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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 174-178

www.thepharmajournal.com
Received: 15-09-2017
Accepted: 17-10-2017

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Influence of linseed oil supplementation on egg cholesterol content, fatty acid profile, and shell quality

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Abstract
An experiment was conducted to evaluate the effect of supplementing different levels of linseed oil in the laying hens' diet on cholesterol content, fatty acid profile and shell quality of egg during a period of 16 weeks. One hundred forty White Leghorn layers were randomly allocated into seven experimental groups having 5 replications of 4 birds in each and sited in individual cages from 22 to 38 weeks of age. The laying hens of control group (T_1) were fed a basal diet formulated as per [7] standards. The layers of treatment groups T_2, T_3, T_4, T_5, T_6 and T_7 were fed basal diet supplemented with linseed oil at levels of 1, 2, 2.5, 3, 3.5 and 4%, respectively. The results indicated a significant ($p<0.05$) decrease in cholesterol content in layers fed @ 1, 2.5, 3, 3.5 and 4% linseed oil as compared to control. Birds fed linseed oil had significant ($p<0.05$) decrease in palmitic (C: 16), stearic(C: 18) &oleic acid (18:1) being lowest in T_7 (4% linseed oil). Linoleic acid (C18:2) linolenic acid (18:3) & arachidonic acid (C20:4) significantly ($p<0.05$) increased being highest in T_7 (4% linseed oil) and lowest in T_1 (control). The results showed significant ($P<0.05$) decrease in egg shell thickness in treatment groups $T_6$and T_7 as compared to T_1, T_2& T_3.Egg shell weight percentage decreased but, egg shell weight increased due to increased level of linseed in the diet. Thus, from the present study it can be concluded that supplementation of linseed oil at different levels in laying hens' diet significantly ($P<0.05$) decreased egg cholesterol, egg shell thickness, egg shell weight percentage and saturated fatty acid whereas there was significant increase in polyunsaturated fatty acid (PUFA) and egg shell weight.

Keywords: Hens, Linseed oil, Linoleic acid, Linolenic acid PUFA, and shell quality

Introduction
The egg is considered a functional food [27] as it is a source of protein, vitamins, and lipids, such as phospholipids and polyunsaturated fatty acids [3, 16]. In the production of eggs rich in n-3 fatty acids, there is an increasing interest in maximizing the use of feedstuffs containing these nutrients because there is a correlation between their levels in feeds and in the yolk. The egg is naturally poor in linolenic acid, and does not contain eicosapentoenoic (EPA) and docosahexaenoic (DHA) fatty acids.Omega-3 polyunsaturated fatty acids (PUFA n-3) have been extensively studied in human health, as well as n-6 fatty acids, which are considered essential in human diets. Linolenic acid (LNA, 18:3, n-3) can be metabolically converted into docosahexaenoic (DHA, 22:6, n-3) and eicosapentaenoic (EPA, 20:5, n-3) fatty acids, but the enzymes involved in this process are common to the elongation and desaturation of linolenic acid (LNA), and therefore the competition with n-6 fatty acids reduces the amount of converted LNA.

One of the main dietary sources of long-chain n-3 polyunsaturated fatty acids is fish, which is rich in EPA and DHA. Oilseed oils, particularly linseed oil, are rich in linolenic acid, a precursor of those fatty acids [8]. PUFA(Polyunsaturated fatty acids),compared to the standard eggs, can be done by feeding the layers diets which include linseed oil.Efforts have been focused on increasing the n-3 polyunsaturated fatty acids (PUFA) content of eggs by the inclusion of dietary sources of these fatty acids into the hens'ration [12]. Oil plants and some legumes can serve as source of oils to be used for supplementation of diets for poultry. Due to increasing public demand for animal products low in fat and cholesterol, studies have been focusing on improving the quality of foods from animal origin. Cholesterol and fatty acid concentrations of egg yolk vary depending on dietary manipulation and pharmacological agents as well as genetics, age and production level of bird. However, increases in the polyunsaturated fatty acid (PUFA) content of eggs by means of omega-3 fatty acid enrichment would also result in an increased susceptibility to lipid oxidation.

This could affect egg quality negatively, mainly due to a decrease in organoleptic properties of eggs, decreasing consumer acceptability toward "enriched" products [12]. It seems that factors such as lipid source and inclusion levels [22], as well as bird age and genotype [23], could influence the metabolic efficiency of dietary fatty acids into egg yolk fatty acids. The divergent information in terms of lipid sources and inclusion levels, as well as their effects on the oxidation stability of end products produced (eggs), clearly indicates the need for more research. The purpose of this investigation was to study the effect of supplementation of linseed oil on the egg fatty acid profiling, cholesterol and egg shell quality in layers.

Materials and Methods

A total of one hundred and forty single comb White Leghorn hens of commercial strain, 22-23 weeks of age, in the first phase of their production cycle with an average weight of 1737 ± 44.28 g were randomly divided in to seven treatment groups, having five replications with four birds in each replication. The laying hens of control group (T_1) were fed a basal diet formulated as per BIS [7] standards, its ingredient and composition has been given in Table 1. The layers of treatment groups T_2, T_3, T_4, T_5, T_6 and T_7 were fed basal diet supplemented with linseed oil at levels of 1%, 2%, 2.5%, 3%, 3.5% and 4%, respectively. Hens were fed the experimental diet for sixteen weeks of experimental period beginning at 22 weeks of age and continued up to 38 weeks of age. The hens were offered feed and water *ad libtum* through linear feeder and waterers. For each replicate group, cholesterol and fatty acid profile were estimated at the end of experiment. Total lipids from sample were extracted according to the method of [1] and Cholesterol of extracted fat from egg yolk was estimated by using "ERBA Kit" in Automatic Analyzer.

For fatty acid profile the yolks from three eggs were separated for each replicate, pooled, homogenized and fat separation by the method of [1]. Methyl ester was prepared by the method of [15].Then fractionation of methyl ester by using gas chromatograpy. All the diets were analyzed for proximate principles [4]. Egg shell thickness measured by screw gauge. Egg shell weight was taken after removal of shell membrane using electronic weighing balance. Shell thickness was measured by using Screw Gauge. For this purpose membrane removed pieces of shell were collected from three places, the average shell thickness was taken as the final reading. Egg shell weight percent was calculated by dividing eggshell weight by egg's weight, multiply by 100. The data were analyzed using completely randomized design [25].

Results and Discussion

Ingredient and chemical composition of ration of control group

(% DM basis) of experimental ration of control group has been given in table 1. The CP and metabolizable energy content of diet were 19.04 % and 2697.17 (Kcal/Kg), respectively.

Table 1: Ingredient and chemical composition of ration for layers of control group

Feed ingredients	Percentage
Maize	50
Groundnut cake	7
Soybean Meal	13
DORP	12
Rice Polish	5
Fish Meal	6
Mineral Mixture	3
Salt	0.5
Shell Grit	3.5
Chemical composition	**% DM basis**
CP	19.04
CF	6.74
EE	3.61
NFE	62.81
Ash	7.80
Metabolizable energy*(Kcal/Kg)	2697.17

* calculated value (BIS,2007), Feed additive included Spectromix-10g (Each g contained vitamin A- 82,500 IU, vitamin D_3 12,000 IU, vitamin B2- 50mg, and vitamin K- 10mg.), Spectrimix-BE-10g (Each g contained vitamin B1- 80mg, vitamin B_6– 16mg, Niacin-120mg, vitamin B_{12}- 80mg, Calcium Pantothenate- 80mg, vitamin E -160mg, L-lysine HCl- 10mg, DL- Methionine -10mg, and Calcium-260mg) per 100 Kg of ration.

Cholesterol

The mean values of total cholesterol in egg yolk were 13.57, 12.43, 13.08, 13.03, 12.97, 12.91 and 12.05 mg per g of egg yolk in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively (Table 2). The findings clearly indicate that, there was significant ($P<0.05$) decrease in the mean values of cholesterol, from 13.57 mg/g in control group to 12.05 mg/g in treatment group T_7. In dietary treatments T_2, T_3, T_4, T_5 and T_6 had no significant difference among themselves. However the value gradually decreased with increase in the level of linseed oil in diet. These findings are in consistent agreement with [2] who found that yolk cholesterol level decreased linearly with the increase in the level of flaxseed in the diet and the highest yolk cholesterol content was seen in the control group. The author hypothyzed that the decrease in cholesterol could arise because of the crude fiber content of the diet as the diet contained the highest crude fiber of all the diets used in the trial. Fig. 1 represents mean values of cholesterol in the egg yolk. Thus, it can be concluded that dietary inclusion of linseed oil reported a trend of reduction in yolk cholesterol.

Table 2: Mean values of cholesterol in egg yolk of layers under different treatments.

Treatments	Cholesterol (mg/g egg yolk)
T_1	$13.57^{a} \pm 0.18$
T_2	$12.93^{b} \pm 0.30$
T_3	$13.08^{ab} \pm 0.07$
T_4	$12.97^{b} \pm 0.10$
T_5	$12.91^{b} \pm 0.05$
T_6	$12.97^{b} \pm 0.09$
T_7	$12.05^{c} \pm 0.37$
CD	0.58

The mean values in same column with different superscripts differ significantly ($P< 0.05$)

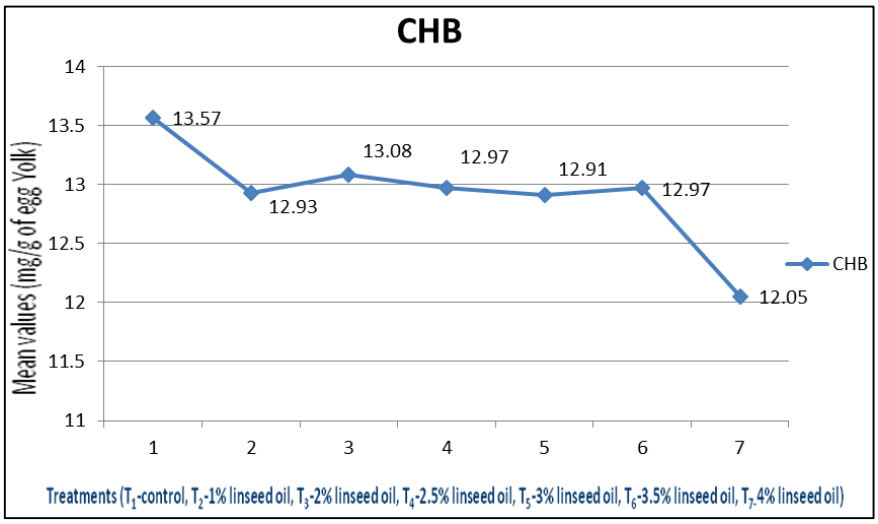

Fig 1: Mean values of cholesterol (CHB) in egg yolk of layers under different dietary treatments.

Fatty acid profile

The mean values of palmitic acid (C: 16), in egg yolk were 34.21, 33.50, 32.00, 31.06, 30.25, 29.30 and 28.35 in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively (Table 3). Results revealed that values of palmitic acid decrease significantly (P<0.05) with the increasing level of linseed oil. The mean values of Stearic acid (C: 18) were 13.29, 12.17, 11.05, 11.00. 10.16, 9.03 and 8.19 in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. Study depicted that stearic acid decreases significantly (P<0.05) with the increasing level of linseed oil. Thus saturated fatty acids (Palmitic and Stearic) decrease with increasing level of linseed oil. The mean values of Oleic acid (18:1) were 38.12, 37.40, 37.45, 37.09, 36.46, 36.56 and 36.10 for T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. The present study showed that there was significant (P<0.05) decrease in the level of oleic acid as the level of linseed oil increased. The mean values of Linoleic acid (C18:2) were 12.13, 13.23, 14.45, 14.61, 15.05, 15.68 and 16.12 in dietary treatments T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. Study depicted that Linoleic acid increased significantly (p<0.05) with the increasing level of linseed oil. The mean values of linolenic acid (18:3) were 1.52, 2.71, 4.10, 5.26, 7.08, 8.35 and 9.79 for T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. The present study showed that there was significant (P<0.05) increase in the level of Linolenic acid as the level of linseed oil increased. The mean values of Arachidonic acid (C20:4) in egg yolk were 0.74, 0.89, 0.93, 0.98, 1.00, 1.08 and 1.45 in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. Results reviled that Arachidonic acid (C20:4) increased significantly (P<0.05) with the increasing level of linseed oil. The present study showed that there was significant decrease in the n6: n3 ratio from T_1 to T_7. In agreement with present findings [2, 5, 9, 10, 11, 14, 18, 19, 24, 26, 28, 29, 31] reported that linseed oil supplementation in ration of layer increases PUFA and decreases n6:n3. This decrease in saturated fatty acid in egg yolk is due to the capability of hen to deposit added polyunsaturated fatty acid instead of saturated fatty acids.

Shell thickness, shell weight and shell weight percent

The collective mean values (22-38 weeks) of egg shell thickness were 0.373, 0.367, 0.371, 0.363, 0.363, 0.351 and 0.349 mm, (Table 4) in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. The result findings shows that there was significant (P<0.05) decrease in egg shell thickness in treatment groups $T_6$and T_7as compared to T_1, T_2 and T_3. During weeks 24-26, 28-30, 30-32 and 32-34 the mean values of shell thickness were non- significant. Collective mean of egg shell thickness decreased with the use of (1%, 2%, 2.5%, 3%, 3.5% and 4%) level of linseed oil in treatment T_2, T_3, T_4, T_5, T_6 and T_7 as compared to T_1 (0% linseed oil). Study depicted that during 22-24 weeks egg shell thickness was significantly higher in T_3 and T_4 as compared to T_5, T_6 and T_7. During weeks 26-28, 34-36 and 36-38 shell thickness decreased statistically/numerically in T_2, T_3, T_4, T_5, T_6 and T_7 as compared to T_1 (control diet). Shell quality, in terms of shell thickness and shell strength, is a significant factor in egg appearance, as well as being very important in egg handling. The collective mean values (22-38 weeks) of egg shell weight and egg shell weight % were 5.33, 5.45, 5.52, 5.52, 5.44, 5.51 and 5.56 g (Table 5) and 10.17, 10.30, 10.42, 10.04, 9.89, 9.86 and 9.71 percent (Table 6) in treatment groups T_1, T_2, T_3, T_4, T_5, T_6 and T_7, respectively. The results depicted that there was significant (P<0.05) increase in egg shell weight and decrease in shell weight percent over the different experimental periods, however, the mean values show variable significance under different dietary levels of linseed oil feeding during progressive weeks. The mean values of shell weight during 22-24, 26-28, 28-30 and 30-32 weeks and shell weight percent in week 22-24 of age were found to be non-significant. With respect to the whole period, there was significant (P<0.05) increase in egg shell weight under various linseed oil regimes (T_4, T_6 and T_7) as compared to the control (T_1) group. Egg shell weight percent was found highest in T_3 and lowest in T_7. Thus, the result findings clearly indicate that there is a notable increase in egg shell weight and decrease in shell percent with the supplementation of linseed oil in the ration of layer hens. Egg shell weight percent during 30-32 and 32-34 weeks was significantly lower in T_6 and T_7 as compared to other dietary treatments. Present findings are in agreement with the findings of [11, 20, 17, 29]. On contrary [13] reported that 2, 4 and 6% linseed supplementation significantly (P < 0.01) increased the shell thickness [9]. Reported that egg shell thickness remained similar in the hens fed 5 % linseed oil as compared to the controls. [11] Demonstrated that the hens fed 5 or 10 % linseed oil produced eggs with unchanged shell weight. Linseed oil in the ration of layers did not produce any visible change in egg shell quality and shell weight [21]. In a study by [6] eggshell thickness remained unchanged by feeding flaxseed. Decrease in egg

shell thickness may be due to increase in PUFA in diet which in turn bind with calcium to form calcium salt of fatty acid in gut and non-availability of calcium occurs. Egg shell weight is higher in treatment group T_6 and T_7 as compared to control group. Egg shell weight is significantly higher in treatment group T_6 and T_7 as compared to control group due to larger size of egg which leads to thin shell proportionately. Egg shell Weight percentage is least in treatment group T_7 because egg weight is maximum in T_7.

Table 3: Mean values of different fatty acids % in egg yolk of layers under different treatments.

Treatment	C:16 (palmitic acid)	C:18 (Stearic Acid)	C18:1 (Oleic acid)	C18:2 (Linoleic acid)	C18:3 (Linolenic acid)	C20:4 (Archidonic acid	n6:n3
T1	34.21^{a}±0.01	13.29^{a}±0.01	38.12^{a}±0.01	12.13^{g}±0.01	1.52^{g}±0.01	0.74^{f}±0.01	8.46
T2	33.50^{b}±0.01	12.17^{b}±0.01	37.40^{b}±0.01	13.23^{f}±0.01	2.71^{f}±0.01	0.89^{e}±0.01	5.21
T3	32.00^{c}±0.04	11.05^{c}±0.04	37.45^{c}±0.02	14.45^{e}±0.02	4.10^{e}±0.01	0.93^{d}±0.01	3.75
T4	31.06^{d}±0.01	11.00^{c}±0.01	37.09^{d}±0.01	14.61^{d}±0.01	5.26^{d}±0.01	0.98^{c}±0.01	2.96
T5	30.25^{e}±0.02	10.16^{d}±0.02	36.46^{e}±0.01	15.05^{c}±0.01	7.08^{c}±0.01	1.00^{c}±0.01	2.26
T6	29.30^{f}±0.01	9.03^{e}±0.01	36.56^{f}±0.01	15.68^{b}±0.01	8.35^{b}±0.02	1.08^{b}±0.02	2.00
T7	28.35^{g}±0.04	8.19^{f}±0.04	36.10^{g}±0.01	16.12^{a}±0.01	9.79^{a}±0.01	1.45^{a}±0.01	1.79
CD	0.06	0.06	0.04	0.04	0.04	0.04	

The mean values in same column with different superscripts differ significantly (P< 0.05).

Table 4: Mean values of egg shell thickness (mm) during progressive age (weeks) under different dietary treatments.

Weeks/ Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	CD
22 – 24	0.390^{ab}±0.01	0.390^{ab}±0.002	0.402^{a}±0.01	0.400^{a}±0.002	0.381^{bc}±0.01	0.370^{cd}±0.002	0.358^{d}±0.002	0.01
24 – 26	0.367±0.001	0.371±0.001	0.377±0.01	0.370±0.01	0.359±0.01	0.364±0.002	0.351±0.002	NS
26 – 28	0.360^{a}±0.01	0.344^{ab}±0.01	0.351^{ab}±0.01	0.342^{ab}±0.01	0.342^{ab}±0.01	0.327^{b}±0.01	0.329^{b}±0.002	0.03
28 – 30	0.353±0.01	0.339±0.01	0.344±0.01	0.331±0.01	0.331±0.02	0.328±0.01	0.328±0.01	NS
30 – 32	0.381±0.01	0.366±0.03	0.384±0.02	0.389±0.01	0.378±0.01	0.378±0.01	0.363±0.01	NS
32 – 34	0.381±0.00	0.376±0.01	0.365±0.01	0.360±0.01	0.379±0.01	0.352±0.01	0.355±0.01	NS
34 – 36	0.371^{ab}±0.00	0.372^{a}±0.01	0.368^{ab}±0.01	0.364^{ab}±0.01	0.366^{ab}±0.00	0.351^{b}±0.01	0.353^{b}±0.01	0.01
36 – 38	0.379^{a}±0.01	0.377^{a}±0.01	0.379^{a}±0.01	0.353^{bc}±0.01	0.369^{ab}±0.00	0.342^{c}±0.01	0.353^{bc}±0.01	0.02
Mean	0.373^{a}±0.00	0.367^{a}±0.01	0.371^{a}±0.00	0.363^{ab}±0.00	0.363^{ab}±0.00	0.351^{bc}±0.00	0.349^{c}±0.00	0.009

The mean values in same row with different superscripts differ significantly (P< 0.05)

Table 5: Mean values of egg shell weight (g) during progressive age (weeks) under different dietary treatments.

Weeks/ Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	CD
22 – 24	4.94±0.39	5.16±0.17	5.23±0.16	5.15±0.10	5.12±0.26	5.06±0.10	5.30±0.11	NS
24 – 26	5.12^{c}±0.26	5.53^{abc}±0.10	5.77^{ab}±0.09	5.98^{a}±0.15	5.52^{bc}±0.10	5.83^{ab}±0.14	5.91^{ab}±0.19	0.45
26 – 28	5.63±0.12	5.72±0.13	5.73±0.06	5.61±0.04	5.72±0.08	5.76±0.05	5.77±0.05	NS
28 – 30	5.44±0.14	5.49±0.07	5.56±0.08	5.53±0.06	5.42±0.08	5.57±0.12	5.57±0.08	NS
30 – 32	5.48±0.10	5.45±0.05	5.56±0.05	5.52±0.08	5.53±0.03	5.60±0.04	5.60±0.01	NS
32 – 34	5.28^{b}±0.06	5.48^{a}±0.06	5.49^{a}±0.06	5.40^{ab}±0.05	5.40^{ab}±0.05	5.42^{ab}±0.09	5.43^{ab}±0.11	0.20
34 – 36	5.33±0.05	5.38±0.05	5.43±0.05	5.41±0.05	5.33±0.03	5.38±0.08	5.47±0.06	NS
36 – 38	5.46^{ab}±0.04	5.39^{b}±0.07	5.40^{b}±0.02	5.53^{a}±0.04	5.49^{ab}±0.04	5.49^{ab}±0.01	5.44^{ab}±0.04	0.12
Mean	5.33^{b}±0.06	5.45^{ab}±0.04	5.52^{a}±0.04	5.52^{a}±0.04	5.44^{ab}±0.04	5.51^{a}±0.05	5.56^{a}±0.04	0.12

The mean values in same row with different superscripts differ significantly (P< 0.05)

Table 6: Mean values of egg shell weight percent during progressive age (weeks) under different dietary treatments.

Weeks/ Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	CD
22 – 24	9.41±0.43	9.87±0.45	9.94±0.50	9.62±0.28	10.01±0.49	9.75 ±0.41	10.15 ±0.29	NS
24 – 26	9.67^{b} ±0.66	10.51^{ab} ±0.39	11.11^{a} ±0.57	10.98^{ab} ±0.38	10.22^{ab} ±0.25	10.69^{ab} ±0.28	10.86^{ab}±0.50	1.31
26 – 28	11.03^{a}±0.45	11.0^{a}±0.26	10.87^{a}±0.13	10.55^{ab}±0.26	9.92^{b}±0.22	10.01^{b}±0.19	10.0^{b}±0.13	0.74
28 – 30	10.32^{ab}±0.4.9	10.48^{ab}±0.21	10.61^{a}±0.24	9.53^{c}±0.14	9.53^{c}±0.25	9.82^{bc}±0.15	9.46^{c}±0.14	0.74
30 – 32	10.58^{a}±0.42	10.27^{a}±0.13	10.24^{a}±0.11	10.16^{a}±0.16	10.23^{a}±0.06	10.09^{ab}±0.09	9.58^{b}±0.07	0.54
32 – 34	9.87^{a}±0.19	10.14^{a}±0.10	10.20^{a}±0.10	9.97^{a}±0.12	9.82^{a}±0.02	9.37^{b}±0.11	9.19^{b}±0.21	0.39
34 – 36	10.22^{a}±0.43	10.18^{a}±0.24	10.10^{ab}±0.31	9.29^{bc}±0.25	9.32^{bc}±0.10	9.48^{abc}±0.25	9.28^{c}±0.28	0.81
36 – 38	10.14^{a}±0.16	9.98^{ab}±0.08	10.26^{a}±0.21	10.24^{a}±0.08	10.07^{ab}±0.25	9.64^{b}±0.14	9.16^{c}±0.15	0.47
Mean	10.15^{abc}±0.16	10.30^{ab}±0.10	10.42^{a}±0.12	10.04^{bc}±0.11	9.89^{cd}±0.09	9.86^{cd}±0.10	9.71^{d}±0.12	0.20

The mean values in same row with different superscripts differ significantly (P< 0.05)

Conclusion

It was concluded that supplementation of different levels of linseed oil in hens' diet decreased egg yolk cholesterol, n6: n3 ratio, egg shell thickness and increased beneficial omega fatty acid, egg shell weight and egg shell weight percentage.

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