	**Zn serum**	**Se serum**	**Fe serum**
Diet + Omega 3	1								
Diet + Vitamin E	0.632	1							
	0.0002								
Diet + metal source	0.447	0.707	1						
	0.013	<0.0001							
Fe egg	0.1286	0.109	0.233	1					
	0.4983	0.566	0.214						
Se egg	−0.844	−0.642	−0.523	−0.220	1				
	<0.0001	0.0002	0.003	0.243					
Zn egg	−0.230	0.112	0.104	0.530	0.114	1			
	0.222	0.556	0.584	0.003	0.549				
Zn serum	−0.063	0.057	−0.131	0.095	−0.063	−0.242	1		
	0.740	0.766	0.489	0.619	0.742	0.197			
Se serum	−0.010	0.215	0.355	−0.171	−0.023	−0.338	0.310	1	
	0.959	0.253	0.054	0.367	0.905	0.068	0.096		
Fe serum	−0.030	−0.274	−0.152	−0.104	0.031	−0.553	0.671	0.475	1
	0.874	0.144	0.424	0.584	0.871	0.002	<0.0001	0.008	

4.4. Effect dietary treatments on egg health (Qualitative, nutritional, and metabolic) indices

In recent years, the demand for healthy foods has increased because of its relationship to improving consumer health and increasing resistance to diseases and epidemics that afflict him. Thus, enhancing the egg health indices are a very interesting result of the present study. The TI in egg yolk in 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 % FO+VE+NSeZnFe experimental groups was significantly greater than the control group, while AI, HypoCI, and EHI did not differ from control group. TI and AI are the most employed nutritious indices for evaluating fatty acid content since they clearly demonstrate important implications (Chen and Liu, 2020). The TI and AI indices of the present study are similar between groups and are comparable with those reported by Attia et al. (2015). A healthy egg was characterized by low HypoCI, AI, and TI, which indicate a delaying of atherosclerosis and, consequently the risk of cardiovascular disease (Attia et al., 2015; Mutungi et al., 2008).

It could be hypothesized that Δ6-desaturase is engaged in the n-3 PUFAs pathway rather than the n-6 PUFAs pathway, which inhibits the conversion of linoleic acid to ARA (Jia et al., 2008), leading to a decrease in n-6 PUFAs concentration and n6/n3 PUFAs ratio in eggs from hens fed FSCM.

The ratio of PUFAs to SFAs is a commonly used indicator for analyzing the effects of a particular diet on cardiovascular health, assuming that all PUFAs lower low-density lipoprotein and total cholesterol, but all SFAs can increase serum cholesterol. Consequently, this is a direct indicator: higher levels indicate a greater benefit (or the opposite effect) of eating a particular egg (Simopoulos, 2008). In this case, the group with 7.5 % WFS +1.5 %FO +VE+ ISeZnFe, showed a significantly higher ratio between PUFAs and SFAs (positive effect), so that the atherogenic and thrombogenic indices were lower and the HH ratio was higher in the omega-3 treated groups (7.5 % flaxseed +1.5 % fish oil) than in the control group. This is related to the omega-3 meals, which correlate significantly positively with the PUFAs and the H:H ratio of the fatty acid profile of the chicken egg yolk and show a strong negative correlation with the thrombogenic index of the fatty acid profile of the hens eggs.

A lower ratio of n-6PUFAs/n-3PUFAs is desirable to reduce the risks of chronic diseases, which are prevalent in developing countries and in Western societies (Timmis et al., 2022). Thus, all omega-3 meals (7.5 % flaxseed and 1.5 % fish oil) had a lower n-6PUFAs/n-3PUFAs index than the control group, resulting in a negative association between omega-3 and the n-6PUFAs/n-3PUFAs index.

Since LA and ALA fatty acids cannot be synthesized in the human body and must be consumed in the diet, the LA:ALA ratio was used to evaluate infant nutrition. They also compete for the same desaturase and elongase enzymes that enable the synthesis of PUFAs. Due to the low conversion rate of ALA, decreasing the LA:ALA ratio improves the levels of certain omega-3 fatty acids such as EPA and DHA (Das, 2006); consequently, the LA:ALA ratio could be considered the first step in estimating PUFAs. In agreement with Ryman, Packiriswamy (Ryman et al., 2017), omega-3 supplementation increased the ALA content of egg yolk and decreased the LA/ALA ratio with increasing dose. There were strong positive correlations between the omega-3 treated groups (7.5 % flaxseed +1.5 % fish oil), particularly the 7.5 %WFS +1.5 %FO+VE+ NSeZnFe treated group, with egg yolk ALA content, resulting in strong negative correlations between omega-3 and the LA:ALA ratio. However, vitamin E intake or metal intake had no effect on the ALA content in egg yolk, resulting in a weak negative correlation between vitamin E intake or metal intake and the LA:ALA ratio.

EPA and DHA are n-3 PUFAs that benefit human health by preventing cardiovascular disease, inflammation, and reproductive health (Ochi and Tsuchiya, 2018). Here, EPA was only present in birds fed omega-3 diets and was highest in the 7.5 % WFS +1.5 %FO +VE+ ISeZnFe group. In addition, the levels of DHA and EPA% plus DHA% were highest in birds treated with omega-3 diet without metal supplementation, lowest in birds treated with omega-3 diet without metal supplementation and lowest in the control group. This suggests that EPA, DHA and FLQ levels are weakly negatively correlated with the omega-3 diet and not related to vitamin E or the metal source in the diet.

The UI contains information on the degree of unsaturation of the individual FAs. In this study, the UI was positively correlated with the omega-3 diet and not with vitamin E or the metal source in the diet, so that the groups treated with omega-3 without additional metal source had a higher UI than the groups treated with omega-3 plus different metal sources. This index plays a role in determining oxidative feed stability in humans and animals and in determining certain oxidative protective measures (Crupi and Cuzzocrea, 2022; Untea et al., 2023). In this case, the egg yolk of hens treated with omega 3 (7.5 % WFS+1.5 % FO) had a higher UI value than that of hens treated with other groups, indicating a higher probability of autoxidation of FAs but a higher value of healthy fat in their egg yolk. Omega-3 fatty acids correlated positively with the UI value, as there was a positive correlation between omega-3 fatty acids and tri- and pentaenoic acid. From a nutritional perspective,

the nutrient indices are considered (Chen et al., 2016). Regardless of the intake of vitamin E and/or minerals, the intake of omega-3 fatty acids (7.5 % flaxseed and 1.5 % fish oil) significantly improved the nutritional indices (NVI, EHI, hypocholesterolemic index, and HPI were best in the group treated with 7.5 % WFS +1.5 % FO +VE+ ISeZnFe; and FLQ was best in the groups treated with 7.5 % WFS+1.5 % FO with/without vitamin E). Regardless of the intake of vitamin E and/or dietary mineral sources, the intake of omega-3 fatty acids (7.5 % flaxseed and 1.5 % fish oil) correlated positively with the lipid quality index of eggs, the FLQ, and negatively with the TI. An atherogenic index is a more specific index for determining the atherogenicity of foods than PUFAs/SFAs (Dal Bosco et al., 2016). With the exception of stearic acid, which is not considered proatherogenic due to the ability of humans to desaturate stearic acid to oleic acid, there were no correlations between dietary vitamin E, dietary mineral sources, and/or dietary omega-3 fatty acids with the atherogenic index. Saturated lauric, myristic and palmitic acids promote lipocyte adhesion to circulatory and immune system cells and aggregation of atherogenic plaques, while decreasing esterified fatty acid and phospholipid levels (Omri et al., 2019). In this study, there were strong positive correlations between the atherogenicity index and the saturated fatty acids myristic and palmitic, while atherogenicity did not correlate with lauric. In contrast to the PUFAs:SFAs index, a lower value indicates that the meal has bette nutritional properties. In agreement with Chen et al. (2004), they suggested that the HPI directly indicates that this index is the exact opposite of the atherogenic index. Thus, the best HPI values at both FSCM levels were significantly better in the nutritional value of FAs and lower in the atherogenic and thrombogenic index compared to the control. Thus, the HPI is used to assess the nutritional value of FAs and the effects of fats on cardiovascular disease. The thrombogenicity index is used together with the atherogenicity index to further describe the thrombogenic capacity of FAs. Supplementation of feeds with lower thrombogenicity is therefore beneficial for public health, which indirectly means that omega-3 PUFAs have lower thrombogenicity. The HH ratio focuses on the relationships between dietary FAs and low-density lipoproteins in blood in relation to the hypocholesterolemic FAs (oleic acid and PUFAs) and hypercholesterolemic FAs (lauric acid, myristic acid, palmitic acid) (Chen et al., 2016). In this study, the group treated with 7.5 % WFS +1.5 %FO +VE+ ISeZnFe was able to increase hypocholesterolemic FAs as it contained the highest PUFAs (there was a very positive correlation between dietary omega-3 PUFAs and PUFAs), while the group treated with 7.5 % WFS +1.5 % FO +VE+ OSeZnFe decreased hypercholesterolemic FAs, resulting in a weak correlation between omega-3 PUFAs and HH ratio. The fatty acid elongase and thioesterase activities of egg yolk were comparable in all treatments, as 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. The intake of omega-3 fatty acids, vitamin E or metals in the diet did not correlate with elongase and was only weakly negatively correlated with thioase. Thus, elongase was similar in the different experimental groups, but the 5 % WFS +1.5 % FO +VE+ ISeZnFe group had higher levels of Δ9-desaturase, which converted saturated C16:0 + C:18:0 fatty acids to monounsaturated C16:1 + C:18:1 fatty acids, than the 7.5 % WFS +1.5 %FO +VE+ OSeZnFe group. This suggests that the inorganic metal source in the 7.5 % WFS +1.5 % FO +VE treated group may be the result of increased Δ9-desaturase activity, which is higher than the organic metal source. The Δ5-desaturase plus Δ6-desaturase did not correlate with dietary levels of omega-3 PUFAs, vitamin E or organic metal sources. Energy expenditure (β-oxidation cycle) can be derived from the ratios of n-3 PUFAs and ALA (Houten and Wanders, 2010). In our case, the hens that did not receive omega 3 in the diet had significantly higher kinetic activity compared to the omega 3 treated groups, and their yolks had a higher oxidative status than a glycolytic status. It was shown that there was a negative correlation between index activity and the intake of omega-3 PUFAs, vitamin E, or metal sources in the diet. Others (Failla et al., 2021; Dal Bosco et al., 2012) agree. They found that hens with higher kinetic activity had more oxidative than glycolytic states. Therefore, the activity index takes into account the energy consumed by the locomotor activity of the hens during their lifetime, considering all nutritional and qualitative effects on their yolk fatty acids.

5. Conclusions

Based on the results of the present study, it might be concluded that inclusion of 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 % FO+VE+NSeZnFe in hens' diets had a positive effect on antioxidative properties in blood serum and egg contents. Interestingly, dietary inclusion of 7.5 %WFS+1.5 %FO+VE+OSeZnFe, or 7.5 %WFS+1.5 % FO+VE+NSeZnFe enhanced ALA, EPA, DHA, and total n-3 PUFAs concentrations in eggs, whereas n-6/n-3 PUFAs ratio was decreased significantly. Moreover, egg health indices were also improved. Based on these findings, the SBM in treatments' diets was replaced by only 1.40 %, which is considered a promising replacement rate.

Author contributions

Conceptualization, Y.A.A., R.A.A; methodology, Y.A.A., R.A. A., A.A. Al-S., EL-S. O.S.H.., V.T. and M.J.O.; software, Y.A. A., S.A.N, and M.J. O.; data collection, M.J.O.; EL-S. O.S.H., and A.A.Al-S.; investigation, H. A.S., Y.A.A., M.J.O.; K.A.A., S.A.N., A.A.A, A.A.Al-S., M.M.Q., G.P., V.T. and EL-S. O.S.H.; resources, A.A.Al-S., EL-S. O.S.H., and M.J.O.; writing—original draft preparation, Y.A.A., N.M.A., T.A.E. and M.J.O.; writing—review and editing, Y.A.A., T.A.E., V. T., H.A.S., A.A.A., K.A.A., M.J.O., and A.A.Al-S.; project administration and supervising, Y.A.A., and R.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.

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Institutional review board statement

This work was done with general humane treatment of animals that does not cause distress, suffering, pain, or harm, as reported by Royal Decree No M59 in 14/9/1431H and institutional approval code ACUC-22–1–2.

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Data are available upon official request from the principal investigator and with the permission of the funding agent.

CRediT authorship contribution statement

Youssef Attia: Writing – review & editing, Writing – original draft, Conceptualization. **Ahmed A.Al Sagan:** Data curation. **El-Sayed O.S. Hussein:** Investigation. **Marai J. Olal:** Investigation. **Tarek A. Ebeid:** Formal analysis. **Abdulaziz A. Alabdullatif:** Investigation, Formal analysis. **Rashed A. Alhotan:** Methodology, Investigation. **Mohammed M. Qaid:** Software, Investigation. **Vincenzo Tufarelli:** Writing – review & editing, Writing – original draft. **Gianluca Pugliese:** Writing – review & editing. **Khaild A. Asiry:** Investigation. **Sameer A. Nagadi:** Visualization. **Heba A. Shehta:** Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

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