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Egg Quality, Sensory Attributes, and Protein Metabolites of Laying Hens Fed Whole Flaxseed, Fish Oil, and Different Sources of Trace Elements

Youssef Attia [1], Ahmed A. Al Sagan [2], El-Sayed O.S. Hussein [3], Marai J. Olal [4], Tarek A. Ebeid [5], Abdulaziz A. Alabdullatif [3], Rashed A. Alhotan [3], Gamaleldin M. Suliman [3], Mohammed M. Qaid [3], Vincenzo Tufarelli [6], Heba A. Shehta [7] and Marco Ragni [8]

[1] Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Damanhour 22713, Egypt
[2] King Abdulaziz City for Science and Technology, Riyadh 12354, Saudi Arabia
[3] Department of Animal Production, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia
[4] Admin & Animal Nutrition Technical Services, Danisco Animal Nutrition and Health (IFF), Riyadh 11451, Saudi Arabia
[5] Department of Poultry Production, Faculty of Agriculture, KafrEl-Sheikh University, KafrEl-Sheikh 33516, Egypt
[6] Department of Precision and Regenerative Medicine and Jonian Area (DiMePRe-J), Section of Veterinary Science and Animal Production, University of Bari 'Aldo Moro', Bari 70010, Italy;
[7] Regional Center for Food and Feed, Agricultural Research Center, Giza 12619, Egypt
[8] Department of Soil, Plant and Food Science, University of Bari 'Aldo Moro', Bari 70125, Italy

This study evaluated the effects of whole flaxseed (WFS), fish oil (FO), and different sources of Se, Zn, and Fe (inorganic, organic, and nano-source) on egg production, quality, sensory attributes, and serum protein metabolites in laying hens. A total of 144 hens were divided into six groups with six replicates of four hens each. Hens were fed six diets as follows: 1) control diet; 2) 7.5%WFS+1.5%FO; 3) 7.5%WFS+1.5%FO+175 mg/kg vitamin E (VE); 4) 7.5%WFS+1.5%FO+175 mg/kg VE + inorganic sources of Se, Zn, and Fe (ISeZnFe); 5) 7.5%WFS+1.5%FO+175 mg/kg VE + organic sources of Se, Zn, and Fe (OSeZnFe); 6) 7.5%WFS+1.5%FO+175 mg/kg VE + nano-source of Se, Zn, and Fe (NSeZnFe) from 40–50 weeks of age. Laying hens fed 7.5% WFS, 1.5% FO, and different sources of trace elements in their diets had no negative effects on laying rate, egg weight, egg mass, feed intake, feed conversion ratio, body weight change, or survival rate compared to that of hens fed the control diet. Dietary treatments did not negatively affect the external and internal egg characteristics or egg sensory attributes. Feeding 7.5%WFS+1.5%FO+VE+ISeZnFe positively influenced yolk color in fresh eggs. Dietary treatments had a significant impact on egg nutritional composition, with the highest levels of macronutrients found in eggs from hens fed the 7.5%WFS+1.5%FO+VE+NSeZnFe treatment. The highest plasma globulin concentrations were observed in hens fed organic and nano-source trace elements. The same diets reduced plasma uric acid levels. Based on these findings, the inclusion of organic or nano-source trace minerals in diets containing WFS and FO positively affected egg quality and hen protein metabolites.

Key words: egg quality; laying hens; protein metabolites; trace elements; whole flaxseed.

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Introduction

Proteins are among the most important and expensive ingredients in commercial poultry feed. Due to high costs and unpredictable supply, some protein sources are unable to meet the rising demand, especially from poultry and aquaculture feed manufacturers[1]. Recently, traditional protein feed resources have been subject to a demand-supply mismatch, resulting in increased feed costs and decreased sustainability of livestock production. In ad-

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Correspondence: Prof. Vincenzo Tufarelli, Department of Precision and Regenerative Medicine and Jonian Area, University of Bari 'Aldo Moro', 70010 Bari, Italy (E-mail: vincenzo.tufarelli@uniba.it); Prof. Marco Ragni, Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari 70125, Italy (E-mail: marco.ragni@uniba.it)

dition, in regions where soybeans are not grown, there is a movement to replace them with alternative sources of crop byproducts and vegetable protein for animal nutrition[2].

Poultry nutrition has received special attention in poultry diets, with less expensive feedstuffs receiving additional weight. In recent decades, there has been greater focus on discovering ways to recycle feed manufacturing byproducts. Owing to its high protein level and important amino acid content, soybean by-product, a renewable resource, is considered a viable raw material for substituting or supplementing animal, poultry, and fish meal[1]. Therefore, there is a growing interest in identifying alternatives to soybean meal, such as other plant protein feeds and feed byproducts. Another purpose of using crop co-products is to reduce feed costs and environmental pollution. Moreover, these co-products are important sources of bioactive molecules with significant advantages for human and animal health and the environment[3].

Marine products, including fish oil (FO) and plant products, which enhance the nutritive value of poultry products (eggs and meat), are sources of omega-3 polyunsaturated fatty acids (n-3 PUFAs)[1,4,5]. Linseed or flaxseed (*Linum usitatissimum* L.) is an important global annual crop valued as a feed additive and food because of its fiber and oil content. Globally, linseed production of approximately 8,7000,000 tons in 2016 was priced at approximately \$70.2[6]. Recently, there has been a growing interest in using flaxseed as a nutrient source for both humans and animals because of the high levels of protein (20%–25%), oil (35%–45%), metabolizable energy (3,800–3,960 kcal/kg), fiber (20%–25% including 10% soluble fiber), and ash (3%–4%)[7,8]. Whole flaxseed (WFS) generally provides approximately 40% oil, including 9%–10% of saturated fatty acids (mainly as palmitic and stearic acids), 20% monounsaturated fatty acids (mainly oleic acid), and PUFAs ((mainly α-linolenic acid (ALA, 18:3, n-3))[9]. However, WFS contains many anti-nutritional factors (ANFs), such as cyanogenic glycosides, tannins, phytic acid, trypsin inhibitors, anti-vitamin B6, and non-starch polysaccharides, such as mucilage[10,11]. These ANFs impair digestion and absorption and increase the viscosity of ingesta, which ultimately limits the use of WFS in poultry nutrition[12]. Therefore, various techniques, including physicochemical and biological methods, have been used to reduce ANFs[1,12,13].

Several studies have shown that WFS and its products may be used in poultry production to enhance egg and meat quality[14]. Flaxseed, flaxseed meal, and flaxseed oil are the primary sources of ALA in broiler chickens, laying hens[6], and Japanese quail[15]. *n*-3 enriched eggs and poultry meat are successful strategies to reduce the incidence of lifestyle diseases and enhance the human health index[16]. WFS (10%–20%) in the diets of laying hens increased ALA levels in egg yolk by 10–20 fold[2]. Furthermore, Scheideler et al.[17] have observed that 15% WFS in the diet increases the yolk ALA content by up to 7.1 g/100 g of fatty acids compared to that of a control diet. However, there is inconsistency in the recommended WFS levels in chicken rations, including 2%–15% for broilers[18,19] and up to 10% for laying hens[2,14]. Leeson et al.[20] have observed that 20% ground flaxseed in the diet has a negative effect on laying performance, compared to that of zero and 10% treatments. Nam et al.[21] have revealed that the inclusion of 10% WFS in the diet has no significant effect on the performance of broilers. In addition, Gonzalez-Esquerra and Leeson[22] have concluded that single-comb White Leghorn roosters tolerate up to 10% WFS in the diet in comparison to that of broiler chicks, which have a significantly lower tolerance, manifesting as diarrhea. Similarly, Al-Nasser et al.[14] have reported that 10% WFS in the diet increases n-3 PUFAs and has no undesirable impact on laying performance. Recently, Radanović et al.[23] have studied the impact of feeding mixtures with both 5% linseed oil and fish oil n-3 PUFAs. They found that consuming n-3 PUFA-enriched eggs had a positive effect on some blood biochemical indicators compared to that of feeding diets with 5% soybean oil. In addition, Aguillón-Páez et al.[24] have observed that dietary treatments with full-fat sunflower and full-fat flaxseed or seeds have no significant effect on egg quality parameters; however, the rate of lay and total egg production are significantly lower in birds receiving sunflower seeds.

Trace minerals, such as Zn, Se, and Fe, are crucial for many physiological and biochemical functions, including production and reproduction, antioxidative status, immune status, and lipid metabolism[25]. Optimal mineral concentrations are essential for laying hens because of their roles in egg production, eggshell formation, egg quality, immune response, and antioxidative properties[26]. Trace minerals are present in the form of inorganic compounds, organic compounds, and nano-particles. Organic sources are more bioavailable and stable than inorganic sources, allowing the addition of feed containing lower mineral amounts[27]. In a recent study, Arbabi-Motlagh et al.[28] have shown that organic Zn and Se supplementation in oxidized fat diets enhances hen productive performance, egg oxidative stability, and yolk fatty acids. To date, there has been a growing interest in using elemental nanoparticles owing to their low toxicity, high bioavailability, and high catalytic efficiency in broilers[29], laying hens[22], and rabbits[30].

Therefore, the present study investigated the effects of diets containing WFS and FO with different sources of trace elements (inorganic, organic, or nano-source of Se, Zn, and Fe) on egg production, egg quality, sensory attributes, and plasma metabolic profiles of laying hens.

Materials and Methods

Ethical Approval

This study was performed using general humane care of animals that refrained from causing suffering, distress, harm, or pain, as stated by Royal Decree number M59 in 14/9/1431H and Institutional Approval No. ACUC-22-1-2.

Experimental Design and Treatments

A total of 144 forty-week-old Hisex white-shell egg laying hens with a mean body weight of 1703 ± 28.7 g were used. The trial lasted for 10 weeks (40–50 weeks of age). The first 14 days (adaptation period) were not included in the study. Throughout the study, laying hens were housed in floor pens of 1.5 × 0.6 × 2.0 m, with six hens/pen and six replicates/treatment. Each pen had a feeder and drinker and food and water were provided *ad libitum.* All pens were furnished with wood shavings and hens were kept in an environmentally controlled room at 22−24 °C with a 16 h light /8 h dark daily cycle. The feeding trial design consisted of six experimental treatments, each containing eight replicates (three hens per replicate), housed in floor pens. Hens were fed six diets as follows: 1) control diet; 2) 7.5%WFS+1.5%FO; 3) 7.5%WFS+1.5%FO+175 mg/kg vitamin E (VE); 4) 7.5%WFS+1.5%FO+175 mg/kg VE + inorganic sources of Se, Zn, and Fe (ISeZnFe); 5) 7.5%WFS+1.5%FO+175 mg/kg VE + organic sources of Se, Zn, and Fe (OSeZnFe); and 6) 7.5%WFS+1.5%FO+175 mg/kg VE + nano-sources of Se, Zn, and Fe (NSeZnFe). Diets were supplemented with 0.20 mg/kg inorganic Se + 55 mg/kg inorganic Zn from Zn oxide (72% Zn) + 441 mg/kg inorganic Fe from ferrous carbonate (40% ferrous) (WFSFOVE-Inorganic-SeZnFe), 7.5% WFS + 1.5% FO + 0.20 mg/kg organic Se (from Bio-Sel, Se yeast, *Saccharomyces cerevisiae*, 0.2% with selenomethionine, min 70%) + 55 mg/kg organic Zn from Zn chelate of glycine hydrate + 441 mg/kg organic Fe from ferrous chelate of glycine hydrate (WFSFOVE-Organic-SeZnFe), or 7.5% flaxseed + 1.5% FO + 0.2 mg/kg Nano Se + 55 mg/kg Nano-Zn + 441 mg/kg Nano Fe (WFSFOVE-Nano-SeZnFe), respectively, from a nano source containing 100% concentration of each element. Organic Se was added as Bio-Sel 2000 Se yeast (0.2% Se, 70% selenomethionine; IBEX International). The organic sources of Zn and Fe were products of Wspieramy Nature (Kolejowa, Poland), as Glystatr-25% Zn and Glystar®-Fe-17%, respectively. The nano compounds were prepared by mechanical grinding (using a ball mill) by interaction with ascorbic acid (the number of acid equivalents was equal to the number of salt equivalents and therefore equal to the number of prepared nano equivalents), which performed the bio-reduction of ions and converted them into charge less nanoparticles with mechanical grinding that reduced the size of the nanoparticles to reach an average size from 1–100 nm. The nanoparticles were chemically stable compounds that were not affected by long-term storage; since the number of equivalents was equal in the preparation, there was no increase in acid or salt during the reaction, and therefore the concentration of the prepared nanoparticles was 100%. Since the compound was stable, it was added to the feed mixture throughout the production cycle, but for safety, if the production cycle was 45 days, it was added every 15 days. Whole flaxseed was purchased from a commercial supplier and used in the experiments (Table 1). The nutritional composition and fatty acid content of the experimental diets (Table 2) were computed using Centraal Veevoerder Bureau feedstuff[31] analytical values and previously reported WFS chemical assessments.

Laying Performance, Egg Quality and Sensory Attributes

Egg weight (g), egg mass (g/hen/d), laying rate (%), feed consumption (g/hen/d), and feed conversion ratio (FCR) (g/g) were assessed on a replicate basis and calculated over the course of the study. At 50 weeks of age, 28 eggs were randomly collected from each treatment on two production days (4 eggs/replicate). The quality of fresh eggs was assessed using half of the eggs, while the other half were kept for 21 days at a temperature below 25 °C. The eggs were then subjected to an estimation of egg quality, which included both internal and external measurements[32,33]. Both the interior and external qualities of eggs were evaluated[34]. Egg sensory attributes were measured (n = 7 eggs/treatment) using twenty untrained panelists to evaluate sensory characteristics. Ten eggs from each treatment were hard-boiled for 10 min, or until the water reached the boiling point, by covering the eggs in a saucepan of water in a single layer. The eggs were submerged in water at 18 °C for 5 min after the pot was removed from the hotplate. The hot water was removed and the eggs were peeled, divided lengthwise into four equal sections, and placed on plastic trays. The panelists rated the eggs according to their appearance, yolk color, albumen color, flavor, and general acceptability using a continuous unstructured line intensity scale (nine-point hedonic scale) ranging from 9 (strongly like) to 1 (strongly dislike).

Egg Nutritional Composition

A total of seven egg samples were randomly collected per treatment (one egg per replicate) and used to determine egg chemical composition. Using the Association of Official Analytical Chemistry techniques 934.01, 942.05, and 954.01, respectively, the proximate analyses of eggs, including crude protein (CP), ether extract (EE), and crude ash, were conducted[35]. The egg gross energy (GE) was determined[36]. Using the following formula, the nitrogen-free extract (NFE) was determined as: NFE = [100 − (moisture + CP + EE + CF + crude ash)].

Blood Biochemical Indicators

Blood samples (n = 7) were randomly collected from each treatment group to represent all treatment replicates for the hens at 40 weeks of age. Blood serum was separated by centrifugation at 1500 g × for 10 min. The serum total protein, albumin, uric acid, and creatinine were determined[6] using commercial kits manufactured by Diamond Diagnostics Inc. (Holliston, MA, USA). Serum globulin concentration = serum total protein - serum albumin. The albumin-to-globulin and uric acid-to-creatinine ratios were calculated.

Statistical Analysis

Data were analyzed using a one-way analysis of variance statistical analysis software program[37]. Each replicate was considered the experimental unit. Tukey's *post-hoc* test was used to compare significant differences among the means of the treatments. All percentage data were transformed into log10 values and normalized prior to analysis.

Results

The data presented in Table 3 show the effects of 7.5% WFS, 1.5% FO, and different sources of Se, Zn, and Fe on laying performance measurements in laying hens. Laying hens fed dietary treatments had no detrimental effect on laying rate, egg weight, egg mass, feed intake (FI), FCR, body weight change, or survival

Table 1. **Fatty acid profile of whole flaxseed and fish oil used in the formulation of experimental diets for laying hens.**

Chemical name		Flaxseed	Fish oil
Common Name	**Systematic Name**	**%**	**%**
Adipic acid	Hexanedioic acid	-	0.02
Lauric acid	Dodecanoic acid	-	0.25
Tridecylic acid	Tridecanoic acid	-	0.63
Myristic acid	Tetradecanoic acid	-	5.39
Pentadecylic acid	Pentadecanoic acid	-	0.98
Palmitic acid	Hexadecanoic acid	8.90	11.89
Margaric acid	Heptadecanoic acid		1.46
Stearic acid	Octadecanoic acid	7.75	5.07
Nonadecylic acid	Nonadecanoic acid	-	0.44
Arachidic acid	Eicosanoic acid	0.22	-
Heneicosylic acid	Heneicosanoic acid	-	0.40
Behenic acid	Docosanoic acid	-	1.81
Tricosylic acid	Tricosanoic acid	-	0.23
Lignoceric acid	Tetracosanoic acid	-	0.29
Cerotic acid	Hexacosanoic acid	-	0.06
ΣSFA		**16.9**	**28.9**
Myristoleic acid		-	0.17
Palmitoleic acid	9-Hexadecenoic acid	-	8.50
	11-Octadecenoic acid	-	13. 8
Oleic Acid	9-Octadecenoic acid	-	6.49
	cis-11-Eicosenoic acid	-	3.47
	13-Docosenoic acid	-	2.45
	15-Tetracosenoic acid	-	1.17
	6-Hexadecenoic acid	-	0.34
	9-Eicosenoic	-	2.08
	Petroselinic acid	-	0.13
ΣMUFA		**-**	**38.6**
	5,11,14,17-eicosatetraenoic acid	-	1.05
Eicosapentaenoic acid (EPA)	*cis*-5,8,11,14,17-eicosapentaenoic acid	-	19.5
Heneicosapentaenoic acid (HPA)	6,9,12,15,18-heneicosapentaenoic acid	-	0.94
Docosahexaenoic acid (DHA)	4,7,10,13,16,19-docosahexaenoic acid	-	8.70
α-Linolenic acid (ALA)	9,12,15-octadecatrien-1-ol, (Z,Z,Z)	61.9	0.59
	Squalene	-	0.13
Σ*n*-3 PUFA		**61.9**	**30.9**
EPA+DHA		-	29.2
Linoleic acid	9,12-octadecadienoic acid (Z,Z)-	21.2	-
γ-Linolenic acid	Gamolenic Acid	-	1.62
Σ*n*-6 PUFA		**21.2**	**1.62**
Σ*n*-6 PUFA/Σ*n*-3 PUFA		0.342	0.052
ΣUFAs		83.1	71.1
ΣSFAs + ΣUFAs		**100**	**100**

ΣSFA=sum of saturated fatty acids; ΣMUFA=sum of monounsaturated fatty acids; Σ n-3 PUFA=sum of n-3 polyunsaturated fatty acids; EPA=eicosapentaenoic acid; DHA=ocosahexaenoic acid

rate compared to those of hens fed the control diet.

Dietary treatments had no significant effect on egg grade, egg shape index, and shell thickness, whereas in fresh eggs, shell percentage and shell weight per unit surface area (SWUSA) were significantly affected by dietary treatments (Table 4). The highest shell percentage and SWUSA values were observed in

Table 2. **Composition and chemical analysis of experimental diets fed to laying hens from 40–48 weeks of age.**

	Diets					
Ingredients	**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	64.250	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
Calcium diphosphate	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
Vitamin-mineral 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	0.200	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[4]	0.95	4.60	4.60	4.60	4.60	4.60
Vitamin E, mg/kg[5]	0.0	0.0	350	350	350	350
Se, mg/kg	0.0	0.0	0.0	4.4	100	0.20
Zn, mg/kg	0.0	0.0	0.0	76.4	220	55
Fe, mg/kg	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
Calculated analysis						
ME, kcal/kg	2700	2701	2701	2701	2701	2701
Ca, %	4.04	4.04	4.04	4.04	4.04	4.04
P available, %	0.346	0.34	0.34	0.34	0.34	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 PUFA %	1.37	1.53	1.53	1.53	1.53	1.53
C18-3 PUFA, %	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
Vitamin E	25.5	25.5	200.5	200.5	200.5	200.5

[1]The 7.5%WFS+1.5%FO+VE+ISEZNFE-diets contain 7.5% flaxseed + 1.5% fish oil + 175 mg/kg Vit E + 0.2 mg/kg inorganic Se as sodium selenite (4.5% Se) + 55 mg/kg inorganic Zn from Zn oxide (72% Zn) + 441 mg/kg inorganic Fe from Fe carbonate (40% Fe). [2]The 7.5%WFS+1.5%FO+VE+OSEZNFE-diets contain 7.5% flax seed + 1.5% fish oil + 175 mg/kg Vit E+ 0.2 mg/kg organic Se from Bio-Sel, Se yeast (*Saccharomyces cerevisiae*, 0.2% with selenomethionine, min 70%) + 55 mg/kg organic Zn from Zn chelate of glycine hydrate + 441 mg/kg organic Fe from Fe chelate of glycine. Organic Se was added as Bio-Sel 2000 Se yeast (0.2% Se, 70% selenomethionine; IBEX International). The organic sources of Zn and Fe are products of Wspieramy Nature (Kolejowa, Poland), as Glystatr-25% Zn and Glystar®-Fe-17%, respectively. [3]The 7.5%WFS+1.5%FO+VE+OSEZNFE-diets contain 7.5% flax seed + 1.5% fish oil + 175 mg/kg Vit E + 0.2 mg/kg nano Se + 55 mg/kg nano-Zn + 441 mg/kg nano Fe, respectively, from nano sources containing 100% concentration of each element. [4]Building sand was added to improve crushing of the entire flaxseed in the gizzard. [5]Provided per kg diet: Vit. A 1200000 IU/kg, Vit D3 3000.000 IU/kg, VE 25.5 mg/kg, VC 30 mg/kg, Vit K3 (MNB) 2.05 mg/kg, Vit. B1 2.00 mg/kg, Vit. B2 6.00 mg/kg, Vit. B6 3.00 mg/ kg, Vit. B12 0.03 mg/kg, Niacin 30.00 mg/kg, folic acid 1.00 mg/kg, pantothenic acid 10.00 mg/kg, biotin 0.10 mg/kg, cobalt 0.40 mg/kg, copper 10 mg/kg, iodine 1.50 mg/kg, iron 9 mg/kg, manganese 80 mg/kg, Se 0.30 mg/kg, Zn 80 mg/kg, methionine 0.15%. [2]Se, Zn, and Fe have been added to replace the same amount of sand.

WFS = Whole flaxseed; FO = Fish oil; VE = Vitamin E (175 mg/kg); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

Table 3. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on laying performance.**

Dietary treatments	Laying rate, %	Egg weight, g	Egg mass, g/h/d	Feed intake, g/h/d	FCR, g/g	Body weight change, g	Survival rate, %
Control	90.3	63.0	56.9	124.4	2.19	52.7	100
7.5%WFS+1.5%FO	82.9	65.2	54.1	117.6	2.17	30.3	100
7.5%WFS+1.5%FO+VE	91.2	63.8	58.3	116.1	1.99	14.0	100
7.5%WFS+1.5%FO+VE+ISeZnFe	85.6	61.9	52.9	116.9	2.22	26.3	100
7.5%WFS+1.5%FO+VE+OSeZnFe	82.1	63.1	51.9	122.2	2.38	76.7	100
7.5%WFS +1.5%FO+VE+NSeZnFe	92.7	63.2	58.7	121.0	2.08	64.0	100
RMSE	7.61	1.84	5.04	8.43	0.254	78.1	0.0
P value	0.089	0.087	0.109	0.465	0.164	0.716	0.0

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

Table 4. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on egg shape index and eggshell quality of fresh and stored eggs.**

Dietary treatments	Egg grade	Shape index, %	Shell weight, %	Shell thickness, µm	SWUSA, mg/cm^2
		Fresh eggs			
Control	1.67	74.7	8.74^{a}	355	112.8^{ab}
7.5%WFS+1.5%FO	1.47	73.6	8.57^{a}	353	111.2^{ab}
7.5%WFS+1.5%FO+VE	1.93	75.4	8.96^{a}	361	115.0^{a}
7.5%WFS+1.5%FO+VE+ISeZnFe	1.87	74.2	8.56^{a}	346	110.4^{ab}
7.5%WFS+1.5%FO+VE+OSeZnFe	2.27	74.5	8.98^{a}	357	114.9^{ab}
7.5%WFS+1.5%FO+VE+NSeZnFe	1.87	75.0	8.33^{b}	350	106.5^{b}
RMSE	1.076	3.89	0.622	22.3	7.97
P value	0.437	0.518	0.027	0.327	0.028
		Stored eggs			
Control	2.80	74.9	10.42^{a}	350^{ab}	127.7^{a}
7.5%WFS+1.5%FO	3.30	75.7	9.44^{ab}	360^{ab}	118.3^{ab}
7.5%WFS+1.5%FO+VE	3.40	80.2	9.96^{ab}	359^{ab}	124.1^{ab}
7.5%WFS+1.5%FO+VE+ISeZnFe	3.30	76.3	9.72^{ab}	347^{ab}	119.6^{ab}
7.5%WFS+1.5%FO+VE+OSeZnFe	3.20	76.9	10.18^{ab}	371^{a}	126.2^{a}
7.5%WFS+1.5%FO+VE+NSeZnFe	3.10	76.5	9.18^{b}	341^{b}	112.8^{b}
RMSE	0.654	4.99	0.861	22.1	9.84
P value	0.390	0.257	0.022	0.050	0.013

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

hens fed the 7.5%WFS+1.5%FO+175 mg VE/kg treatment. In stored eggs, dietary WFS, FO, and different sources of Se, Zn, and Fe significantly affected shell percentage, shell thickness, and SWUSA. The highest values of shell thickness and SWUSA were observed in the 7.5%WFS+1.5%FO+VE+OSeZnFe treatment (Table 4).

The effects of the dietary treatments on the yolk and albumen quality characteristics of fresh and stored eggs are listed in Tables 5 and 6, respectively. The treatments had no significant influence on yolk percentage, albumin height, Haugh unit (HU), or egg weight change percentage in fresh or stored eggs. However, dietary WFS, FO, and different sources of Se, Zn, and Fe had a sig-

Table 5. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on egg yolk quality of fresh and stored eggs.**

Dietary treatments	Yolk weight, %	Yolk color	Yolk index, %	Yolk to albumin ratio
		Fresh eggs		
Control	26.9	8.07^{ab}	43.7 ^{ab}	0.418
7.5%WFS+1.5%FO	25.8	7.93^{ab}	43.7 ^{ab}	0.393
7.5%WFS+1.5%FO+VE	26.6	8.20^{ab}	42.6 ^{ab}	0.413
7.5%WFS+1.5%FO+VE+ISeZnFe	26.8	9.07^{a}	40.3^{b}	0.415
7.5%WFS+1.5%FO+VE+OSeZnFe	26.5	7.46^{ab}	47.4^{a}	0.411
7.5%WFS+1.5%FO+VE+NSeZnFe	26.2	7.13^{b}	46.4^{ab}	0.400
RMSE	1.71	1.52	0.146	0.0.35
P value	0.439	0.018	0.043	0.536
		Stored eggs		
Control	35.3	9.70	66.3	0.650^{a}
7.5%WFS+1.5%FO	30.6	9.80	64.1	0.511^{b}
7.5%WFS+1.5%FO+VE	32.7	10.11	63.7	0.571^{b}
7.5%WFS+1.5%FO+VE+ISeZnFe	32.2	10.20	62..3	0.55 ^{b}
7.5%WFS+1.5%FO+VE+OSeZnFe	30.9	9.70	65.2	0.525^{b}
7.5%WFS+1.5%FO+VE+NSeZnFe	35.8	9.70	62.1	0.651^{a}
RMSE	3.80	0.705	0.037	0.069
P value	0.112	0.415	0.529	0.023

[a,b] 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); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

nificant effect on yolk color and yolk index in fresh eggs, as well as the yolk-to-albumin ratio and albumin percentage in stored eggs. Incorporation of 7.5%WFS+1.5%FO+VE+ISeZnFe into laying hen diets produced the greatest yolk color in fresh eggs. The 7.5%WFS+1.5%FO+VE+OSeZnFe diet produced the highest yolk index in fresh eggs. Dietary treatment had no significant effect on egg flavor, general acceptance, albumin color, or yolk appearance in fresh eggs (Table 7).

The results presented in Table 8 show that dietary WFS, FO, and different sources of Se, Zn, and Fe significantly affected egg chemical composition. The highest values of chemical composition were observed in the 7.5%WFS+1.5%FO+VE+NSeZnFe treatment.

As shown in Table 9, different levels of FSC have no effect on serum protein metabolites, uric acid, creatinine, or their ratios. The highest plasma globulin concentrations were observed in hens fed the 7.5%WFS+1.5%FO+VE and 7.5%WFS+1.5%FO+VE+NSeZnFe treatments. However, the lowest plasma uric acid concentrations were observed in hens fed the 7.5%WFS+1.5%FO+VE+OSeZnFe and 7.5%+1.5%FO+VE+NSeZnFe treatments.

Discussion

The findings presented in Table 3 show that laying hens fed 7.5%WFS, 1.5%FO, and different sources of Se, Zn, and Fe in their diets have no detrimental effects on laying performance compared to those of hens fed the control diet. These results are consistent with those of previous studies[1,38,39], which show that laying hens withstand up to 10% WFS in the diet without adversely affecting laying performance indicators. Bean and Leeson[38] have shown that 10% dietary WFS has no significant effect on egg weight or production in brown and white laying hens. Similarly, Scheideler and Froning[40] have confirmed that laying hens tolerate up to 15% dietary WFS without any negative effects on egg-laying performance. Mattioli et al.[39] have stated that 10% extruded flaxseed in the diet does not affect egg-laying performance, including egg-laying rate, egg weight, egg yield, FCR, or final body weight of laying hens. Hosseini et al.[41] have assumed that feeding with flaxseed (270, 180, 90, and 0 g/kg) has no significant effect on egg mass, FI, or FCR. Therefore, it was reasonable to predict that dietary WFS had no negative influence on yolk formation or egg production rates. The percentage of WFS used in the diet may be increased up to 15% using additional treatments, such as adding enzyme mixtures or heat. Moghadam et al.[42] have observed that 15% heated flaxseed in the diet enhances hen day egg production and egg mass, whereas FI and egg weight are not affected. Jia et al.[43] have shown significant improvements in egg production in hens fed 15% flaxseeds and multi-carbohydrate enzymes. Aguillón-Páez et al.[24] have stated that dietary inclusion of full-fat flaxseed seeds increases egg quality compared to that of hens fed full-fat sunflower seeds.

Table 6. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on the albumin quality traits of fresh and stored eggs.**

Dietary treatments	Albumin, %	Albumin height, mm	Haugh unit score	Egg weight change, %
		Fresh eggs		
Control	64.3	5.72	69.8	-
7.5%WFS+1.5%FO	65.6	6.44	75.4	-
7.5%WFS+1.5%FO+VE	64.4	5.19	64.5	-
7.5%WFS+1.5%FO+VE+ISeZnFe	64.6	5.43	65.8	-
7.5%WFS+1.5%FO+VE+OSeZnFe	64.5	4.59	56.9	-
7.5%WFS+1.5%FO+VE+NSeZnFe	65.5	5.45	65.3	-
RMSE	1.82	2.09	22.5	-
P value	0.216	0.273	0.378	-
		Stored eggs		
Control	54.3^{b}	3.34	49.8	-9.90
7.5%WFS+1.5%FO	59.9^{a}	2.63	37.9	-8.94
7.5%WFS+1.5%FO+VE	57.3^{ab}	2.41	35.7	-8.69
7.5%WFS+1.5%FO+VE+ISeZnFe	58.1^{ab}	2.08	32.8	-9.39
7.5%WFS+1.5%FO+VE+OSeZnFe	58.9^{ab}	2.42	35.1	-9.66
7.5%WFS+1.5%FO+VE+NSeZnFe	55.0^{ab}	2.63	41.5	-10.50
RMSE	3.92	0.984	15.7	2.97
P value	0.015	0.122	0.196	0.782

$^{a–c}$ 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); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

Table 7. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on the egg sensory attributes of fresh eggs.**

Dietary treatments	Yolk appearance	Albumen color	Egg flavor	Overall acceptance
Control	7.62	7.19	7.13	7.63
7.5%WFS+1.5%FO	7.37	7.75	7.63	7.94
7.5%WFS+1.5%FO+VE	7.56	7.69	7.00	7.19
7.5%WFS+1.5%FO+VE+ISeZnFe	7.81	7.63	7.25	7.56
7.5%WFS+1.5%FO+VE+OSeZnFe	7.69	7.63	7.50	7.57
7.5%WFS+1.5%FO+VE+NSeZnFe	7.31	7.19	6.94	7.20
RMSE	1.45	1.48	1.48	1.39
P value	0.921	0.795	0.738	0.646

$^{a–c}$ 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); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

The different sources of Se, Zn, and Fe had no detrimental effects on laying performance. These data are consistent with those reported in several previous studies[44–46]. Kim et al.[45] have illustrated that the addition of an inorganic and organic mineral premix has no negative influence on egg weight, hen day egg production, FI, or FCR compared to those of hens fed the control diet. In addition, Kannan et al.[46] have shown that dietary inorganic and organic Fe at 100, 200, and 300 mg/kg has no significant effect on egg production rate, FI, or egg weight in laying hens. In a study, Buckiūnienė et al.[44] have concluded that different dietary Fe sources have no significant effect of laying performance, including rate of egg production, FI and FCR in laying hens. Estradiol-17β stimulates yolk lipoprotein precursor synthesis, such as vitellogenin and very-low-density lipoprotein (VLDL) in the liver, which are then absorbed by the developing yolk via an endocytosis mediated receptor[47].

Table 8. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on chemical composition of whole edible egg parts.**

Dietary treatments	Dry matter, %	Crude protein, %	Ether extract, %	Ash, %	NFE, %	GE, cal/g
Control	50.6^{b}	17.0^{ab}	31.3^{b}	0.971	0.800	350^{d}
7.5%WFS+1.5%FO	50.6^{ab}	16.8^{ab}	31.6^{ab}	0.895	0.800	369^{c}
7.5%WFS+1.5%FO+VE	50.4^{ab}	16.5^{b}	31.7^{ab}	0.912	0.803	388^{b}
7.5%WFS+1.5%FO+VE+ISeZnFe	50.7^{ab}	16.8^{ab}	31.7^{ab}	0.917	0.813	392^{ab}
7.5%WFS+1.5%FO+VE+OSeZnFe	50.5^{ab}	16.6^{b}	31.7^{ab}	0.926	0.788	397^{ab}
7.5%WFS+1.5%FO+VE+NSeZnFe	51.5^{a}	17.3^{a}	32.1^{a}	0.927	0.802	400^{a}
RMSE	0.481	0.309	0.413	0.059	0.021	5.97
P value	0.004	0.002	0.105	0.375	0.518	<.0001

[a,b,c] Means within a column within each factor not sharing similar superscripts are significantly different, $P < 0.05$; NFE = nitrogen free extract; GE = gross energy; RMSE = Root mean square error; WFS = Whole flaxseed; FO = Fish oil; VE = Vitamin E (175 mg/kg); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

Table 9. **Effect of whole flaxseed, fish oil, and different sources of Se, Zn and Fe fed to laying hens from 40–48 weeks of age on the levels of serum protein fractions.**

Dietary treatments	Total protein, g/dL	Albumin, g/dL	Globulin, g/dL	Albumin/globulin	Creatinine, mg/dL	Uric acid, mg/dL	Uric acid/creatinine ratio
Control	5.23^{a}	3.73^{a}	1.50^{b}	2.48^{a}	1.054^{a}	3.01^{a}	2.86^{abc}
7.5%WFS+1.5%FO	5.22^{a}	3.72^{a}	1.50^{b}	2.48^{a}	1.055^{a}	3.00^{bc}	2.84^{d}
7.5%WFS+1.5%FO+VE	5.21^{b}	3.70^{b}	1.52^{a}	2.45^{b}	1.045^{b}	2.99^{bc}	2.87^{a}
7.5%WFS+1.5%FO+VE+ISeZnFe	5.20^{c}	3.69^{c}	1.49^{b}	2.47^{a}	1.044^{b}	3.00^{b}	2.87^{a}
7.5%WFS+1.5%FO+VE+OSeZnFe	5.19^{c}	3.71^{b}	1.49^{b}	2.50^{a}	1.049^{b}	2.98^{c}	2.85^{cd}
7.5%WFS+1.5%FO+VE+NSeZnFe	5.21^{b}	3.71^{b}	1.51^{a}	2.44^{b}	1.047^{b}	2.98^{c}	2.85^{bc}
RMSE	0.008	0.004	0.007	0.013	0.004	0.006	0.011
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

[a–d] 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); ISeZnFe = Inorganic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); OSeZnFe = Organic source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg); NSeZnFe = Nano-source of Se (0.20 mg/kg), Zn (55 mg/kg), and Fe (441 mg/kg).

Plasma Zn levels indicate vitellogenin in the bloodstream[48]. The present study found that the incorporation of 7.5%WFS, 1.5%FO, and different sources of Se, Zn, and Fe did not alter plasma levels of VLDL and Zn (data not shown). Therefore, dietary 7.5%WFS+1.5%FO+VE+OSeZnFe did not negatively affect folliculogenesis or the growth of ovarian follicles, which, in turn, was associated with the rate of egg production in the current study.

Dietary treatment had no effect on external egg quality features, including egg grade, egg shape index, and shell thickness (Table 4). These results correspond with those of Mattioli et al.[39], who have shown that the inclusion of 10% extruded flaxseed in the diet has no significant effect on shell quality traits. Bean and Leeson[38] have observed that 10% WFS in the diet has no significant effect on shell weight, albumin height, or shell thickness in laying hens. Likewise, Hosseini et al.[41] have shown that dietary extruded flaxseed at–0–2700 g/kg in 90 g/kg increments has no effect on shell thickness or strength. Moghadam et al.[42] have observed that 15% heated flaxseed in the diet has no significant effect on shell weight or thickness. Similarly, Panaite et al.[49] have shown that 5% WFS supplemented with different levels of dried tomato waste (2.5%, 5.0%, and 10.0%) in the diet does not affect shell weight, shell thickness, or shell-breaking strength in laying hens. These data suggest that WFS had no negative effects on eggshell formation and did not interfere with the supply of calcium required for shell formation. This assumption has been confirmed by the results of Caston et al.[50], who have postulated that 20% WFS in the diet has no influence on eggshell deformation or absolute shell weight. In addition, Ebeid[34] and Bozkurt et al.[51] have reported similar outcomes when hens are fed flaxseed oil or FO as a n-3 PUFAs source. In the literature, different sources of Fe[44] and Zn[27] have no significant effects on external egg quality parameters or sensory attributes. However, Ogbuewu and Mbajiorgu[52] have indicated that dietary Zn supplementation enhances eggshell thickness in laying hens. Arbabi-Motlagh et al.[28] have reported that dietary organic Zn (20 mg/kg) plus organic Se (0.2 mg/kg) in oxidized fat diets is beneficial for improving shell quality characteristics (shell thickness and shell breaking strength) in laying hens.

The results in Tables 5 and 6 show that dietary treatments have no significant effect on yolk percentage, albumin height, HU, or egg weight change percentage in both stored and fresh eggs. These results are in agreement with previous findings[2,50] reporting that dietary WFS has no negative effect on yolk weight or relative yolk weight. Furthermore, adding 10% WFS to the diets of laying hens does not significantly influence the albumin percentage, albumen height, or HU score, according to other studies[38,53]. Recently, Hosseini et al.[41] have postulated that graded amounts of dietary extruded flaxseed have no significant effect on the HU score. Similarly, Mattioli et al.[39] have shown that 10% extruded flaxseed in the diet does not affect the HU score, whereas the percentage of albumin increased. Additionally, 15% heated flaxseed in the diet has no significant effect on HU, albumin weight and thickness, or yolk weight, height, and width[42].

As shown in Table 5, dietary WFS, FO, and different sources of Se, Zn, and Fe have a significant effect on yolk color and yolk index in fresh eggs, as well as the yolk-to-albumin ratio and albumin percentage in stored eggs. Incorporation of 7.5%+1.5%FO+VE+ISeZnFe into laying hen diets produced the greatest yolk color in fresh eggs. The 7.5%+1.5%FO+VE+OSeZnFe diet produced the highest yolk index in fresh eggs. Similarly, Westbrook and Cherian[54] have revealed that the addition of a 0.05%–0.1% enzyme mixture to 10% flaxseed diet enhances yolk weight. The yolk color results are in agreement with those of Moghadam et al.[42], who have observed that 15% heated flaxseed in the diet improves yolk color score, and this improvement may be related to enhanced digestibility and consequently more utilization of antioxidants and pigmentation in flaxseeds. Furthermore, dietary flaxseed oil and/or FO supplementation significantly positively affects egg yolk color[34]. Cachaldora et al.[55] have reported similar findings, demonstrating that n-3 PUFA oil-enriched diets improve yolk color compared to that of the control diet. Dietary carotenoids are fat-soluble; therefore, intestinal absorption is expected to increase in tandem with lipid absorption[34]. Notably, improving yolk color has a positive effect on customer choice of eggs produced by chickens fed flaxseed, which is an additional advantage of raising n-3 PUFA levels[50].

Dietary WFS, FO, and different sources of Se, Zn, and Fe had no significant effect on yolk appearance, general acceptance of albumin color, or flavor in fresh eggs. These results agree with those of Hayat et al.[56], who have revealed that adding 10% WFS to the diet of laying hens has no adverse effects on egg sensory characteristics and that eggs are acceptable to untrained panelists and consumers. In addition, Aguillón-Páez et al.[24] have reported that dietary inclusion of full-fat sunflower or full-fat flaxseed seeds has no significant effect on egg quality parameters. Likewise, Buckiūnienė et al.[44] have reported that dietary rapeseed oil plus organic or inorganic Se plus VE has no undesirable effect on egg sensory characteristics (odor, taste, color, and acceptability), and texture traits (hardness, cohesiveness, springiness, and chewiness). Dietary Se and Zn improve antioxidative properties and reduce lipid peroxidation[25,26,48], which is associated with enhanced oxidative stability of egg yolks, leading to improved sensory attributes. Imran et al.[57] have shown that most untrained evaluators do not discriminate between *n*-3-enriched eggs and controls that come from laying hens fed a 10% extruded flaxseed meal in their diet. Mazalli et al.[58] have reported no unpleasant fishy flavor in hard-boiled eggs obtained from chickens fed 9% WFS. Professional panelists, however, detect variations in organoleptic properties in eggs produced by laying hens fed flaxseed meal, especially at high levels[57]. Hence, it is critical to emphasize the importance of careful consideration when using WFS in the diet of laying hens.

The findings presented in Table 8 show that dietary WFS, FO, and different sources of Se, Zn, and Fe have significant effects on the chemical composition of eggs, including dry matter, CP, EE, and GE. However, few studies have investigated this aspect. Mattioli et al.[39] have observed that 10% extruded flaxseed in the diet has no influence on egg chemical proximate analyses, including dry matter, EE, ash, and CP, whereas egg cholesterol content is reduced.

As shown in Table 9, different levels of FSC have a significant effect on serum protein metabolites, uric acid, creatinine, and the uric acid:creatinine ratio. Similarly, Hosseini et al.[41] have hypothesized that adding extruded flaxseed to the diet has no significant effect on total protein, albumin, globulin, or albumin:globulin ratio in hens. In addition, Shafey et al. [59] have observed that adding 5% or 10% flaxseed meal to laying hen diets have an insignificant effect on serum protein metabolites in laying hens. The current study found that the highest plasma globulin concentrations were observed in hens fed the 7.5%WFS+1.5%FO+VE and 7.5%WFS+1.5%FO+VE+NSeZnFe treatments. These results may be attributed to the role of trace mineral nanoparticles in improving protein and immunoglobulin synthesis[60].

In conclusion, feeding laying hens up to 7.5% WFS had no negative effects on laying performance indices, quality characteristics, sensory attributes, chemical composition of eggs, or blood plasma biochemical parameters. Furthermore, 7.5%WFS+1.5%FO+VE+OSeZnFe in the diet enhanced shell thickness, SWUSA, yolk color, yolk index, and plasma globulin concentration in laying hens.

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Author Contributions

Conceptualization: Y.A.A.; methodology: Y.A.A., M.A.-H., A.A.Al-S., EL-S. O.S.H., and M.J.O.; software: Y.A., N.M.A., and M.J.O.; data collection: M.J.O., EL-S. O.S.H., and A.A.Al-S.; investigation: Y.A.A., M.J.O., N.M.A., A.A.Al-S., M.J.O., G.M.S., EL-S., and O.S.H; resources: A.A.Al-S., EL-S., O.S.H., T.A.E., and M.J.O.; writing—original draft preparation: Y.A.A., T.A.E., N.M.A., and M.J.O.; writing—review and editing: Y.A.A., N.M.A., M.J.O., T.A.E., V.T., M.R., and A.A.Al-S.; project administration and supervising: Y.A.A.; funding acquisition: Y.A.A. All the authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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