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REGULAR ARTICLES

Dietary flaxseed cake influences on performance, quality, and sensory attributes of eggs, serum, and egg trace minerals of laying hens

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Abstract

Nowadays, there is a global shortage in feed supply for animal nutrition; however, there are a considerable amount of agro-industrial co- and by-products that may offer a reasonable solution. Flaxseed cake (FSC) is a by-product of flaxseed for oil extraction rich in n-3 α-linolenic acid (ALA). Thus, the dietary inclusion of FSC on laying performance, egg quality, and serum and egg trace elements (Se, Zn, and Fe) was evaluated using Hisex White hens. The hens were distributed to three equal experimental treatments and provided diets including 0%, 5%, or 10% FSC from 48 to 58 weeks of age. Findings clarified that up to 10% FSC in the laying hen diet had no detrimental effect on laying rate, egg mass, and feed utilization. It was found that FSC resulted in a valuable source of protein, energy, macro- (Ca and P), micro- (Se, Zn and Fe) elements, and essential amino acids, with arginine being the highest. Dietary FSC did not negatively influence the egg quality traits, as well as egg sensory attributes. Including 5% or 10% FSC in diet did not significantly affect serum total protein and renal function in terms of creatinine, uric acid, and uric acid-to-creatinine ratio. Different FSC levels did not influence the chemical composition of eggs and trace elements in serum and eggs. It could be concluded that FSC is a valuable feedstuff that can provide a good source of energy, protein, amino acids, and macro- and micro-elements for hens' nutrition. The inclusion of up to 10% of FSC in hens diet did not adversely influence egg laying performance, egg quality of both fresh and stored eggs, sensory attributes, and nutritional composition, as well as Se, Zn, and Fe in serum and eggs due to balanced nutrient profile of FSC.

Keywords Flaxseed cake · Laying hens · Egg quality · Serum biochemistry · Trace elements

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Introduction

Recently, traditional feedstuffs, like soybean meal, have been suffering from a long-term imbalance between stock and request, leading to a rise in feed cost and a decrease in the maintainability of the poultry and livestock industry (Hafez and Attia, 2020). Also, in countries that do not grow soybeans, there is a trend to substitute it with alternative sources of vegetable protein and crop by- or co-products in animal production (Volek et al., 2018). Both agro-industrial by-/co-products can also reduce feed costs and environmental pollution (Tufarelli et al., 2022). Moreover, the co-products could be a reservoir of valuable metabolites with essential biological effects (Brunetti et al., 2022), adding extraordinary value to final products and also safeguarding the environment and human and animal health (Vastolo et al., 2022).

Enhancing meat and eggs with omega-3 fatty acid sources increases the nutritive value and the selling value of poultry products and meets the consumer desire for added-value animal products (Ebeid et al., 2008a; Beheshti Moghadam and Cherian, 2017; Maina et al., 2023). Cold-pressed flaxseed cake (FSC) is a valuable by-product for animal and poultry nutrition and has a considerable amount of omega-3 fatty acids (Attia, 2003; Xu et al., 2022). It had significant levels of residual oil (~ 10%) and crude protein (~ 30–34.9%) when cold pressing extraction of oil was used at about 35 °C (Chen et al., 2014; Attia et al., 2022a), and minerals (Bernacchia et al., 2014; Shakeel et al., 2017). However, flaxseeds contain non-starch polysaccharides (NSP), such as mucilage, and numerous anti-nutritional factors, such as tannins, cyanogenic glycosides, anti-vitamin B_6, trypsin inhibitors, and phytic acid, which are concentrated in the flaxseed meal (Xu et al., 2022). These anti-nutritional factors limit FSC use in poultry diets. Therefore, several methods are used for destroying such anti-nutritional factors in flaxseed, including the physicochemical way (boiling, soaking in warm water, extruding, solving, roasting, microwaving, and autoclaving) and biological processes (microbial and enzymatic fermentation) (Qota et al., 2002; Alzueta et al., 2002; Attia et al., 2022a).

Dietary inclusion of FSC in layer diets might enhance the laying performance, egg quality characteristics, yolk and plasma lipid profiles, antioxidative status, and immunity (Attia et al., 2022a). It was reported that dietary inclusion of 10% or 20% whole flaxseeds in laying hen diets elevated ALA concentration in egg yolk by 10- and 20-fold, respectively (Caston and Leeson, 1990). Similarly, Scheideler et al. (1998) observed that dietary 15% whole flaxseeds increased yolk ALA content to 7.07 g/100 g of fatty acids compared to a control diet (0.26 g/100 g of fatty acids). In addition, total antioxidant status and thiobarbituric acid reactive substances of 30% genetically modified FSC were substantially improved (Matusiewicz et al., 2014). However, there is a discrepancy in the suggested level of FSC in poultry rations, containing 2–15% for broiler chickens (Qota et al., 2002; Attia, 2003) and 6–15% for laying hens (Richter et al., 1998; Al-Nasser et al., 2011; Halle and Schöne, 2013) depending by FSC type. It was reported by Nam et al. (1997) that the inclusion of 10% flaxseed had no noteworthy impact on the growth of broilers. Also, Al-Nasser et al. (2011) found that dietary 10% flaxseed increased omega-3 fatty acids and had no negative impact on layer performance. Most previous studies used soaked-treated FSC, thermal processing, or vitamin B6 supplementation, which increased the feed cost (Imran et al., 2015; Panaite et al., 2021; Attia et al., 2022a). In addition, the effect of FSC on serum and egg trace minerals (Se, Zn, and Fe) is absent in the literature; however, these minerals are of particular interest for human nutrition and health (Robberecht et al., 2020; Frydrych et al., 2023). It could be hypothesized that laying hens might tolerate up to 10% un-soaked FSC as an alternative feed ingredient. Therefore, the present study aimed to fill the gap of using different levels of untreated FSC (0%, 5%, or 10%) on egg production, quality and sensory attributes, serum protein profile, and both serum and egg trace mineral (Se, Zn, and Fe) profiles in commercial laying hens.

Material and methods

Ethical approval

This work was conducted according to the guidelines of human treatment of animals that keep animal welfare as cited by Royal Decree number M59 in 14/9/1431H and institutional approval number ACUC-22–1-2.

Preparation of flaxseed cake

A brown variety of flaxseed was purchased from a local supplier in Riyadh, Saudi Arabia, and then cold-pressed for oil extraction using an electric screw-pressed machine at ~ 40 °C. The by-product obtained (flaxseed cake, FSC) was then grounded into 2-mm particles and included in laying hen diets.

Experimental design

A total of 63 Hisex layers with an average body weight of 1712 ± 43.6 g were used in the present study. The trial lasted 10 weeks, from 48 to 58 weeks of age. The initial two weeks served as a period of adaptation to diets and were not included in data collection. The experimental design contained three dietary treatments, each having seven replicates (three hens/each), and each replicate was housed in 1 × 1 m floor pens furnished with wood shaving as a litter material. Each pen was provided with a 50-cm-round tube feeder and one stainless steel cube drinker. Laying hens were fed diets including 0%, 5%, and 10% FSC, respectively. The dietary FSC levels were chosen based on previous results by Caston and Leeson (1990), Scheideler et al. (1998), Matusiewicz et al. (2014), and Attia et al. (2022a). However, lower levels were used herein due to using cold-pressed raw FSC without any treatment to overcome the anti-nutritional factors in FSC (Shakeel et al., 2017; Xu et al., 2022). The proximate chemical analysis and mineral and amino acid profile of FSC were reported in Table 1 and 2. The composition of the experimental diets was displayed in Table 3. The nutritional makeup and fatty acid contents of experimental diets were computed based on the analytical values of ingredients (CVB Feed Table, 2018) and the actual analyses of FSC (Table 1 and 2).

Laying performance and egg quality

The performances of laying hens [i.e., feed intake (g/hen/d), rate of laying (%), egg weight (g), egg mass (g/hen/d), and FCR (g/g)] were assessed on the replicate basis and estimated for 50–58 weeks of age. At 58 weeks old, four eggs were randomly gathered from each replicate (28 eggs/treatment) for two consecutive production days. The eggs were divided into two equal parts: one part was used for the determination of egg quality of fresh eggs, and the other portion was stored at ~ 25 °C for 21 days and subjected to determine egg quality after storage. The egg quality measurements were assessed as reported in previous studies (Wesley and Stadelman, 1959; Ebeid et al., 2012). The external egg quality traits included egg shape index, shell weight and percentage, shell weight per unit surface area (SWUSA), and shell thickness. The internal egg quality characteristics assessed were albumen and yolk weight and yolk-to-albumen ratio. The albumen height was measured to estimate the Haugh unit score. The yolk index was calculated by dividing the yolk height by its diameter and multiplying it by 100. The egg yolk color score was investigated using the Roche yolk color fan (Ebeid, 2011).

Egg sensory attributes were measured as reported by Attia et al. (2022a) using seven eggs per treatment by recruiting 20 untrained panelists with no self-cited egg allergy or intolerance. The panelists gauged the sensory properties and were told not to smoke or eat for at least two hours prior to the assessment. The panelists assessed the eggs on a constant unstructured line intensity scale (9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely) in terms of edible egg appearance, yolk color, albumen color, flavor, and overall acceptability.

Table 1 Proximate chemical composition, amino acids, and mineral contents of flaxseed cake

Nutrients	Present study	Literature	Laying hen requirements
Dry matter,%	93.29	89.35[1]–90.3[2]	90.0
Crude protein,%	34.46	27.78[1]–43.3[2]	18.8
Ether extract,%	9.38	29.37[1]–1.67[2]	1.00–1.10
Crude fiber,%	1.61	7.02[1]	3–5%
Ash,%	4.77	3.40[1]–6.40[2]	NR
Nitrogen-free extract,%	41.24	21.78[1]–48.7[2]	NR
Gross energy, kcal/kg	10.48	12.6[3]	12.13
Ca, g/kg	1.65	4.3[4]	4.06
P, g/kg	3.70	9.0[4]	0.31
Se, µg/kg	196.7	NR	80
Zn, mg/kg	42.7	68[4]	44
Fe, mg/kg	87.1	175[4]	56
Mn, mg/kg	21.6	40[4]	25
Cu, mg/kg	11.6	19[4]	NR

[1]Gutiérrez et al. (2010)

[2]Mueller et al. (2010)

[3]AMEn value

[4]Feedipedia (2022)

NR, not reported

Chemical composition of flaxseed cake, diets, and eggs

Samples of flaxseed cake ($n = 3$), diets ($n = 3$), and eggs ($n = 7$) per treatment were gathered to determine the proximate chemical composition on a triplicate basis. The crude protein (CP), ether extract (EE), ash, and crude fiber (CF) were determined according to AOAC methods: 934.01, 954.01, 942.05, and 978.10, respectively (AOAC, 2007). The moisture content was assessed using the method ISO 6496–1999. The nitrogen-free extract (NFE) was calculated by the difference of using the following equation: $NFE = [100 - (moisture + CP + EE + CF + ash)]$. The gross energy (GE) was assessed using an adiabatic bomb calorimeter (Parr Instrument Co., Moline, IL; USA), according to McGill et al. (2004).

The amino acid (AA) profile of FSC was analyzed using freeze-dried samples that were ground through a 1-mm mesh screen and immediately prepared for the analysis of CP (AOAC 2007) and AA. Samples were hydrolyzed for 24 h with 6NHCl at 110 °C to determine AA by High-Speed Amino Acid Analyzer (Hitachi LA8080 Amino-SAAYA). Amino acid profiles were determined according to AOAC International (2007), with methionine and cysteine being analyzed as methionine sulfone and cysteic

Table 2 Amino acids composition of the current flaxseed cake sample compared with literature value and laying hen requirements (National Research Council, 1994)

Amino acids (mg/100 g) as fed basis	Present study	Literature[1]	Laying hen requirements[2]
Essential amino acids			
Methionine	0.59	0.65	0.38
Methionine + cystine[3]	1.50	1.26	0.73
Lysine	1.17	1.36	0.86
Threonine	1.11	1.33	0.59
Tryptophan[3]	0.20	0.55	0.20
Arginine	2.34	3.27	0.88
Histidine	0.53	0.85	0.21
Phenylalanine	1.41	1.64	0.59
Phenylalanine + tyrosine	2.09	2.49	1.04
Iso-leucine	1.21	1.50	0.81
Leucine	1.72	2.05	1.03
Valine	1.38	1.77	0.88
Total essential amino acids	13.25	16.43	7.23
Nonessential amino acids			
Alanine	1.38	1.60	NR
Aspartic acid	2.38	3.21	NR
Glycine	1.66	2.05	NR
Proline	0.28	1.43	NR
Serine	2.44	1.64	NR
Asparagine	2.14	NR	NR
Glutamine	5.27	NR	NR
Glutamic acid	6.46	6.96	NR
Total nonessential amino acids	22.01	16.89	NR
Total amino acids	35.26	33.32	7.23

[1]Feedipedia (2022)

[2]As a percent of the diet

[3]Cystine and tryptophan, being partly destroyed under acid hydrolysis

NR, not reported

acid after performic acid oxidation. Cystine and tryptophan were partly lost under acid hydrolysis. Nitrogen analysis was carried out with a Leco N analyzer with Pump 1 buffer solutions and RG, ammonia filtration at 20–80 °C, TDE3 reactor at 125–140 °C, detector at 440–570 nm, and Pump 2 ninhydrin reagent and washing solution. All samples were assayed in triplicate.

To determine the content of trace elements (Ca, P, Se, Zn, Fe, Mn, and Cu) in flaxseed cake, samples were dried in an oven at 105 °C until constant weight; then, a 2-g sample was put in a flask and digested with a mixture of concentrated HNO_3 and H_2SO_4 (3:1 v/v) (AOAC, 2007). The digestion process was continued until the solution became clear. The samples were transferred to another flask and diluted to 25 mL with distilled water (Pawan et al., 2006). The estimation of trace elements was assessed using a Varian ICP-Optical Emission Spectrometer (ICP-OEM): Varian 720-ES, as described by Olajire and Ayodele (1997).

Blood biochemical constituents and trace elements

Blood samples ($n = 7$/treatment) were collected at week 58. The serum separated the blood after centrifugation at 1500 g × 10 min to represent all treatment replicates, which were randomly gathered at blood serum total protein, albumin, creatinine, and uric acid, which were determined according to Attia et al. (2022a) using the commercial kits produced by Diamond Diagnostics (El-Montazah St. Heliopolis, Cairo, Egypt; http://www.diamonddiagnostics.com). The globulin content was estimated as the difference between the total protein and albumin. The albumin-to-globulin ratio and uric acid-to-creatinine ratio were computed.

On serum and whole edible eggs, the contents Se (Prohaska et al., 2000), Zn (de Blas et al., 1994), and Fe (Ronald et al., 1983) were determined ($n = 7$ per treatment) using a Varian ICP-Optical Emission Spectrometer (Varian 720-ES, ICP-OEM, Leuven, Belgium).

Table 3 Ingredients and chemical analysis of the experimental diets fed to laying hens

Ingredients, %	Flaxseed cake level, %		
	0	5	10
Yellow corn	64.5	61.5	58.47
Soybean meal, 48% CP	24.0	22.0	20.00
Flax seed cake	–	5.0	10.0
Calcium diphosphate	1.30	1.30	1.30
Calcium carbonate	9.00	9.00	9.00
Sodium chloride	0.30	0.30	0.30
Vitamin–mineral premix*	0.50	0.50	0.50
DL-methionine	0.15	0.15	0.18
L-lysine	0.10	0.10	0.10
Sodium bicarbonate	0.10	0.10	0.10
Choline chloride	0.05	0.05	0.05
Determined analysis			
Dry matter %	93.39	94.24	94.54
Crude protein %	16.69	16.74	17.03
Crude fat %	2.58	3.93	5.16
Crude fiber %	3.21	3.61	4.13
Ash,%	17.43	15.90	15.53
Se, ppm	0.367	0.384	0.395
Zn, ppm	83.5	87.8	92.7
Fe, ppm	233.4	244..8	258.0
Calculated analysis			
Metabolizable energy, kcal/kg diet	2731	2722	2700
Calcium %	4.06	4.05	4.0
Phosphorus available %	0.351	0.367	0.344
Methionine %	0.412	0.424	0.503
Lysine,%	0.885	0.886	0.892
C18-2 PUFAs, %	0.486	0.467	0.447
C18-3 PUFAs, %	0.0202	0.0445	0.0691
C18:2/C18:3 ratio	24.1	10.5	6.50

*Three kg of vitamin – mineral premix per ton of feed supplied each kg of diet with vitamin A 12,000 IU; vitamin D3 2000 IU; vitamin E 10 mg; vitamin k3 2 mg; vit. B1 1 mg; vitamin B24 mg; vitamin B6 1.5 mg; pantothenic acid 10 mg; vitamin B12 0.01 mg; folic acid 1 mg; niacin 20 mg; biotin 0.05 mg; choline chloride,50% choline 500 mg; Zn 55 mg; Fe 30 mg; I 1 mg; Se 0.1 mg; Mn 55 mg; and ethoxyquin 3000 mg

Statistical analysis

The normality of data, appropriate sample size, and differences among treatments were statistically analyzed by one-way ANOVA (SAS Institute User's Guide, 2012). The experimental unit was the replicate. Tukey's post hoc test was used to compare the significant differences among treatment means. All percentages were transformed to $\log_{10}$ for data distribution normalization prior to perform statistical analysis.

Results

Chemical composition, trace minerals, and amino acid profile of flaxseed cake

Results in Table 1 and 2 reported the proximate chemical composition, macro- and micro-elements and amino acids profiles, laying hens requirements, and literature values. The results showed considerable nutrients: 34.46% CP, 9.38% EE, and 41.24% NFE. The flaxseed cake contained a good amount of Ca (1.65 g/kg), P (3.70 g/kg), Se (196.7 μg/kg), Zn (42.7 mg/kg), Fe (87.1 mg/kg), Mn (21.6 mg/kg), and Cu (11.6 mg/kg). The amino acid profile (Table 2) showed 1.50% methionine and cystine, 1.17% lysine, 1.11% threonine, 2.34% arginine, and 0.20% tryptophan. The other essential amino acids were 0.53%, 2.09%, 1.21%, 1.72%, and 1.38% for histidine, phenylalanine, iso-leucine, leucine, and valine, respectively. The nonessential amino acids ranged from 0.28% for proline and 6.46% for glutamic acid. The sum of essential amino acids was 13.25%, and for nonessential amino acids, it was 22.01%.

Effect of flaxseed cake levels on laying performance

Data in Table 4 showed the effect of FSC levels on the performance of laying hens. Although feeding 5% FCS reduced egg rate, laying hens fed 10% FSC in the diet did not have a detrimental effect on laying rate, egg mass, and FCR compared to layers in the control diet. The highest

Table 4 Effect of flaxseed cake levels on the laying performance of Hisex laying hens

Flaxseed cake, %	Laying rate, %	Egg weight, g	Egg mass, g/h/d	Feed intake, g/d	Feed efficiency, g/g	Body weight change, g	Survival rate, %
0	85.7[a]	62.2	53.3[ab]	111.0[b]	2.08[b]	76.2	100
5	78.8[b]	60.3	47.6[b]	121.4[ab]	2.60[a]	87.2	100
10	87.5[a]	64.0	56.0[a]	131.8[a]	2.35[ab]	71.6	100
RMSE	3.13	2.36	0.428	9.85	0.279	63.7	-
p value	0.006	0.085	0.012	0.022	0.041	0.925	-

[a,b]Means within a column within each factor not sharing similar superscripts are significantly different, $P < 0.05$

RMSE, root mean square error

Table 5 Effect of flaxseed cake levels on egg shape index and eggshell quality traits of fresh and stored eggs of Hisex laying hens

Flaxseed cake, %	Egg grades	Egg shape index, %	Shell weight, %	Shell thickness, µm	SWUSA, mg/cm^2
Fresh eggs					
0	1.80	73.5	8.90	353	113.7
5	2.40	76.2	9.04	348	114.1
10	1.80	75.3	8.62	341	111.4
RMSE	0.598	3.54	0.577	29.05	9.90
p value	0.167	0.121	0.452	0.219	0.541
Stored eggs (21 days)					
0	2.80	78.4	9.23	339	114.7
5	3.10	76.9	9.06	353	120.5
10	3.30	73.8	9.07	343	114.4
RMSE	1.07	5.94	0.895	27.1	11.7
p value	0.544	0.227	0.332	0.517	0.437

SWUSA, shell weight per unit of surface area; *RMSE*, root mean square error

Table 6 Effect of different levels of flaxseed cake on egg yolk quality traits of fresh and stored eggs of Hisex laying hens

Flaxseed cake, %	Yolk,%	Yolk color	Yolk index, %	Yolk/albumen ratio
Fresh eggs				
0	29.2^{ab}	8.80	48.5	0.471^{ab}
5	30.6^{a}	9.30	47.9	0.507^{a}
10	27.1^{b}	9.80	47.6	0.430^{b}
RMSE	2.23	1.13	0.243	0.0071
p value	0.005	0.162	0.196	0.0132
Stored eggs				
0	32.0	7.13	52.9	0.545^{b}
5	35.6	7.62	55.3	0.649^{a}
10	33.3	6.69	54.6	0.576^{ab}
RMSE	5.38	1.72	7.95	0.009
p value	0.351	0.312	0.265	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

Table 7 Effect of flaxseed cake levels on the albumen quality traits of fresh and stored eggs of Hisex laying hens

Flaxseed cake, %	Albumen, %	Albumen height, mm	Haugh unit	Egg weight change,%
Fresh eggs				
0	61.9^{ab}	5.05	64.7	-
5	60.4^{b}	4.25	57.2	-
10	64.4^{a}	5.21	68.0	-
RMSE	3.45	1.47	17.3	-
p value	0.003	0.175	0.227	-
Stored eggs				
0	58.7	3.48	51.4	−8.17
5	54.8	2.63	37.7	−6.75
10	57.6	2.71	37.4	−7.11
RMSE	5.29	1.19	20.51	3.52
p value	0.247	0.229	0.235	0.649

^{a,b}Means within a column within each factor not sharing similar superscripts are significantly different, $P<0.05$

RMSE, root mean square error

significant feed intake was obtained in hens fed 10% FSC compared to the control diet. There was no significant effect of dietary 5 or 10% FSC on egg weight, body weight change, and survival rate of hens.

Effect of flaxseed cake levels on egg quality characteristics

Different levels of FSC did not significantly affect egg grade, egg shape index, shell weight and thickness, and SWUSA of fresh and stored eggs, as reported in Table 5.

The effect of FSC levels on yolk and albumen characteristics in fresh and stored eggs was illustrated in Table 6 and 7. Diet including 10% FSC did not significantly affect yolk weight, index, yolk-to-albumen ratio, albumen percentage and height, Haugh unit score, and egg weight change of both fresh and stored eggs. However, the incorporation of 5% FSC in the diet increased yolk percentage and yolk-to-albumen ratio in stored eggs compared to control. Even though the differences were not significant ($P=0.162$), there was an enhancement trend in yolk color due to the addition of 5% or 10%

Table 8 Effect of flaxseed cake levels on the egg sensory attributes of fresh eggs of Hisex laying hens

Flaxseed cake, %	Yolk appearance	Albumen color	Egg flavor	General acceptance
0	7.63	7.19	7.13	7.63
5	7.31	7.44	7.38	7.37
10	6.75	6.63	6.81	6.75
RMSE	1.79	1.901	1.791	1.744
p-value	0.381	0.471	0.676	0.352

RMSE, root mean square error

Table 9 Effect of flaxseed cake levels on the chemical composition of eggs of Hisex laying hens

Flaxseed cake, %	Dry matter, %	Crude protein, %	Ether extract, %	Ash, %	NFE, %	GE, cal/g
0	48.3^{ab}	15.9	30.4	0.818	1.18	3.24
5	48.1^{b}	15.8	30.1	0.845	1.36	3.18
10	48.9^{a}	16.5	30.3	0.861	1.24	3.17
RMSE	0.135	0.436	0.262	0.058	0.028	0.067
p value	0.001	0.061	0.272	0.448	0.209	0.246

^{a,b}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

Table 10 The effect of flaxseed cake levels on blood serum biochemical parameters of Hisex laying hens

Flaxseed cake, %	Total protein, g/dl	Albumin, g/dl	Globulin, g/dl	A:G	Creatinine, mg/dl	Uric acid, mg/dl	U:C
0	4.46	3.82	0.635	6.04	10.24	31.0	4.79
5	4.47	3.84	0.61	6.26	10.29	31.1	5.06
10	4.45	3.30	0.619	6.20	10.23	31.0	4.96
RMSE	0.017	0.013	0.019	1.84	0.514	0.823	0.233
p value	0.606	0.189	0.166	0.143	0.174	0.653	0.168

RMSE, root means square error; *A:G*, albumin-to-globulin ratio; *U:C*, uric acid-to-creatinine ratio

Table 11 The effect of flaxseed cake levels on blood serum and yolk trace elements of Hisex laying hens

Flaxseed cake, %	Serum trace minerals			Egg trace minerals		
	Se, mg/l	Zn, mg/l	Fe, mg/l	Se, ppm	Zn, ppm	Fe, ppm
0	1.40	1.61	124.2	8.67	77.9	119.6
5	1.41	1.62	123.9	7.94	64.2	119.0
10	1.42	1.64	122.5	8.30	77.0	119.5
RMSE	0.003	0.006	0.004	0.525	16.70	0.537
p value	0.084	0.289	0.865	0.087	0.312	0.217

RMSE, root means square error

FSC in the diet. Feeding of FSC did not significantly affect yolk appearance, albumen color, egg flavor, and general acceptance in fresh eggs (Table 8). The findings presented in Table 9 showed that dietary FSC did not significantly impact egg dry matter, protein, lipids, ash, NFE, and GE.

Effect of flaxseed cake levels on blood parameters and serum and egg trace minerals

As reported in Table 10, dietary FSC did not significantly influence serum total protein, albumin, globulin, albumin-to-globulin ratio, creatinine, uric acid, and uric acid-to-creatinine ratio. Table 11 showed that the inclusion of 5% or 10% of FSC in laying hens diets did not significantly affect Se, Zn, and Fe contents in blood serum as well as in egg yolk.

Discussion

Nutritive value of flaxseed cake

The nutritional value of flaxseed cake showed a substantial amount of protein, lipid, energy value (gross energy = 10.48 kcal/kg, Table 1), and macro-elements (Ca and P) as trace elements such as Se, Zn, Fe, and Mn that can contribute to the nutrients and minerals requirements of laying hens (National Research Council, 1994). These values indicated that FSC is a valuable protein, energy, and mineral source for animal nutrition. These results agree with those reported by Gutiérrez et al. (2010), Mueller et al. (2010), Bernacchia et al. (2014), Feedipedia (2022), and Sharma and Saini (2022). These studies indicated that flaxseed is a source of valuable nutrients and bioactive substances such as omega-3, arginine, cyanogenic glycosides, phenolic compounds, and lignans (Singh et al., 2011; Mattioli et al., 2017; Rahimlou and Hejazi, 2022).

The AA profiles meet or excess the laying hens' requirements based on National Research Council (1994) recommendations and agree with those reported by Feedipedia (2022) for cold-pressed FSC and those for flaxseed cited by Kaur et al. (2017) and Sharma and Saini (2022). The findings showed that FSC is a rich source of essential AA, in particular arginine, leucine, phenylalanine, and valine, while tryptophan was the lowest. All nonessential AAs were found in FSC, with glutamic acid and glutamine being the greatest. The current results showed that FSC is a valuable source of both essential and nonessential AA for poultry nutrition, and the essential AAs were 37.6% of the total AAs of flaxseed (Attia, 2003; Attia et al., 2022a, b; Feedipedia, 2022; Sharma and Saini, 2022).

Laying hens performance

Laying hens fed 10% FSC in diet had no detrimental effect on the rate of laying, egg weight and mass, FCR, body weight change, and survival rate. These results demonstrated the safety of feeding layer hens 10% of unprocessed FSC during 50–58 weeks of age. However, for unknown reasons, hens fed 5% FSC reduced laying performance, which warrants further investigation. These findings followed those of the previous studies by Al-Nasser et al. (2011), Ahmad et al. (2017), and Attia et al. (2022a). These authors documented that layers could tolerate up to 15% FSC without adversely disturbing indices of performance. Moreover, Bean and Leeson (2003) noted that dietary inclusion of 10% flaxseed did not significantly impact egg weight and laying rate in Brown and White laying hens. Likewise, Roth-Maier et al. (1998) explained that the tolerance of laying hens to flaxseed appeared greater than broilers. Recently, Hosseini et al. (2023) postulated that dietary-extruded flaxseed (270 g/kg, 180 g/kg, 90 g/kg, and 0 g/kg) had no marked impact on feed intake, egg mass, and FCR in laying hens. It might be expected that dietary FSC had no adverse effect on yolk formation and, consequently, the rate of egg production. Indeed, yolk lipoprotein precursors, including vitellogenin and vLDL, are hepatically synthesized under the influence of 17-β-estradiol and taken up by developing yolk through receptor-mediated endocytosis (Attia et al., 2016). Plasma Zn concentration indicates circulating vitellogenin (Ebeid et al., 2008b). The current trial showed that dietary inclusion of 5–10% FSC did not alter vLDL (33.0–33.3 mg/dL) and serum Zn concentrations. Therefore, it might be speculated that dietary FSC had no adverse effect on folliculogenesis (ovarian follicular development), which is consequently related to the egg production rate in the current study. On the other side, other studies reported a negative effect of dietary 15% flaxseed on egg production rate (Aymond and van Elswyk, 1995; Antruejo et al., 2011; Halle and Schöne, 2013). As in Table 2, the highest significant feed intake was reported in laying hens fed 10% FSC compared to the control diet. This might be related to the high tolerance of laying hens to unprocessed FSC, which might enhance the acceptance of the by-product. This assumption agreed with Imran et al. (2015), who proved that extruded flaxseed meal included 86% and 76% less hydrocyanic substances and tannins, respectively, compared to unprocessed flaxseed. In addition, flaxseed contains bioactive substances that would improve the antioxidant status and performance of animals such as phenolic compounds (Singh et al., 2011; Mattioli et al., 2017; Rahimlou and Hejazi, 2022).

Egg quality characteristics

Different dietary levels of FSC did not significantly affect external egg traits in fresh and stored eggs (Table 5). The current results agreed with those of Panaite et al. (2021), who illustrated that adding 5% FSC to the layer diet did not affect eggshell percentage and eggshell breaking strength, while eggshell thickness was considerably increased. Also, Bean and Leeson (2003) noted that dietary incorporation of 10% flaxseed did not significantly affect shell weight, albumin height, and shell thickness in brown and white laying hens. Similarly, Hosseini et al. (2023) postulated that dietary-extruded flaxseed at a graded level from 0 to 270 g/kg by 90 g /kg intervals had no significant impact on shell thickness and strength. These data might suggest that FSC had no negative implications for eggshell formation and did not interfere with the sufficient supply of Ca for shell formation. These results demonstrated the safety of FSC for mineral metabolism for bone and eggshell formation. In this regard, data in Table 1 showed that FSC contained valuable amounts of Ca, P, Fe, Zn, Mn, and Se, which can support animal health status under marginal feeding and stress conditions. This hypothesis is also confirmed by Caston et al. (1994), who demonstrated that dietary 20% flaxseed had no significant influence on eggshell quality. Also, Bozkurt et al. (2008) and Ebeid (2011) reported similar results when hens fed flaxseed or fish oil as a n-3 PUFA source. On the other hand, Halle and Schöne (2013) proved that eggshell percentage significantly declined in laying hens provided 5–15% FSC compared to rapeseed cake.

Results displayed in Table 6 and 7 illustrated that dietary 10% FSC did not significantly affect yolk weight, index, yolk-to-albumen ratio, albumen percentage and height, Haugh unit score, and egg weight change in fresh and stored eggs. These results indicated that unprocessed FSC had no adverse influence on egg quality. In addition, flaxseeds had bioactive substances, including proteins, cyclolinopeptides, and cyanogenic glycosides, which may also produce biological actions that may improve internal egg quality. These findings are in line with those of Caston and Leeson (1990) and Caston et al. (1994), which indicated that dietary flaxseeds had no detrimental effect on yolk weight and yolk percentage. Additionally, the albumen yield and Haugh unit score were not significantly affected by adding 10% flaxseed to the diet of laying hens (Bean and Leeson, 2003; Panaite et al., 2021). Furthermore, graded levels of dietary-extruded flaxseed did not significantly impact the Haugh unit (Hosseini et al., 2023). It was found that the addition of 5% FSC in layer diets increased yolk percentage and yolk-to-albumen ratio in fresh eggs in comparison to control (Table 6). These findings supported those of Halle and Schöne (2013), who stated that increasing the dietary level of FSC (5%, 10%, and 15%) decreased yolk and increased the albumen. Similarly, Bean and Leeson (2003) noted that dietary inclusion of 10% flaxseed reduced yolk weight and yolk percentage in laying hens. Caston et al. (1994) observed that the weight of yolk was notably lowered due to 20% flaxseed addition, and this reduction might be attributed to the low ME content of the flaxseed diet (2970 kcal/kg). However, Shang et al. (2004) revealed that raising the n-6/n-3 ratio PUFAs (6:1, 8:1, and 14:1) enhanced albumen % in laying hens. Conversely, increasing the n-6/n-3 PUFAs ratio from 0.6:1 to 7.8:1 did not significantly affect the weight of yolk, albumen, and color of yolk (Grobas et al., 2001).

The numerical increase in yolk color observed herein due to the addition of 5% or 10% FSC in the layer's diet could be due to increasing pigmentation intake of FSC diets. Following several previous studies (Halle and Schöne, 2013; Panaite et al., 2021; Attia et al., 2022a), current results indicated that a diet containing 10% soaked FSC exhibited the highest increase in yolk color, which can meet consumer desire for darker egg yolk color. Also, Halle and Schöne (2013) revealed that dietary FSC improved yolk color compared to hempseed and rapeseed cakes. Recently, Panaite et al. (2021) demonstrated that increasing the dietary level of FSC produced either a darker yolk color or similar to the control diet, which might be associated with the greatest consumption of FSC pigments. Moreover, dietary flaxseed oil and/or fish oil supplementation enriched egg yolk color (Ebeid, 2011). Similar findings were also reported by Cachaldora et al. (2008), who found that the color of yolk was enhanced in diets enriched with n-3PUFA oils. Carotenoids included in the feed ingredients are fat-soluble; thus, their intestinal absorption is expected to increase in matching with lipids absorption (Ebeid, 2011). It was noteworthy to indicate that the beneficial effect of enhancing yolk color on customer preference for eggs produced from layers provided FSC is the extra advantageous outcome of elevating n-3 PUFA (Caston et al., 1994; Attia et al., 2022b).

It seems that stored eggs had lower egg quality than fresh eggs, and FSC had no harmful eggs of the quality of sorted eggs. This indicated that FSC is a safe feedstuff for feeding laying hens and did not affect egg quality after the post-harvest period may be due to its bioactive substances, such as phenolic compounds (Singh et al., 2011; Mattioli et al., 2017; Rahimlou and Hejazi, 2022). Different levels of FSC did not significantly affect eggs' sensory attributes, including yolk appearance, albumen color, egg flavor, and general acceptance in fresh eggs, demonstrating the safety of FSC on the taste and aroma of eggs. These results are in line with those by Hayat et al. (2010), who showed that dietary inclusion of 10% flaxseed in layer diets had no negative effect on sensory or organoleptic traits of eggs and that eggs are acceptable to the consumers and untrained panelists. Similarly, Imran et al. (2015) showed that most untrained evaluators did not distinguish differences between the control and omega-3-enriched eggs laid by hens fed a 10% extruded flaxseed meal in the diet. Furthermore, Mazalli et al. (2004) documented that no unfavorable fishy flavor was detected in hard-cooked eggs from hens fed 9% flaxseed in the diet. However, professional panelists can perceive differences in sensory or organoleptic attributes in eggs produced by laying hens fed flaxseed meal, mainly when used at high levels (Hayat et al., 2010; Imran et al., 2015). Therefore, emphasizing that much attention should be taken when using FSC in laying hen diets is essential.

Blood serum profile and trace elements

Dietary levels of FSC did not influence serum protein profiles, total protein, albumin, globulin, albumin-to-globulin ratio, and renal function indices (in terms of creatinine, uric acid, and uric acid-to-creatinine ratio). This demonstrated the safety of FSC on protein metabolism and renal function. Similarly, Shafey et al. (2015) observed that dietary supplementation of 5% and 10% FSC in laying hens diet did not harm serum total protein, albumin, globulin, and albumin-to-globulin ratio in laying hens. Likewise, dietary-extruded flaxseed showed no harmful impact on blood protein profile and albumin-to-globulin ratio in laying hens (Hosseini et al., 2023).

Including 5% or 10% of FSC did not significantly affect blood serum and yolk contents of the trace elements Se, Zn, and Fe. Similarly, various fatty acid sources did not significantly affect serum minerals (Van Elswyk, 1997). It is well known 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). It is well documented that Se, Zn, and Fe are of special interest in human nutrition as antioxidants, antiviral, and health-enhancing factors (Robberecht et al., 2020; Frydrych et al., 2023). Therefore, dietary FSC did not interfere with trace minerals metabolism, leading to no detrimental effect on lipid metabolism and antioxidative properties of egg and serum of laying hens.

Conclusions

Based on the results of the present study, we concluded that FSC resulted in a valuable source of protein, energy, Ca and P, trace elements (Fe, Zn, Mn, Cu, and Se), and essential amino acids. In addition, feeding laying hens up to 10% FSC did not adversely influence laying hens' productive traits, egg quality characteristics, egg sensory attributes, egg nutritional composition, and blood serum biochemical parameters including trace elements. Further studies are still needed to support the obtained results and to investigate the effect of dietary 10% FSC on immune response and antioxidative properties in laying hens.

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

Declarations

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