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Effects on Performance, Egg Quality Criteria and Cholesterol Level of Adding Different Ratios Flaxseed Oil Instead of Sunflower Oil to Compound Feed of Laying Hens

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ABSTRACT
This study was conducted to examine the effect on performance, egg quality criteria and egg cholesterol level of adding different ratios of flaxseed oil instead of sunflower oil to the compound feed of laying hens. A total of 210, 30-week old Lohmann LSL laying hens were acquired for the study. The study groups consisted of a control group (5% sunflower oil (SO) + 0% flaxseed oil (FO) and trial 1 (4% SO + 1% FO), trial 2 (3% SO + 2% FO), trial 3 (2% SO + 3% FO), trial 4 (1% SO + 4% FO) and trial 5 (0% SO + 5% FO) groups. The study lasted for 8 weeks. The feed consumption was not different among the experimental groups. Feed utilization rate was higher in group 2 compared to those of other between 0-8 weeks ($P<0.001$). Between weeks 0 to 8, all trial groups were found have significantly higher levels of linoleic acid, one of the fatty acids found in yolk, compared to the control group ($P<0.001$). Additives were not found to affects levels of cholesterol in yolk, with no significant differences found between groups. In short, sunflower oil and flaxseed oil added to laying hen rations did not create any differences in terms of egg quality criteria or egg cholesterol levels, but higher levels of flaxseed oil added to the rations resulted in linearly higher levels of linolenic acid content of yolk, and use of the two oil additives together increased egg yields.

Keywords: Egg quality criteria, Laying hens performance, α-linolenic acid, Vegetable oils, Yolk cholesterol

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1. Introduction

The need to provide a healthy and balanced diet to the world's growing population has made animal husbandry one of the most important economic sectors in countries. Quality foods have balanced amounts of nutrients such as protein, fat, carbohydrate, minerals and vitamins, which can be found in foods of vegetable and animal origin in varying amounts. Animal products such as meat, milk and eggs play an important role in human nutrition, and there has been a growing trend in recent years to pay more attention to the quality of the food we consume. The growing public demand for low-fat and low-cholesterol animal products has led to a number of studies into improving the quality of foods of animal origin (Choupani et al. 2013), and foremost among these foods are eggs.

Chicken eggs are one of the most important animal products for human nutrition. Similar to other farm animals, poultry use basic organic nutrients like carbohydrates, fats and proteins to meet their energy needs. In terms of level of use, carbohydrates come first, followed by fats (Özdoğan & Sarı 2001). In other words, compound feeds mostly rely on carbohydrates for energy. That said, carbohydrate-rich grains alone cannot meet the needs of animals with high energy needs such as poultry, and so energy-rich fats and oils are used to make up the shortfall in energy, and usually fats and oils that are not suitable for human consumption. All poultry feed must have a minimum fat content of 1% is it is to provide a sufficient amount of such essential fatty acids as linoleic and linolenic acids, to improve the taste of compound feeds and to prevent powdering, alongside other economic or nutritional considerations (Omidi et al. 2015).

Fats participating in the rations are divided into two main groups, being either saturated or unsaturated (Çakmakçı & Kahyaoğlu 2012). Of the saturated fatty acids, acetic and butyric acids are formed as the final products of carbohydrates; capric, caproic and caprylic acids are found in vegetable oils, and in very small concentrations, in butter; lauric acid is found in coconut oil, cinnamon, palm kernel, and bay leaf; myristic acid is found in coconut oil and palm kernel; and palmitic and stearic acids are found in all animal fats and vegetable oils (Ergün et al. 2014). Of the unsaturated fatty acids, palmitoleic acid and oleic acid are found in vegetable oils and animal fats; linoleic acid is found in sunflower seeds, soybeans, corn, peanuts, and cotton seeds;

linolenic acid is found in flaxseed oil and fish oil; and arachidonic acid is found in peanuts and animal phospholipids (Ergün et al. 2014).

To obtain eggs rich in linoleic and linolenic fatty acids, which are needed by humans but have to be obtained through foods because the human body cannot synthesize them, vegetable oils rich in these fatty acids are added to the rations of laying eggs. Sunflower oil and flaxseed oil are the two most common oils used for this purpose.

Sunflower oil is rich in linoleic acid (omega 6) but poor in linolenic acid (omega 3). Flaxseed oil is one of the richest sources of α-linolenic acid (ALA)-a type of omega-3 (n-3) fatty acid that accounts for around 55% of all fatty acids (İşleroğlu et al. 2005). The fat content and quality of flaxseed varies depending on the plant variety and hereditary characteristics. Moreover, environmental factors such as temperature, soil conditions, cultural practices and plant diseases can affect both the fat content and quality. The greatest variation in the composition of fatty acids can be observed in oleic acid (14-60%), linoleic acid (3-21%) and linolenic acid (31-72%) (İşleroğlu et al. 2005). Zelenka et al. (2007) report that some flax varieties are rich in linolenic acids, whereas others are rich in linoleic acids.

Eseceli & Kahraman (2003) found that adding vitamins E and C to hen rations with sunflower oil and fish oil increased some performance parameters, but the qualities of the source of oil in the ration had a more significant effect on performance compared to the addition of vitamins E and C. Mazalli et al. (2004) fed laying hens mixed rations with 3% animal fats and vegetable oils, including sunflower oil and flaxseed oil, as well as ground flaxseed treated with two different levels of vitamin E (12 IU/kg and 100 IU/kg), and reported that the treatment did not produce any significant effects on performance or egg quality criteria.

Cholesterol is a natural yolk component, and an egg weighing 60 to 62 g is expected to contain 200 to 220 mg of cholesterol under normal conditions, but studies by different researchers report a range of 122 to 408 mg (Jacob & Miles 2000; Vorlova et al. 2001; Yegani & Nilipour 2003; Raj Manohar 2015; Rakıcıoğlu 2016). In a study on functional egg production, 2% flaxseed oil added to laying hen rations was found to lower the cholesterol level of eggs significantly (Küreç 2009).

The present study investigates the effects on performance, egg quality criteria and cholesterol of using varying rates of flaxseed oil rather than sunflower oil in layer rations, the former of which has greater linolenic acid content than the latter.

2. Material and Methods

2.1. Animals, rations and experimental design

The animal materials used in the study were 210 Lohmann LSL type white egg-laying hens aged 30 weeks, purchased free of disease from a private poultry business in Adana, Turkey. The raw materials for the feeds used in the study were bought on the open market in Van. The cold-pressed flaxseed oil used in the trial was obtained from the plant products company Doğa Bitki Ürünleri Gıda Ticaret Ltd. Şti. in Antalya.

The trial was conducted at the Laying Hens Trial Unit of the Van Yüzüncü Yıl University Research and Application Farm. The hens were kept in two-tier battery cages for laying hens for the trial. The site was illuminated with fluorescent lights for 16 hours a day during the trial and kept dark for 8 hours a day.

The 210 hens used in the study were divided into six groups of 35 hens each, and each group was further divided into five subgroups consisting of seven hens. The hens were group-fed ad libitum, keeping trial feed containing approximately 18.5% crude protein and 2800 kcal/kg metabolic energy in the feeders at all times during the egg laying period, taking care to keep all groups under identical conditions. Fresh water was provided continuously using a drip watering system. The oil content of the feeds was 5% for all groups. In the control group, all of this was sunflower oil, whereas in the trial groups, sunflower and flaxseed oils were used in different ratios, with one trial group receiving only 5% flaxseed oil. The trial lasted for 8 weeks. Table 1 reports the treatments for all groups.

Table 1- Groups and treatments (Total oil content of the ration 5%)

Groups	*Sunflower content of the ration (%)*	*Flaxseed content of the ration (%)*
Group 1 (Control)	5	0
Group 2 (Trial 1)	4	1
Group 3 (Trial 2)	3	2
Group 4 (Trial 3)	2	3
Group 5 (Trial 4)	1	4
Group 6 (Trial 5)	0	5

Isonitrogenic and isocaloric rations were prepared in the compound feed preparation unit of the poultry house for laying hens of the Van Yüzüncü Yıl University Research and Application Farm. The nutrient requirements of laying hens were determined according to NRC (NRC 1994). The compositions and nutrient contents of the feeds were reported in the Table 2 and fatty acid

composition of the sunflower and flaxseed oils used in the trial (%) are reported in Table 3. The chemical compositions of the trial feeds were determined using the methods explained in AOAC (1984), in the laboratory of the Department of Animal Feeding and Feeding Diseases of the Van Yüzüncü Yıl University Faculty of Veterinary Medicine. The metabolizable energy (ME), calcium, available phosphorus, methionine+cysteine, lysine and sugar values of the ration used in the trial was determined by calculation (Jurgens 1996). Starch analysis was made according to Karabulut & Canbolat (2005). The metabolic energy contents of sunflower and flax oil included in the rations were determined as 8840 and 8842 kcal/kg, respectively. Thus, while the participation rate of the raw materials in the experimental rations did not change, only the fat ratios were changed gradually (Table 1). Trial rations were prepared by adding oil sources at different rates to a basic ration (Jurgens 1996). Fatty acid compositions of the sunflower and flaxseed oils, and the yolks samples were performed according to Cherian & Quezada (2016) by using GC-FID device.

Table 2- Components and chemical composition of the ration used in the trial, %

Ingredients	*Basic ration*
Yellow corn	45.05
Wheat	5.00
Wheat bran	1.50
Sunflower oil	5.00
Flaxseed oil	0.00
Soybean meal (48%)	15.05
Sunflower meal (36%)	15.15
Meat and bone meal	2.50
Dicalcium phosphate	4.00
Limestone	5.90
Antioxidant*	0.10
Salt	0.40
Vitamin mix**	0.175
Mineral mix***	0.175
Total	100
Chemical composition	
Dry matter (%)	91
Crude protein (%)	18.5
Metabolic energy (kcal/kg)#	2800
Crude fat (%)	6.53
Crude ash (%)	13.93
Crude fiber (%)	3.97
Calcium (%)#	3.62
Available P (%)#	0.93
Methionine + cysteine (%) #	0.65
Lysine (%) #	0.86
Starch (%)	34.37
Sugar (g/100 g)#	3.24

*: Contains 10.000 mg of antioxidant (Ethoxyquin E324, BHA E320, E330) per kilogram; **: Contains 12.000.000 IU Vitamin A, 2.500.000 IU Vitamin D_3, 30.000 mg Vitamin E, 4.000 mg Vitamin K_3, 3.000 mg Vitamin B_1, 6.000 mg Vitamin B_2, 30.000 mg Vitamin B_3, 10.000 mg Vitamin B, 5.000 mg Vitamin B_6, 15 mg Vitamin B_{12}, 1.000 mg Folic acid, 50 mg D-Biotin H_2, 50.000 mg Vitamin C, 300.000 mg Choline, 3.000 mg Canthaxanthin, 1.500 mg apo ester and 10.000 mg antioxidant per 1.75 kg; ***: Contains 80.000 mg Mn, 60.000 mg Fe, 60.000 mg Zn, 5.000 mg Cu, 2.000 mg I, 500 mg Co and 150 mg Se per 1.75 kg; #: Calculated value (Jurgens 1996)

Table 3- Fatty acid composition of the sunflower and flaxseed oils used in the trial

Number of carbons	*Chemical name*	*Flaxseed oil (%)*	*Sunflower oil (%)*
C14:0	Myristic acid	-	0.07
C16:0	Palmitic acid	5.0	6.5
C16:1n-9	Palmitoleic acid	0.2	0.1
C17:0	Margaric acid	0.1	0.05
C17:1	Heptadecenoic acid	0.1	0.05
C18:0	Stearic acid	2.6	3.8
C18:1n-9	Oleic acid	46	27.6
C18:2n-6	Linoleic acid	19	60.5
C18:3n-3	Linolenic acid	25	0.1
C20:0	Arachidic acid	0.5	0.2
C22:0	Behenic acid	0.3	0.8
C24:0	Lignoceric acid	-	0.3

2.2. Performance parameters

The hens in each subgroup were group-fed, and average feed consumption by each subgroup was measured with weekly weighing. Daily egg yield records were kept for each subgroup. Feed conversion rate was calculated from feed consumption and egg mass values as amount of feed consumed (g)/egg mass (g).

2.3. Egg quality characteristics

Every two weeks and for a total of four times throughout the duration of the study, 20 eggs were selected at random from each group (4 from each subgroup), making a total of 120 eggs, and egg weight, eggshell thickness, egg shape index, albumen index, and Haugh unit parameters were examined.

Once a week, all eggs from each group were weighed after being kept at room temperature for 24 hours. Samples were taken from the pointy, blunt, and middle sections of broken eggshells, and their thicknesses were measured using a micrometer after removing the membrane. The arithmetic mean of these values was recorded as eggshell thickness. The egg width and length were measured with a digital caliper and egg shape index was calculated from the data (Card & Nesheim 1972).

Shape index = (egg width/egg length) x100

The eggs were cracked on a glass table. The heights of yolk and albumen were measured using a tripod micrometer, while yolk diameter and albumen width were measured using a caliper, after waiting for 10 minutes to avoid large changes. These values were used to calculate the albumen index and Haugh unit based on the following equations (Şenköylü 2001):

Albumen index = (height of the thick albumen/(width+length/2)) x100

Haugh unit = 100 Log (height of the thick albumen + 7.57 – 1.7 egg weight$^{0.37}$)

2.4. Yolk cholesterol content

Following the trial, 15 eggs from each group (three from each subgroup) were boiled, and the cholesterol content of their yolks was examined (Boehringer Mainheim Gmbh Biochemica 1989; Küçükersan 2004).

For cholesterol analysis, yolks were mashed and homogenized. 0.1 g of yolk were put in a glass tube, and 4 mLof isopropyl alcohol were added. The mixture was mixed in a vortex until it became homogeneous, and filtered through a filter paper. For the cholesterol kit, glass tubes were marked sample, standard, and blind. 2 mL of cholesterol kit were added to all tubes, which were then kept in a 37 ºC water bath for 2 minutes. At the end of this period, 0.02 mL (20µl) kit was added to the sample tubes, 0.02 ml cholesterol standard was added to the standard tube, and 0.02 mL water was added to the blind tube. All tubes were kept in a 37 ºC water bath for 10 minutes. Results were read on the spectrophotometer (Schimadzu UV-1201V) at a wavelength of 520 nm. The optic density (OD) values read were evaluated using the following formulas:

$$\text{Cholesterol content of the extract (mg/g)} = \frac{\text{Sample OD}}{\text{Standard OD}} \times \text{Concentration of the standard}$$

$$\text{Yolk cholesterol content (mg/g)} = \frac{(\text{Cholesterol content of the extract} \times 100) \times 4}{\text{Sample weight (g)}}$$

2.5. Statistical analysis

All of the data obtained during the study was subjected to a variance analysis (SAS 1982). Inter-group differences were examined using Duncan's Multiple Comparison test (Steel & Torrie 1980).

3. Results

Looking at trial data for the entire 8-week period, no statistically significant differences were found between the groups in terms of feed consumption (Table 4). Trial group 2 had a higher feed utilization rate compared to the control group for weeks 0 to 8 ($P<0.001$, Table 4). Trial groups 3 and 5, in particular, were found to have feed utilization rates that were very similar to one another and better than other trial groups at 6. weeks. In general, mean egg yields was considerably higher in the intervention groups when compared to the control group in weeks both 5 and 0-8 ($P<0.05$, $P<0.001$, Table 4). Only egg yield in trial group 2 was similar to that of the control group in weeks both 5 and 0-8. In terms of average egg weights, statistically significant differences were found between groups at weeks both 7 and 0-8. Trial 2 group had higher egg weight than other groups in 0-8 weeks period. Egg quality criteria for trial groups given in Table 5. An overall analysis of the findings of the study of egg quality criteria identified no statistically significant differences between the groups. Total fatty acid content of yolks for groups are given

in Table 6. The present study found significant differences between the groups (P<0.001) in terms the total fatty acid content of the eggs, with the exception of linoleic acid content. Linoleic acid levels may fluctuate depending on the amount of sunflower oil used in trials. Yolk cholesterol content of groups are given in Table 7. No significant differences were found between the groups in terms of egg cholesterol content.

Table 4- Performance parameters for trial groups

	Feed consumption, g/day/hen						
Weeks	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
1	116.18±1.80	120.80±3.91	118.38±1.16	116.78±0.84	119.62±1.25	118.32±1.30	0.599
2	113.61±4.82	116.00±2.61	118.59±4.09	124.41±3.76	114.21±3.90	120.46±3.55	0.362
3	110.62±10.58	114.08±1.58	111.48±2.43	116.04±2.79	113.68±1.27	109.02±2.86	0.915
4	121.62±4.67	118.41±1.86	121.00±2.66	123.28±6.73	117.64±1.89	121.81±2.43	0.894
5	119.43±1.98	119.58±2.54	116.44±2.04	118.53±3.79	118.42±1.78	118.25±2.45	0.960
6	124.48±1.90	118.25±4.01	121.26±4.20	114.38±5.26	118.25±1.28	106.40±5.32	0.060
7	120.09±7.75	118.48±1.58	120.48±4.66	119.31±3.24	118.28±1.97	111.36±3.54	0.692
8	125.90±1.79	123.90±1.94	116.02±2.76	123.25±3.10	120.77±0.77	126.63±3.67	0.069
0-8	119.56±1.02	117.52±0.75	117.08±1.01	117.94±0.86	117.61±0.73	116.67±1.17	0.599
	Feed conversion rate, kg feed/kg egg						
1	2.21±0.03	2.42±0.14	2.39±0.07	2.25±0.11	2.37±0.14	2.40±0.15	0.729
2	2.27±0.08	2.21±0.03	2.48±0.10	2.34±0.12	2.19±0.03	2.27±0.06	0.147
3	2.14±0.13	2.16±0.09	2.17±0.08	2.03±0.05	2.23±0.15	1.99±0.05	0.547
4	2.37± 0.12	2.13±0.06	2.33±0.10	2.23±0.05	2.20±0.05	2.22±0.05	0.269
5	2.20±0.05	2.17±0.07	2.24±0.06	2.10±0.08	2.19±0.05	2.12±0.04	0.605
6	$2.22±0.02^{b}$	$2.07±0.06^{c}$	$2.42±0.04^{a}$	$2.05±0.05^{c}$	$2.12±0.03^{bc}$	$2.05±0.07^{c}$	0.001***
7	2.13±0.15	2.06±0.07	2.35±0.07	2.25±0.07	2.11±0.03	2.55±0.25	0.117
8	2.19±0.03	2.19±0.05	2.23±0.06	2.26±0.03	2.21±0.09	2.37±0.08	0.329
0-8	$2.21±0.03^{b}$	$2.17±0.03^{b}$	$2.33±0.03^{a}$	$2.19±0.03^{b}$	$2.20±0.05^{b}$	$2.25±0.05^{ab}$	0.020*
	Egg yield, %						
1	87.76±1.44	91.84±1.18	85.71±2.58	93.37±2.26	92.35±1.93	90.31±1.74	0.089
2	85.20±1.74	92.35±2.10	88.44±1.36	90.31±2.26	89.80±2.89	91.43±0.76	0.251
3	88.27±2.68	96.94±1.02	88.98±2.47	96.60±1.36	87.25±6.68	94.29±1.00	0.271
4	90.48±3.40	95.92±1.18	86.74±5.11	92.25±1.19	91.84±2.58	94.70±1.53	0.270
5	$83.67±2.04^{b}$	$96.43±0.51^{a}$	$89.79±2.96^{ab}$	$94.90±3.06^{a}$	$91.84±2.76^{a}$	$96.33±0.76^{a}$	0.025*
6	90.31±3.37	94.69±2.29	88.78±3.17	94.39±2.93	93.47±3.06	89.39±2.92	0.561
7	88.98±3.33	92.35±3.16	88.16±3.62	86.39±1.80	93.88±2.14	92.86±1.02	0.527
8	87.24±2.93	93.37±3.94	87.24±3.67	93.37±1.28	94.96±0.00	92.86±3.17	0.178
0-8	$87.95±0.94^{b}$	$94.09±0.84^{a}$	$88.06±1.02^{b}$	$92.61±0.81^{a}$	$92.13±1.12^{a}$	$92.83±0.71^{a}$	0.001***
	Egg weight, g						
1	56.94±0.61	57.30±0.79	58.43±0.68	56.66±0.51	57.13±0.64	56.95±0.66	0.459
2	58.30±0.67	58.54±0.71	59.43±0.70	57.54±0.53	58.42±0.61	57.65±0.60	0.352
3	58.07±0.54	58.58±0.69	59.65±0.69	58.33±0.58	58.50±0.65	58.11±1.01	0.662
4	58.87±0.58	58.60±0.72	59.58±0.65	57.80±0.66	58.84±0.71	58.54±0.66	0.589
5	59.95±0.79	59.41±0.72	60.23±0.68	58.84±0.66	59.68±0.62	58.79±0.78	0.652
6	59.64±0.74	58.99±0.65	59.75±0.62	59.85±0.78	59.96±0.61	58.34±0.81	0.589
7	$61.10±0.69^{ab}$	$59.59±0.68^{bc}$	$61.95±0.80^{a}$	$59.64±0.71^{bc}$	$59.85±0.72^{abc}$	$58.28±0.86^{c}$	0.019*
8	60.46±0.64	60.65±0.78	61.53±0.79	59.83±0.78	59.60±0.65	58.81±0.63	0.140
0-8	$59.19±0.25^{b}$	$58.98±0.26^{b}$	$60.06±0.26^{a}$	$58.51±0.24^{bc}$	$59.03±0.24^{b}$	$58.17±0.27^{c}$	0.001***

Values with different letters in the same row were found to be different from one another; (*): P<0.05, (***): P<0.001

Tablo 5- Egg quality criteria for trial groups

	Shape index						
Weeks	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
2	76.31±0.23[a]	76.49±0.43[a]	76.14±0.43[a]	76.12±0.51[a]	74.68±0.15[b]	75.41±0.39[ab]	0.022*
4	76.32±0.33	74.86±0.86	75.94±0.28	75.14±0.42	75.77±0.58	75.45±0.45	0.406
6	75.80±0.29	75.51±0.57	76.28±0.46	76.64±0.53	75.22±0.19	76.00±0.83	0.458
8	75.24±0.24	75.26±0.36	76.55±0.62	76.25±0.62	76.92±0.54	75.48±0.46	0.093
	Eggshell thickness, mm×100						
2	34.60 ±0.71	34.72 ±0.30	34.88 ±0.26	35.04 ±0.35	34.84 ±0.41	34.96 ±0.80	0.992
4	33.48 ±0.74	32.92 ±0.68	33.04 ±0.35	32.68 ±0.44	33.40 ±0.82	34.16 ±0.59	0.624
6	33.68 ±0.38	32.80 ± 0.90	34.32 ±0.76	34.40 ±0.62	34.08 ±0.22	34.24 ±0.61	0.472
8	34.24 ±0.64	34.20 ±0.37	33.28 ±0.44	33.96 ±0.57	34.32 ±0.37	34.76 ±0.82	0.582
	Albumen index						
2	7.57 ±0.27	8.13 ±0.25	7.71 ±0.33	7.86 ±0.37	8.15 ±0.28	7.65 ± 0.27	0.621
4	8.67 ±0.42	8.98 ±0.33	8.74 ±0.51	8.81 ±0.26	8.85 ±0.32	8.66 ±0.36	0.988
6	8.65 ± 0.23	9.96 ±0.17	9.66 ±0.45	9.40 ±0.30	9.05 ±0.50	9.27 ± 0.37	0.182
8	8.69 ±0.39	9.62 ± 0.42	8.95 ±0.27	9.10 ±0.25	9.68 ±0.41	9.17 ±0.33	0.344
	Yolk index						
2	55.64±0.68	54.16±0.33	55.79±0.72	56.53±0.79	54.41±0.54	55.54±0.86	0.160
4	54.34±0.36	54.70±0.82	55.17±0.51	54.80±0.28	53.71±0.45	55.40±0.51	0.271
6	53.82±0.53	53.41±0.62	53.54±0.60	53.54±0.65	53.25±0.34	53.95± 0.19	0.935
8	52.73± 0.75	52.20±0.78	52.12±0.46	53.70±0.95	52.88±0.35	52.96±0.35	0.579
	Haugh unit						
2	79.52±1.48	79.90± 1.64	78.60±1.55	79.37±1.95	80.52± 1.06	77.66±0.97	0.801
4	83.14±1.74	82.80±1.52	82.79± 2.04	83.44± 1.26	84.29±0.79	82.34± 1.14	0.954
6	82.89±0.99	87.86±0.70	86.74±1.88	85.53±1.06	83.45±1.39	84.44± 0.99	0.063
8	83.01±1.42	85.96±1.62	84.39± 1.11	84.13± 1.10	86.88± 1.53	85.10± 1.29	0.424

Values with different letters in the same row were found to be different from one another; (*): P<0.05

Table 6- Total fatty acid content of yolks in groups (%)

Fatty acids	*Control*	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
Palmitic acid (C16:0)	23.75 ±0.64[a]	23.56 ±0.37[a]	24.49 ±0.42[a]	23.25 ±0.51[a]	23.15 ±0.40[a]	21.80 ±0.24[b]	0.005**
Oleic acid (C18:1n-9)	41.14 ±1.70[c]	42.33 ±0.51[c]	42.74 ±0.91[c]	44.17 ±1.04[bc]	46.60 ±1.00[b]	49.69 ±0.72[a]	0.001***
Linoleic acid (C18:2n-6)	18.07 ±6.07	19.86 ±0.96	18.37 ±1.35	18.03 ±1.21	14.78 ±0.45	13.68 ±0.43	0.355
Linolenic acid (C18:3n-3)	0.25 ±0.04[e]	0.82 ±0.79[d]	1.55 ±0.17[c]	2.38 ±0.27[b]	3.00 ±0.24[a]	3.34 ±0.17[a]	0.001***

Values with different letters in the same row were found to be different from one another; (**): P<0.01; (***): P<0.001

Table 7- Yolk cholesterol content of groups (mg/egg)

Control	*Trial 1*	*Trial 2*	*Trial 3*	*Trial 4*	*Trial 5*	P
244.31 ± 16.69	239.51 ± 12.92	245.52 ± 30.63	217.98 ± 8.08	223.38 ± 11.39	247.23 ± 12.76	0.694

4. Discussion and Conclusions

The findings concerning feed consumption are consistent with the findings of some previous studies in literature. Küreç (2009) found that adding flaxseed oil to rations had no significant effect on feed consumption in the first week but had a significant effect in the second week. The same study reported that adding 1-2% flaxseed oil to the ration increased feed consumption when compared to the control group, whereas adding 4% flaxseed oil resulted in a significant decrease in feed consumption. In a similar study, Küçükersan (2004) found statistically significant differences in feed consumption in weeks 24, 26, 34 and 36, but no significant differences in the other weeks. Feed utilization rate findings are consistent with the findings of Küçükersan (2004), but slightly higher than those reported by Ezhil Valavan et al. (2006) who reported no statistically significant differences in feed efficiency as a result of adding various feed sources. The feed utilization figures reported by Ahmad et al. (2013) were slightly higher than those in the present study; and the feed utilization rates obtained in the present study were slightly higher than those

reported by Kahraman et al. (2004). In a study conducted with 256 laying hens fed rations containing different amounts flaxseed oil (0%, 5%, 10% and 15%), Sarı et al. (2002) found that adding flaxseed oil resulted in improved feed utilization rates.

The overall findings of the present study concerning egg yield are similar to those reported by Küçükersan (2004), Ezhil Valavan et al. (2006) and Gürbüz et al. (2012). In a similar study, Balevi & Coskun (2003) found that egg yield at the end of 56 days was 69.96% for the group fed a ration containing sunflower oil, and 76.81% for the group fed a ration containing flaxseed oil. The egg yield figures reported Balevi & Coşkun (2003) are lower than those obtained in the present study.

Consistent with the egg weight findings presented here, Kahraman et al (2004) report that adding different sources of oil to rations at varying amounts increases egg weight. Petrovic et al (2012) added 1%, 2%, 3% and 4% flaxseed oil to the compound feed of laying hens, and found that the average egg weights for these groups were 62.5, 60.5, 60.6 and 61.4 g, respectively, compared to 60.4 g for the control group. In the present study, significant differences were found between the groups in week 8 only, but no significant effect was observed compared to the control group. Differences among trial groups may be attributed to linoleic acid contents of the rations with high levels of sunflower oil. Mazalli et al. (2004) added 3% sunflower, flaxseed and fish oils to the rations, as well as two different amounts of vitamin E (12 IU/kg and 100 IU/kg) and found that the treatments did not affect egg weights.

The shape index of the eggs was found to be close to 76, which shows, on the basis of the values reported by Küreç (2009), that the eggs can be classified as round. The finding that additives had no significant effect on the shape index is consistent with the findings of Küçükersan (2004), Küreç (2009) and Oğuz et al. (2016). Similarly, no significant differences were noted between the groups in terms of eggshell thickness, albumen index, yolk index and Haugh unit, which is consistent with previous studies in literature (Küçükersan 2004; Mazalli et al. 2004; Artan 2015; Yassein et al. 2015).

The linolenic acid content, in particular, was observed to increase linearly as the amount of flaxseed oil was increased in the trial groups, when compared to the control group. This finding is consistent with the findings reported by Herkel et al. (2016) in a study their study of flaxseed oil use. What is significant here is that as the amount of flaxseed oil was increased, so did the linolenic fatty acid content by a corresponding amount, indicating transfer to the eggs.

The results obtained in cholesterol values were similar to the results reported by Küçükersan (2004). In a study involving laying hens fed rations containing different amounts of whole flaxseed, Sarı et al. (2002) found that the groups fed rations containing 5%, 10% and 15% flaxseed had lower levels of yolk cholesterol than the control group (0% flaxseed in the ration). The difference here may be attributed to the use of flaxseed.

In conclusion, sunflower oil and flaxseed oil, when added to laying hen rations, produced no difference in egg quality criteria or egg cholesterol levels, while higher ratios of flaxseed oil added to the rations resulted in linearly higher levels of linolenic acid content in the yolk, while use of the two oil additives together increased egg yield. To obtain beneficial effects in the egg yield and linolenic acid content of eggs, it is recommended that a minimum of 1% flaxseed oil should be added to laying hen rations, taking the results of an economic analysis into account.

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