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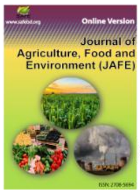

Original Article

Effect of dietary soya oil for optimizing the pullet egg size

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A B S T R A C T

Among a number of factors that influence egg size in laying pullets, the most significant one is the nutrition of the laying hens. Dietary supplementation of oils enriched with linoleic acid, for example, soya oil, has a positive impact on egg size. The current experiment was aimed at assessing the impact of dietary soya oil as a source of linoleic acid on egg quality, egg size in particular, and the laying performance of commercial laying hens. A total of 48 NovoGen-Brown layers aged 22 weeks were allocated at random to three dietary treatments, with four replications per treatment, and each replication consisted of four birds. Three different types of diets namely T_1 (control diet), T_2 (control + 1.5 % soya oil), T_3 (control + 2.5 % soya oil) were fed for 15 weeks. Diets supplemented with 1.5% soya oil and 2.5 % soya oil significantly improved the egg weight and positively influenced egg production. Egg mass was numerically better due to the supplementation of both doses of soya oil at dietary levels. Percent eggshell significantly increased in eggs assessed for quality at 28 weeks in 2.5% soya oil supplemented group. Shape index, yolk color score and boiled egg weight were not affected by soya oil supplementation. Taken all together, it may be concluded that the dietary supplementation of 1.5% soya oil positively influenced laying performance and egg quality characteristics of commercial layers. So, a commercial layer diet supplemented with 1.5 % soya oil may be considered to improve the egg size and other performance parameters as well as the egg quality of laying hens.

Introduction

Eggs are the key source of animal protein with higher biological value and better digestibility than other protein sources. An increase in egg size early in the laying cycle results in increased benefits because consumers show a preference for larger eggs. Three major factors influencing egg size are :methionine, linoleic acid, and supplemental fat **(**Grobas *et al*., 1999; Sohail *et al*., 2002; Harms and Russell 2004). Consequently, any reduction in any of these nutrients in the diet may downgrade the size of eggs and the shell quality as well. Dietary oil supplementation might be taken into consideration to overcome this problem; however, the source of oil has been found to have a significant impact in this regard. Dong *et al*. (2018) compared soya oil, fish oil, or coconut oil from 28 to 47 weeks of age to investigate the comparative effect of these dietary oil on performance, egg quality, and related parameters and found that soya oil and coconut oil treated groups performed better as compared to fish oil. Experimental evidence suggests that the chick has a special demand for linoleic acid, since dietary supplementation with methyl linoleate or linoleic acid concentrates has impact on development. Soya oil is composed of five fatty acids, of which linoleic acid (18:2) is the dominant one (55%), and other fatty acids, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linolenic acid (18:3) account for an average of 10%, 4%, 18%, and 13% of this oil, respectively (Clemente and Cahoon, 2009). The addition of oil as a source of linoleic acid in the diet of laying hens improved egg production performance (Çabuk *et al*., 2006; Gürbüz *et al*., 2011), body weight, shell thickness and yolk color score (Kim *et al*., 2019). Furthermore, Kowalska *et al*. (2021) found that nutritional supplementation with soybean meal (SBM) and narrow-leaved lupin, yellow lupin, and pea resulted in increased egg weight, egg shape index, and reduced eggshell color. When laying hens are fed conjugated linoleic acid, it is efficiently transported into the egg yolk (Shang *et al*., 2005). Linoleic acid insufficiency results in larger livers, increased liver fat, and reduced poultry growth. Fungal infections of the respiratory and digestive tracts have been observed in chicks fed diets deficient in essential fatty acids. Increased susceptibility to *E. coli* infection has also been reported in chicken feed mostly prepared from imported soybeans and soya meal (Hossain *et al*., 2020), thus quality oil is needed to be included in the diet. Soya oil is a good source of linoleic acid that can be used in animal and poultry nutrition. Linoleic acid has a positive impact on egg size (Mandal *et al*., 2017). Several acids with necessary fatty acid activity have now been discovered, with linoleic acid being by far the most significant in terms of nutritional percent. This fatty acid can be metabolized in the body to other fatty acids with essential-like action and is found in large concentrations in vegetable oils (Upadhyaya and Kailash, 2016). As the bird ages, the pressure to increase egg size diminishes rapidly. The nutritional content of the food is essential in young chickens because immature or underweight pullets prefer to utilize nutrients for development at the expense of egg weight. As a result, standard food formulation is necessary to get bigger eggs early in the production cycle. Research by March and MacMillan (1990) concluded that egg weight was consistently greater with the diet, high in linoleic acid. Egg size cannot be maximized if the amount of linoleic acid available is insufficient to sustain the maximal rate of lipoprotein production (Javier *et al*., 2018). While linoleic acid is important for efficient processing, there is no standard or exact dosage or feeding time. Furthermore, in Bangladesh, the usage of linoleic acid considering soya oil as a feed component to improve the size of pullet eggs in laying hens is unique. Therefore, the current study was undertaken in search of the appropriate quantity of linoleic acid derived from soya oil in the diet of laying hen.

Materials and methods

Experimental birds

A total of 48 NovoGen-Brown laying hens of 22 weeks old were reared at BAU Poultry Farm, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. The birds were reared in a three-tier pyramid type layer cage. The area of each pen was the 56 x 60 x 50 cm in length, width and height respectively. Before arranging the experimental birds, the cages were properly cleaned, washed and disinfected, and allowed to dry up. A thermometer and a hygrometer were used to measure the house temperature and relative humidity, respectively. The experimental birds were distributed randomly into three dietary treatments having 4 replications per treatment with four birds per replication.

Birds' husbandry

The feeding trial was conducted in a shed-type open-sided house with a measurement of length 12.5 meter and width of 6.2 meter. The ceiling height of the shed from the floor was 2.3 meter. Feed was supplied twice daily (morning and afternoon) *ad-libitum* basis and safe, clean drinking water was supplied to the birds. Birds were allowed for ten days to acclimatize with housing environment prior to commencement of data collection. During the laying phase, a total of 16 hours of light (natural daylight and artificial illumination) was given. During the cloudy weather or inadequacy of natural light, artificial light was provided to maintain proper lighting duration. The light intensity was maintained between 20 and 30 lux.

Preparation of experimental diet

The diets were prepared as per the standard guidelines of the NovoGen-Brown commercial layer management guide (2019). All feed ingredients were purchased from the local market and mixed manually. The micro-nutrients like lysine, methionine, layer premix, protein concentrate, di-calcium phosphate (DCP), choline chloride, common salt, etc. were mixed very carefully with rice polish and fine particles of crushed maize ensure homogeneous mixing with the whole feed. After mixing, the diet was divided into three equal parts. No addition of soya oil was treated as the control diet T_1 (contained about 1% linoleic acid), a diet supplemented with 1.5% soya oil was treated as $T_{2,}$ (contained about 2% linoleic acid) and addition of 2.5% soya oil was treated as T_3 (contained about 3% linoleic acid).

Record Keeping

Data from the experimental trial were collected on a daily and weekly basis. The initial body weight was recorded before the experiment began, and the final body weight was recorded at the end of the trial. Temperature and humidity were measured twice daily from the beginning of the feeding trial. Following the initial adjustment phase, laying performance, including egg production and egg weight, was recorded daily in the afternoon. At the end of the feeding trial, the average daily feed intake, egg mass output, and feed conversion ratio were calculated. During the trial, eggs from each replication were collected four times at four-week intervals.

Egg quality characteristics

Egg quality parameters were determined for eggs laid by birds of different dietary groups and each time two eggs were collected from each replication. The external (eggshell quality) and internal (albumen and yolk qualities) egg quality parameters were measured using the specific instrument and calculations were done using the procedures as described by Ray (2018). For eggshell qualities, shape index, shell percentage, and shell thickness were measured. Albumen index and Haugh unit were measured for albumen quality, and yolk index, along with yolk color score, were measured for yolk quality parameters. Yolk index and yolk color score were measured as yolk quality of eggs. The yolk index was measured by slide caliper and micrometer, whereas the yolk color score was measured using DSM Yolk Fan^{TM} (an eye estimation method).

Statistical analysis

Data for different variables were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) employing SAS (2009) statistical computer package program. Differences with p-values <0.05 or better were considered to be significant.

Results and Discussion

Laying performance

Table 1 shows the findings of the laying performance of various dietary treatment groups**.** Numerically higher egg production was found in the 1.5% soya oil supplemented group followed by the control and 2.5% soya oil supplemented groups. Jones *et al*. (2000) discovered that hens given high and medium doses of linoleic acid had lower egg production rates than those fed 0 and 0.1 mg linoleic acid/kg, suggesting that linoleic acid had an effect on hen reproductive efficiency. A clear significant effect in the egg weight was detected in 1.5% and 2.5% soya oil fed groups compared to the control. Pariza *et al*. (2000) discovered that linoleic acid greatly affected egg weight, mostly through an effect on yolk weight and lipid content. Al-Daraji *et al*. (2011) reported that adding fish and maize oil to the diet of laying hens resulted in a substantial increase in egg weight. Yan Zhang *et al*. (2017) found that dietary linoleic acid supplementation at all doses was related with considerably greater egg weight. Dong *et al*. (2018) conducted an experiment with soya oil, fish oil and coconut oil, where they found an increased egg weight with soya oil or coconut oil inclusion into diet as compared to fish oil.

The results of egg mass indicated that egg mass output in different treated groups was numerically higher due to the dietary inclusion of linoleic acid at different doses (Table 1). The control group had numerically lowest egg mass output compared to the birds supplemented with linoleic acid containing 1.5% and 2.5% soya oil. Numerically higher egg mass was found in birds that received 1.5% soya oil and 2.5% soya oil compared to the control group. According to the results obtained from this experiment, dietary linoleic acid supplementation had no significant effect on feed intake (Table 1). Similarly, Chamruspollert and Sell (1999) observed that feeding 5.0% CLA decreased feed intake but did not affect rate of egg production, weight of eggs, albumens, or yolks, or body weight gain. The feed efficiency was numerically greater in the treated groups although statically non-significant (P>0.05) (Table 1). Linoleic acid did not influence development but increased feed conversion efficiency in laying hens, (Ostrowsha *et al*., 1999). Tsuzuk *et al*. (2003) found that increasing linoleic acid inclusion levels had no effect on daily feed intake or feed conversion. Yenice *et al*. (2018) showed that combining safflower meal with soya oil in varying proportions (from 3 to 22 percent) in laying hen diets had no effect on livability, body weight increase, egg production, egg weight and mass, feed consumption, or feed conversion. There were no birds that died during the entire feeding trial which also indicates that dietary supplementation of soya oil at the level tested had no adverse effect on bird's health.

Table 1. Effects of different levels of soya oil on the performance of laying hens (24-36 weeks).

Parameters	T_1	T_2	T_3	p-value
HDEP (%)	91.97±2.481	94.13±1.370	90.18±3.005	0.5269
EW (g)	58.47^{b}±0.461	61.58^{a}±0.535	60.55^{a}±0.690	0.0113
EMO (g/b/d)	53.75±1.171	57.94±1.216	54.58±1.677	0.1276
FI (g/b/d)	114±0	114±0	113±0	0.4391
FCR	2.14±0.050	1.97±0.043	2.11±0.068	0.1283

HDEP = Hen Day Egg production; EW=Egg Weight; EMO=Egg Mass Output; FI=Feed Intake; FCR=Feed Conversion Ratio; T_1 = Hand-made layer mash feed with no oil (control); T_2 = Hand-made layer mash feed supplemented with 1.5 % soya oil; T_3 = Hand-made layer mash feed supplemented with 2.5 % soya oil. Means bearing dissimilar alphabets in the similar row differ significantly at the stated level probability.

Eggshell quality

Linoleic acid supplementation in laying pullets enhanced eggshell quality. The results of shape index, shell weight, per cent shell, and shell thickness of birds that received different dietary treatment groups are shown in Table 2 and Figure 1. There were no significant differences in the eggshell quality assessment except in the per cent shell of 28th week, where both the doses of soya oil significantly increased the values compared to the control group (P< 0.01). Other eggshell parameters were either equal to or numerically better than the control group indicating that dietary linoleic acid had no adverse effect on pullet eggshell traits.

Table 2. Effects of different dietary levels of soya oil on eggshell quality (24-36 weeks).

Age (weeks)	Parameter	T_1	T_2	T_3	p-value
24th week	Shape Index	75.91±0.968	78.21±1.345	77.48±0.945	0.3622
	Shell Weight (g)	5.57±0.406	6.17±0.372	6.10±0.083	0.3974
	Per cent Shell	9.97±0.301	10.59±0.428	10.57±0.128	0.3232
	Shell Thickness (mm)	0.35±0.0173	0.38±0.014	0.39±0.0125	0.1420
28th week	Shape Index	76.65±2.373	77.39±1.480	72.02±1.760	0.1536
	Shell Weight (g)	5.03±0.444	5.98±0.153	5.69±0.157	0.1074
	Per cent Shell	9.17^{b}±0.567	10.10^{ab}±0.202	11.11^{a}±0.157	0.0130
	Shell Thickness (mm)	0.33±0.022	0.35±0.008	0.33±0.006	0.6262
32th week	Shape Index	78.16±2.515	77.68±1.251	76.46±1.771	0.8158
	Shell Weight (g)	5.94±0.211	5.95±0.221	5.70±0.282	0.7103
	Per cent Shell	10.90±0.210	10.86±0.516	10.18±0.153	0.2784
	Shell Thickness (mm)	0.39±0.016	0.39±0.011	0.39±0.004	0.9045
36th week	Shape Index	73.93±0.930	77.57±1.476	74.52±2.052	0.2600
	Shell Weight (g)	5.52±0.400	5.90±0.147	5.72±0.197	0.6352
	Per cent Shell	10.05±0.291	10.46±0.357	9.73±0.396	0.3751
	Shell Thickness (mm)	0.38±0.012	0.39±0.006	0.40±0.007	0.3219

Where, T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed + 1.5 % soya oil; T_3 = Hand-made layer mash feed + 2.5 % soya oil. Means bearing dissimilar alphabets in the similar row differ significantly at the stated level probability.

Ribeiro *et al*. (2007) reported no change between treatments for egg shell by utilizing different levels of linoleic acid, 1.93g/100g and 1.48g/100g linoleic acid in the diet of chickens during 27-40 weeks of age, based on egg weight and composition. Al-Daraji *et al*. (2011) found that the addition of oil in the diet of laying birds resulted in a substantial increase in shell weight and shell thickness, which is in line with our results of 28 weeks. The findings of Aziza *et al*. (2013) supported our results. They revealed that brown egg layers fed a corn-soya based diet with 10% camelina or flax meal produced greater egg quality and egg yolk fatty acids and we have also showed that supplementation of layer diet with 1.5% soya oil significantly affect the egg weight of laying pullets. Wang *et al*. (2019) found that including soya oil in the diets had the potential to increase egg freshness and quality. Furthermore, Kowalska *et al*. (2021) found that nutritional supplementation with soybean meal (SBM) and narrow-leaved lupin, yellow lupin, and pea resulted in increased egg weight, form index, and reduced eggshell color.

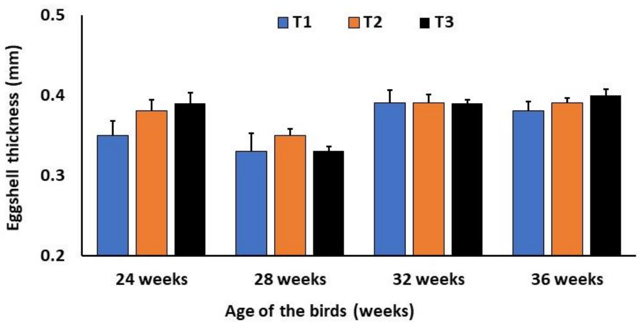

Figure 1. Eggshell thickness of different dietary treatment groups fed soya oil. Where, Where, T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed + 1.5 % soya oil; T_3 = Hand-made layer mash feed + 2.5 % soya oil.

Internal egg quality

Albumen and yolk quality were evaluated as the internal quality of the egg. The results from albumen quality of eggs are presented in Table 3 and Figure 2. The height of albumen is related to the Haugh unit of eggs, and albumen height affects protein quality. The egg's freshness and protein content are thought to improve as the Haugh unit value rises. At 28th week of laying, pullets showed significant differences in both Haugh unit ($p<0.01$) and albumen index ($p<0.05$) compared to the control and 2.5% soya oil supplemented groups.

Table 3. Effects of different dietary levels of soya oil on albumen quality (24-36 weeks).

Age (weeks)	Parameter	T_1	T_2	T_3	p-value
24-week	Albumen Index	0.05±0.014	0.07±0.004	0.07±0.004	0.1079
	Haugh Unit (HU)	64.41±4.940	78.95±1.785	81.87±3.197	0.1522
28-week	Albumen Index	0.08^{a}±0.010	0.08^{a}±0.008	0.06^{ab}±0.002	0.0296
	Haugh Unit (HU)	79.96^{ab}±4.102	83.5^{a}±4.462	69.40^{b}±4.004	0.0154
32-week	Albumen Index	0.04±0.151	0.09±0.012	0.07±0.006	0.3614
	Haugh Unit (HU)	84.72±4.640	86.08±5.870	77.63±3.574	0.4408
36-week	Albumen Index	0.06±0.006	0.08±0.010	0.06±0.005	0.3832
	Haugh Unit (HU)	77.35±3.384	80.26±5.504	74.99±4.002	0.7061

T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed supplemented with 1.5 % soya oil; T_3 = Hand-made layer mash feed supplemented with 2.5 % soya oil. Means bearing dissimilar alphabets in the similar row differ significantly at the stated level probability.

No other statistical differences ($p<0.05$) were observed for the albumen quality of eggs although an increasing trend was followed for the albumen qualities in the 1.5% soya oil treated groups than control and 2.5% soya oil treated groups. Restricting the linoleic acid content of the diet from 1.60 to 1.12 percent had no effect on egg quality or the proportion of egg components, which is consistent with Grobas *et al.* (1999), who discovered that linoleic acid levels in diets for ISA Brown hens ranging from 0.79 to 2.73 percent had no effect on the percentage of broken and dirty eggs, Haugh units, the proportion of egg components and improved the laying hen performance fed soybean meal (SBM) supplementation. Al-Daraji *et al.* (2011) observed that adding oil in the diet of laying hens increased yolk diameter, yolk weight, albumen diameter, and albumen weight. Linoleic acid has been shown to have a significant favorable influence on interior egg quality and overall poultry performance (Ghajarbeygi *et al*., 2015; Wang *et al*., 2019). Kowalska *et al*. (2021) got significantly greater Haugh unit score with dietary addition of soybean meal (SBM) in laying hens in a similar study.

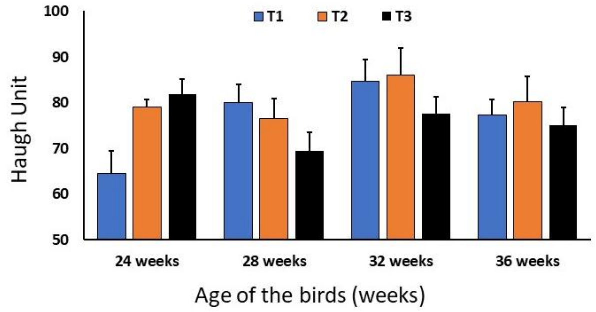

Figure 2. Haugh Unit of different dietary treatment groups fed soya oil. T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed + 1.5 % soya oil; T_3 = Hand-made layer mash feed + 2.5 % soya oil.

The results of yolk quality parameters are presented in Table 4 and Figure 4 which showed that the yolk color score of 32 weeks older laying pullets improved significantly ($P<0.01$). No significant variation was observed from other age groups although a positive effect on the dietary supplementation of soya oil was apparent in the yolk qualities. Soya oil contains few pigmenting agents that might have positively affected yolk coloration.

Table 4. Effects of different dietary levels of soya oil on yolk quality (24-36 weeks).

Age (weeks)	Parameter	T_1	T_2	T_3	p-value
24-week	Yolk Index	0.41±0.020	0.42±0.021	0.41±0.008	0.9736
	Yolk Color Score	4.75±0.478	5.50±0.288	5.125±0.125	0.3227
28-week	Yolk Index	0.36±0.007	0.38±0.012	0.37±0.006	0.4393
	Yolk Color Score	4.75±0.250	5.00±0.408	5.50±0.288	0.2955
32-week	Yolk Index	0.39±0.012	0.40±0.013	0.38±0.012	0.4223
	Yolk Color Score	4.75^{b}±0.250	6.25^{a}±0.250	5.00^{b}±0.408	0.0169
36-week	Yolk Index	0.40±0.000	0.39±0.006	0.39±0.004	0.5217
	Yolk Color Score	5.50±0.288	6.00±0.408	5.50±0.288	0.4997

T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed supplemented with 1.5 % soya oil; T_3 = Hand-made layer mash feed supplemented with 2.5 % soya oil. Means bearing dissimilar alphabets in the similar row differ significantly at the stated level probability.

Al-Daraji *et al.* (2011) concluded that adding oil to the diet of laying hens resulted in a substantial increase in yolk weight, yolk diameter, and yolk weight. Wang Sheng Lin (2015) and Lin YingCai (2015) reported similar findings when they supplemented their diets with linoleic acid, and observed that the yolk color rose as the dietary linoleic acid content increased. Ding *et al.* (2017) hypothesized that the essential oil supply may be a factor in the disparity in yolk fatty acid reactions. Kowalska *et al.* (2021), found that dietary intervention with soybean meal (SBM) enhanced egg yolk color score in laying hens.

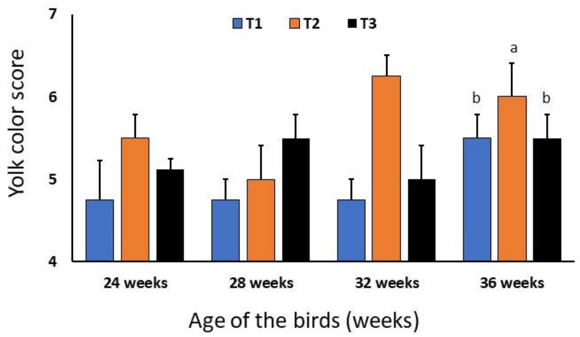

Figure 3. Yolk color score of different dietary treatment groups fed soya oil. T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed + 1.5 % soya oil; T_3 = Hand-made layer mash feed + 2.5 % soya oil.

Table 5. Effects of different dietary levels of soya oil on boiled yolk weight.

Age (weeks)	Parameter	T_1	T_2	T_3	p-value
24-week	Test egg weight (g)	59.10±2.07	60.90±2.11	66.50±3.17	0.144
	Boiled yolk weight (g)	13.74±0.56	13.88±0.42	16.11±1.78	0.285
28-week	Test egg weight (g)	56.34^b±1.39	61.12^a±1.35	61.00^a±1.00	0.041
	Boiled yolk weight (g)	14.69±0.34	14.36±0.84	14.50±0.70	0.939
32-week	Test egg weight (g)	51.15±2.23	56.02±1.97	56.65±1.98	0.176
	Boiled yolk weight (g)	13.72±0.63	13.70±0.28	13.17±0.89	0.803
36-week	Test egg weight (g)	54.16±5.21	60.31±1.13	55.44±1.71	0.404
	Boiled yolk weight (g)	13.22±0.67	14.27±0.44	12.82±0.43	0.190

T_1 = Hand-made layer mash feed (control); T_2 = Hand-made layer mash feed supplemented with 1.5 % soya oil: T_3 = Hand-made layer mash feed supplemented with 2.5 % soya oil. Means bearing dissimilar alphabets in the similar row differ significantly at the stated level probability.

None of the dietary soya oil inclusion in the basal diet influenced boiled egg weight in the experiment ($p > 0.05$) (Table 5). The inclusion of linoleic acid causes an increase in yolk weight which eventually contributed to greater egg weight (Dong *et al*., 2018). However, in our study, we did not find such relationship between yolk weight and egg weight, though dietary soya oil incorporation caused significant improvement in egg weight. From this research, we may suggest that dietary soya oil when included in the diet increases the egg weight of commercial laying hens.

Conclusion

The results of the present study indicate that dietary linoleic acid supplementation had an effect on both laying performance and egg quality. The overall laying performance of the soya oil-treated groups was better than that of the control diet group. Dietary supplementation of 1.5% soya oil seemed to be better as compared to 2.5% soya oil and control groups. Taken together, it may be concluded that dietary supplementation of a layer diet with 1.5% soya oil may be beneficial for improving the pullet egg size and other egg quality parameters.

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Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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