Research Article

Improving Laying Hens Productivity and Lowering Egg Cholesterol Content using Dietary Linseed Oil Supplementation

Abdulaziz A. Alaqil[1]*, Mohammed I. Buhaya[2]

[1]Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia; [2]Department of Accounting, College of Business Administration, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia.

Abstract | Chicken eggs are considered one of the most common high nutritive value animal protein foods sources consumed all over the world. Recently, with the increasing of health-conscious there is a growing interest in producing and consuming functional foods especially for individuals suffering from chronic diseases. The current study aimed to investigate the effect of dietary linseed oil (LO) inclusion, as enriched-source of omega-3 polyunsaturated fatty acids, on egg production performance and egg yolk cholesterol content, blood hematology and biochemical composition of commercial laying hens. A total of 180 Hy-Line brown laying hens 36-week-old were randomly divided into four groups (9 replicates x 5 birds each). The four groups were fed a basal diet formulated to contain; 0, 2, 4 or 6% LO for six weeks. Results showed a significant improvement in egg production performance with dietary LO inclusion ranged from 3 to 4%. Data revealed a significant reduction in egg yolk cholesterol content by 15, 17 and 21% for the 2, 4 and 6% LO groups, respectively, compared to the 0% LO group. Meanwhile, blood high-density lipoprotein was significantly increased, while the low- and very-low-density lipoprotein cholesterols were significantly decreased by LO treatment, compared to the 0% LO group. Dietary LO did not show any adverse effects on the measured blood hematological or plasma biochemical parameters of the layers. It can be concluded that inclusion of LO into layer's diets can be safely used to enhance egg production. Furthermore, LO inclusion might have a beneficial impact on reducing egg cholesterol content which can be considered as an adding value for consumer's general health. The economic efficiency evaluation reviled that diet fortified by 6% LO is the most efficient diet in respect of the current Saudi Arabia market's prices.

Keywords | Laying hens, Linseed oil, Egg production, Blood hematology, Plasma biochemistry, Cholesterol profile, Economic efficiency

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***Correspondence** | Abdulaziz A. Alaqil, Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia; **Email:** aalaqil@kfu.edu.sa
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INTRODUCTION

The demands of high-quality protein of animal origin are constantly increase which impose a huge challenge to cope with. In the last few years, the pandemic crisis of Coronavirus Disease-19 (COVID-19) led to global economic instability and increase food insecurity (Hafez et al., 2021; Attia et al., 2022; McDermott and Swinnen, 2022). For securing sufficient stable protein supply, chicken egg is considered one of the commonly affordable high-quality protein sources. Yet, there is a serious debate on the risk factor of increasing egg consumption and the increasing threat of heart diseases. A number of research studies concluded that high dietary cholesterol or eggs

consumption increased the risk of cardiovascular disease incident (Zhong et al., 2019; Krittanawong et al., 2021). Meanwhile, another research articles clarified that there is no association between reasonable egg consumption (up to an egg a day) and the risk of cardiovascular diseases generation (Qin et al., 2018; Drouin-Chartier et al., 2020). However, egg composite some relatively constant compounds such as; total protein, total lipid and phospholipids as well as other more variable compounds which extremely influenced by the diet such as; fatty acid composition and cholesterol (Lei, 2021). This window of opportunity implies that feed diet manipulation can help in reducing egg cholesterol level which will be of great benefits for consumer health.

Linseed oil (LO) is one of the most common fixed oils known for its high polyunsaturated fatty acids (PUFA) content especially omega-3 (Tavarini et al., 2019; Gutierrez-Luna et al., 2022). Petropoulos et al. (2021) concluded that LO possess antimicrobial and cytotoxic potential with high nutritive value and can be used as a functional food ingredient. The addition of LO to turkey (Stadnik et al., 2018; Szalai et al., 2021) or broiler chicken (Kishawy et al., 2019; Gou et al., 2020; El-Bahr et al., 2021) diets significantly increased muscle meat omga-3 content. In the meantime, Ahmad et al. (2017) reported that linseed has a limited use in commercial layer's diets due to the potential presence of anti-nutritional factors which can have a negative impact on laying hens' egg production performance. However, they stated that LO can be used in laying hen diet to produce omega-3 enriched eggs without any harmful impact on performance. Linseed oil inclusion to layer diet at 2.5% reported to increase the egg yolk PUFA content with no significant change in egg yolk cholesterol content (Batkowska et al., 2021). Furthermore, Duan et al. (2021) suggested that LO inclusion by 2% can be beneficial for the production of functional eggs enriched with docosahexaenoic and α-linoleic acids. However, the optimum amount of LO addition to layer diet for the best egg production with high PUFA content and low cholesterol egg content still unknown. Thus, the aim of the present study was set to investigate the impact of LO inclusion to layer diet at different increasing levels 0, 2, 4 and 6% on egg production performance and egg yolk cholesterol content. In addition, screening the hematological and blood biochemical respond to LO inclusion. Economic efficiency of the experimental diets was studied to evaluate the various economic outcomes of LO addition.

MATERIALS AND METHODS

Ethical statement

Animal care in the present study was compliant with the relevant research ethics guidelines of King Faisal University and the National Committee of Medical and Bioethics. All experimental procedures were approved by the Research Ethics Committee (REC), King Faisal University (KFU-REC-2022-FEB-EA000565).

Experimental design and treatments

One hundred and eighty commercial Hy-Line brown laying hens at the peak of egg production (34-week-old) were symmetrically divided into four experimental groups (9 replicates × 6 hens per replicate) according to the dietary level of linseed oil (LO). The experimental groups were fed for six weeks on a basal diet formulated to have; 0, 2, 4 or 6% LO. The experimental diets were formulated to meet the nutrients' requirement of Hy-Line brown layers (available at https://www.hyline.com/varieties/brown). The experimental diets were formulated to be iso-nitrogenous and iso-caloric. The ingredient and chemical composition of experimental diets are presented in Table 1. Hens were housed in an open-sided layer house and kept in cages (3 hens per cage). The hens had open access to feed and water for the entire experimental period. The lighting program was set to meet 16 h of light and 8 h of dark.

The LO used in the present study was obtained from the Department of Food Sciences, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia. The oil was extracted by cold pressing methodology according to Kasote et al. (2013). The fatty acid profile of the LO used was analyzed using gas chromatography technique according to the method described by Kim et al. (2016) (Table 2).

Productive performance and economic efficiency

During the experimental period, feed intake was recorded weekly. Meanwhile, egg production and egg weight were recorded daily. At the end of the experimental period (6 weeks), egg number, egg weight, total egg mass, feed intake and feed conversion ratio were calculated per hen for each experimental group.

In order to evaluate the economic feasibility of including LO into layer's diet, the costs of the production inputs were calculated in Saudi Arabia riyal (SAR). The prices of the feed ingredients and LO used to formulate the experimental diet as well as labor price and all the management practiced costs were calculate according to the market prices of Al-Ahsa, Saudi Arabia, during the month of May 2022.

Blood sampling and analysis

At the end of feeding trial, 20 blood samples from each experimental group (4 sample per replicate) were collected from the brachial vain and put in two set of heparinized tubes. The first tube was assigned for blood hematology analysis including hematocrit level, total red blood cells

count (RBC's) and total white blood cells count (WBC's) as the method described by (Gehad et al., 2008). Meanwhile, the heterophils to lymphocytes (H/L) ratio was determined according to the method described by Abbas et al. (2020). The second tube of blood samples was centrifuged for 15 min at 3000 rpm and 4°C. The plasma was separated and stored at -20°C pending further analysis.

Table 1: Ingredients and calculated composition of the experimental diets.

Item	Levels of linseed oil			
	0%	2%	4%	6%
Ingredient %				
Corn (IFN 4-02-861)	59.52	59.52	54.24	49.0
Soybean meal, 44% CP (IFN 5-04-596)	20.72	20.72	20.72	20.72
Wheat bran IFN 4-05-190)	0.78	0.78	4.06	7.30
Dicalcium phosphate (IFN 6-26-335)	1.70	1.70	1.70	1.70
Limestone (IFN 6-02-632)	9.10	9.10	9.10	9.10
Salt (IFN 6-04-152)	0.40	0.40	0.40	0.40
Premix layers [1]	0.30	0.30	0.30	0.300
DL-Methionine (IFN 5-03-086)	0.08	0.08	0.08	0.08
Corn gluten meal, 60% CP (IFN 5-02-900)	5.40	5.40	5.40	5.40
Soybean oil (IFN 4-07-983)	2.00	-	-	-
Linseed oil (IFN 5-30-288)	-	2.00	4.00	6.00
Calculated chemical composition				
Crude protein (%)	17.00	17.00	17.00	17.00
ME (Kcal/ Kg)	2800	2800	2800	2800
Calcium (%)	3.82	3.82	3.82	3.82
Available phosphorus (%)	0.40	0.40	0.40	0.40
Lysine (%)	0.80	0.80	0.80	0.80
Methionine+Cystine	0.72	0.72	0.72	0.72
α-Linolenic acid	-	1.07	2.15	3.22

[1]Supplied the following vitamins and minerals per kg diet: 8000 IU vitamin A (IFN 7-05-144); 1500 IU vitamin D_3 (IFN 7-05-699); 4 mg riboflavin (IFN 7-03-921); 10 µg cobalamin (IFN 7-05-146); 15 mg vitamin E (IFN 7-05-150); 2 mg vitamin K (IFN 7-03-077); 500 mg choline (IFN 7-01-228); 25 mg niacin (IFN 7-26-003); 60 mg manganese (IFN 6-03-034); 50 mg zinc (IFN 6-05-551). IFN: International Feed Number (Harris et al., 1981).

Table 2: Fatty acids profile of linseed oil.

Fatty acids [1]	%
Palmitic acid (C16:0)	4.9
Stearic acid (C18:0)	2.6
Arachidic acid (C20:1)	0.8
Oleic acid (C18:1)	15.8
Linoleic acid (C18:2 n-6)	12.3
α-linolenic acid (C18:3 n-3)	62.6

[1](g fatty acid methyl ester per 100 g esters).

The plasma biochemical analyses were performed including total protein, albumin, calcium, and phosphorus. Moreover, plasma total cholesterol and cholesterol fractions were also analyzed including low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), and very low-density lipoprotein cholesterol (VLDLC). All plasma biochemical analyses were determined by quantitative-colorimetric assay using commercial kits (STANBIO Laboratory, Texas, USA). The plasma globulin value for each sample was calculated by subtracting the albumin value from total protein content and the albumin to globulin ratio was then calculated.

Egg yolk cholesterol assay

To determine egg yolk cholesterol content, 30 eggs from each treatment were randomly collected at the end of the experimental period. Yolk was separated and frozen at -20°C until analysis. Firstly, lipids were extracted from egg yolks using chloroform/methanol mixture (2:1, vol/vol) according to the method of AOAC (2005). The total cholesterol was then determined in extracted lipids by enzymatic-colorimetric assay using commercial kits (STANBIO Laboratory, Texas, USA).

Statistical analysis

The data were statistically analyzed using one way ANOVA model that performed by the General Linear Models procedure of SPSS software package (version 22.0; IBM Corp., Armonk, NY, USA, 2013). Significant differences between experimental group means were determined using the Duncan's multiple range test and the probability value was set at $p<0.05$.

RESULTS AND DISCUSSION

Productive performance

Results of productive performance as affected by the level of LO inclusion into laying hens' diets are shown in Table 3. Egg number during the experimental six weeks period was significantly higher by 4.0, 3.1 and 3.7% for the 2, 4 and 6% LO groups, respectively, compared to the 0% LO group. Egg weight was significantly increased in the 4 and 6% LO groups compared to the 2% and the 0% LO groups. While egg mass was significantly higher in the LO added groups compared to the 0% LO group. Feed intake was significantly decreased by the LO inclusion compared to the 0% LO group. Inclusion of LO to layer's diet significantly improved the feed conversion ratio, with the best feeding efficiency observed for the 6% LO group followed by 2% LO and 4% LO groups, compared to the 0% LO group.

Hematology parameters and plasma biochemical assays

No significant differences were found in the hematological parameters values among the four experimental groups (Table 4). Nevertheless, there was a decreasing tendency (P>0.05) in the H/L ratio as the level of LO increase. Furthermore, plasma total protein, albumin, globulin, calcium and phosphorus were not influenced by the LO inclusion into the layers' diets (Table 4). However, higher levels of globulin were observed in the LO added groups compared to the 0% LO group (P>0.05).

Plasma and egg yolk cholesterols

The results of plasma total cholesterol and cholesterol fractions analyses as affected by LO inclusion into layers' diets are shown in Table 5. Plasma total cholesterol was significantly decreased in 4% and 6% LO supplemented groups compared to the 2% LO and the 0% groups. The LO inclusion to layers' diets significantly increased the plasma HDLC levels while decreased the LDLC levels in 2%, 4% and 6% LO groups compared to the 0% LO group. The lowest LDLC level was observed in the 6% LO group followed by the 4% and then the 2% LO groups. The VLDLC levels were significantly decreased by LO supplementation at the level of 4 and 6% compared to the 0% LO groups. Meanwhile, the egg yolk cholesterol content significantly decreased by LO inclusion into the layer's diets at the different experimented levels compared to the 0% LO level (Figure 1). The data implied that the changes in plasma cholesterol levels seem to affect the content of egg cholesterol.

Table 3: Productive performance of laying hens feed on different linseed oil levels.

Traits	Levels of linseed oil			
	0%	2%	4%	LO6%
Egg number/hen/6 wk	37.6±1.0[b]	39.1±1.2[a]	38.8±1.3[a]	39.0±1.1[a]
Egg weight, g	62.7±1.0[b]	62.1±0.9[b]	63.2±0.9[a]	63.4±0.8[a]
Egg mass, g/hen/6 wk	2357.9±10.1[b]	2428.5±12.2[a]	2452.1±11.8[a]	2472.4±12.4[a]
Feed intake/g/hen/d	122.1±5.4[a]	111.9±4.1[b]	112.3±5.1[b]	110.5±6.2[b]
Feed conversion ratio	2.17±0.11[a]	1.93±0.10[b]	1.92±0.10[b]	1.88±0.12[c]

[a, b, c] Means within the same row with different superscript letters significantly differed ($p < 0.05$).

Table 4: Hematology parameters and plasma biochemical assays of laying hens feed on different linseed oil levels.

Traits	Levels of linseed oil			
	0%	2%	4%	6%
HT, %	36.34±3.16	36.45±3.22	36.65±3.21	36.37±3.45
RBC, 10^6/ml	2.88±0.44	3.12±0.64	2.89±0.58	3.22±0.46
TWBC, 10^3/ml	22.55±2.32	22.46±2.68	22.62±2.82	22.39±2.44
H/L ratio	0.55±0.10	0.54±0.10	0.52±0.11	0.51±0.11
Total protein, g/dl	6.15±1.10	6.22±0.88	6.14±1.12	6.54±0.98
Albumin, g/dl	3.51±0.85	3.10±0.62	3.05±0.57	3.14±0.55
Globulin, g/dl	2.64±0.91	3.12±0.61	3.09±0.54	3.40±0.82
A/G ratio	1.33±0.11	0.99±0.12	0.99±0.10	0.92±0.13
Calcium, mg/dl	20.14±2.15	21.45±3.17	21.36±3.12	20.40±3.22
Phosphorus, mg/dl	9.15±3.12	9.22±3.24	9.26±2.15	10.10±3.09

HT: hematocrit; RBC: red blood cells; TWBC: total white blood cells; H/L ratio: heterophils/lymphocytes ratio; A/G ration: albumin/globulin ratio.

Table 5: Plasma cholesterol profile of laying hens feed on different linseed oil levels.

Traits	Levels of linseed oil			
	0%	2%	4%	6%
TC, mg/dl	127.67 ±4.12[a]	124.44 ±3.68[a]	117.98 ±3.55[b]	113.14 ±4.50[b]
HDLC, mg/dl	50.65±3.04[b]	57.66±3.22[a]	57.43±2.78[a]	58.46±3.01[a]
LDLC, mg/dl	70.65±2.16[a]	59.88±3.12[b]	56.64±2.87[b]	50.44±2.24[c]
VLDLC, mg/dl	6.37±0.48[a]	6.90±0.54[a]	3.91±0.67[b]	4.24±0.85[b]

[a, b, c] Means within the same row with different superscript letters significantly differed ($p<0.05$). TC: total cholesterol; HDLC: high-density lipoprotein cholesterol; LDLC: low-density lipoprotein cholesterol: VLDLC: very low-density lipoprotein cholesterol.

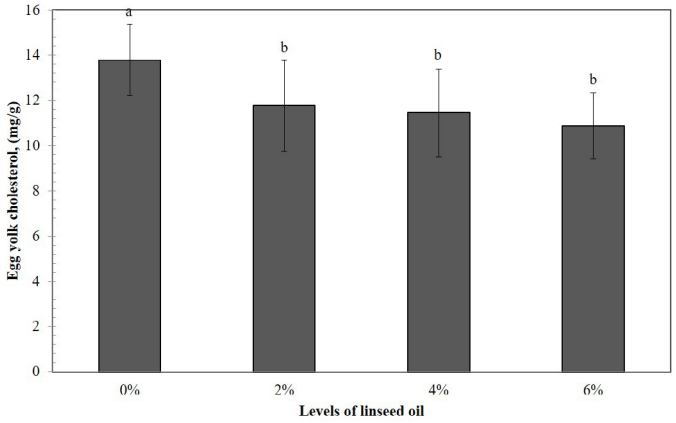

Figure 1: Egg yolk cholesterol content of laying hens' feed on different linseed oil levels.

Economic efficiency

The economic efficiency was calculated for different experimental groups for the 6 weeks experimental period (Table 6). Data reviled that adding LO to layer diet increase the total revenue with the highest economic efficiency observed for the 6% LO group.

Table 6: Economic efficiency calculation of laying hens feed on different linseed oil levels.

Items*	Levels of linseed oil			
	0%	2%	4%	6%
Economic in put				
Labor cost/ bird	0.158	0.168	0.167	0.168
Feeding cost/ bird	11.26	12.40	12.93	13.58
Water cost/ bird	1.99	1.99	2.01	1.99
Egg transportation cost/ bird	1.88	1.95	1.94	1.95
Vaccine & medication cost/bird	1.28	1.33	1.32	1.32
Portion of rearing cost/ experimental period	2.50	2.50	2.50	2.50
Total cost/ bird	19.07	20.34	20.87	21.51
Economic out put				
Egg number/bird/6 weeks	37.6	39.1	38.8	39.0
Egg price/ bird	0.58	0.60	0.62	0.64
Egg revenue/ bird	21.81	23.46	24.06	24.96
Manure revenue/ bird	0.086	0.090	0.089	0.089
Total revenue bird	21.89	23.55	24.15	25.05
Net revenue bird	2.83	3.21	3.27	3.54
Economic efficiency	0.150	0.158	0.157	0.165
Relative Economic efficiency	100	105.3	104.7	110.0

*The economical items were calculated for the entire experimental period (6 weeks). The prices were set in Saudi Arabia riyal (SAR).

The egg production performance noted in the present study indicate that LO fortified diets caused a significant increase in egg production parameters (i.e. egg number, egg weight and egg mass) allied with a significant improvement in feed efficiency. While there are controversial results of the impact of feeding LO on layer's performance (Ahmad et al., 2017), a number of studies indicated a positive effect of LO on egg production performance. Altacli et al. (2022) reported a significant 5.5% increase in egg yield for laying hen feed diet containing 5% LO put as a substitution of sunflower oil. Keten and Matur (2022) also reported that LO supplementation at 2% increased egg production and egg mass of laying hens reared under high density stress. Furthermore, feed efficiency of laying hen was found to increase as LO increase in the diet from 0.5 to 5% in concentration (Ehr et al., 2017).

Little information is known about the influence of dietary supplementation of LO on laying hens' blood hematological profile and biochemical parameters. In the present study, dietary LO inclusion did not affect any of the estimated blood hematological or plasma biochemical parameters. On the contrary, results showed that LO supplementation had plasma hypocholesterolemic effect. Dietary LO supplementation reported to have a hypolipidemic activity in rat fed high fat diet through partaking normal regulation of plasma lipid and cholesterol metabolism in live (Vijaimohan et al., 2006). Gou et al. (2020) demonstrated a reduction in plasma total triglycerides and total cholesterol in yellow-feathered chickens feed 4% LO supplemented diet. In line with the present results, Celebi and Utlu (2006) found a significant decrease in serum VLDLC, LDLC and total cholesterol concentrations, and a significant increase in serum HDLC concentrations of hens fed rations containing 4% LO. Moreover, Fébel et al. (2008) reported that plasma total cholesterol and LDLC significantly decreased when broiler's diet was fortified with 3% LO, with no significant change in HDLC. Furthermore, Švedová et al. (2008) observed a decrease in serum total cholesterol and an increase in HDLC in laying hens fed 3% LO supplemented ration. The mechanism of action of modulating the cholesterol by LO could be explained by decreasing the synthesis rate of apoprotein B or the production of VLDLC and triglycerides in laying hens (Harris et al., 1984). Crespo and Esteve-Garcia (2003) also suggested that the decrease in blood cholesterol concentration of broiler chickens caused by LO supplementation may be due to the suppression of hepatic cholesterol production.

On the other hand, egg yolk cholesterol content (EYC) is one of the limitations of egg consumption especially for those who suffer from cardiovascular diseases (Qin et al., 2018; Zhong et al., 2019). Although most efforts conducted to reduce the cholesterol contents in final products were met with limited success (Grobas et al., 2001), the present study results revealed a significant reduction in EYC associated with the proportion of LO in the diet. Several studies demonstrated a positive effect of LO supplementation to enrich egg with PUFA especially omega-3 α-linolenic acid

(Neijat et al., 2016; Batkowska et al., 2021; Lee et al., 2021). The egg yolk cholesterol content was reported to decrease when hen consumed diet containing 8.64% LO (Yalcyn et al., 2007). Batkowska et al. (2021) found a decreasing tendency in EYC for LO supplemented groups compared to the control (9.35 vs. 10.10 mg/g, respectively). Also, Basmacioğlu et al. (2004) found a significant decrease in egg yolk cholesterol of laying hens receiving 8.64% linseed seed in the diets. Atakisi et al. (2009) also reported that egg yolk cholesterol decreased linearly with the increased level of linseed in the diet of Japanese quail. However, several studies could not find any effect on egg cholesterol for hens fed on diets containing whole linseed (Augustyn et al., 2006; Shapira et al., 2009; Wang and Huo, 2010). The egg yolk cholesterol reduction can be justified by the changes observed in plasma total cholesterol levels which reflected on egg cholesterol deposition.

In conclusion, the present study demonstrated that LO inclusion into laying hens' diet at levels of 2, 4, or 6% can modulate a significant reduction of egg yolk cholesterol with improvement in the egg production performance. Furthermore, the study of the economic efficiency of using LO in layers' diet reviled improvement in both total revenue and net revenue with the increasing levels of LO inclusion with the best efficiency obtained when used LO at 6%. Therefore, under the present experimental condition, the dietary LO inclusion to layer diets, as enriched-source of n-3 PUFA, may be beneficial for producing healthier low cholesterol eggs for consumers and with a relative economic benefit for producers.

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NOVELTY STATEMENT

The present study is one of the first to investigate the effect of linseed oil supplementation on layers performance and egg cholesterol content with special reference to blood hematological parameters and economic efficiency.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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