Chapter 5

EFFECT OF FEEDING FLAXSEED AND ANTIOXIDANTS ON PRODUCTION PERFORMANCE, EGG QUALITY AND LIPID COMPOSITION

5 EFFECT OF FEEDING FLAXSEED AND TWO TYPES OF ANTIOXIDANTS ON PRODUCTION PERFORMANCE, EGG QUALITY AND LIPID COMPOSITION

5.1 ABSTRACT

The present experiment was performed to examine the influence of feeding flax seed and two types of antioxidants [α-tocopherols (Toc), butylated hydroxy toluene, (BHT)] at 3 levels (50, 100, 150 IU or mg/kg) on production performance, egg quality, fatty acid profile, Toc, and egg cholesterol content. Twenty-four week old ISA Brown Leghorn laying hens (n=96) were fed a corn-soybean meal-based diet for 56 days: control (no flax, no antioxidant), 10% flax with no antioxidant or 10% flax + antioxidants. The flax-based diets had no significant effect on egg production, egg weight, egg mass, or feed conversion when compared to the control diet. Feed intake was reduced in hens fed the flax diets ($P<0.05$, except flax +150 mg BHT) as compared to those fed the control diet. Egg weight, yolk weight, shell weight, albumen weight and height, Haugh unit, yolk color and shell thickness were unaffected by feeding flaxseed. Lower levels of saturated fat were observed in eggs from hens fed the flax + Toc and flax + 50 mg BHT diets when compared to hens fed the other diets ($P<0.05$). Eggs from hens fed flax had increased α-linolenic (18:3omega-3), eicosapentaenoic (20:5 omega-3), and docosahexaenoic (22:6 omega-3) acids levels and decreased arachidonic acid (20:4 omega-6) and ratio between total omega-6 to omega-3 when compared to control diet ($P<0.05$). Total omega-6 fatty acids were lowest in eggs from hens fed flax +50 IU Toc, flax +50 mg BHT, flax +100 mg BHT, and flax +150 mg BHT. Total omega-3 fatty acids were highest in eggs from hens fed flax + 50 mg BHT. Flaxseed and antioxidant supplementation had no effect on egg cholesterol content. Inclusion of Toc led to more than 4.5- to 12-fold increases in Toc of eggs from hens fed flax-based diets.

5.2 INTRODUCTION

Omega-3 fatty acids (FA) including α-linolenic (18:3 omega-3), eicosapentaenoic (EPA, 20:5 omega-3), docosapentaenoic (DPA, 22:5 omega-3) and docosahexaenoic acid (22:6 omega-3, DHA) have received considerable attention in the past two decades for their health-promoting effects and their role in growth and development (Simopoulos, 1991; Carlson and Neuringer, 1999; Das, 2006). Dietary omega-3 FA are contributed by marine products (EPA, DPA, DHA) and plant sources such as flax, canola and green vegetables (α-linolenic acid). However, dietary sources do not meet the requirements of these nutrients in the human diet and hence efforts were made to increase the omega-3 FA content of animal-derived foods through dietary manipulation (Kris-Etherton, et al., 2000; Cherian, 2002; Rymer and Givens, 2005; Whelan and Rust, 2006). Marine and plant sources are usually fed to layers to increase the omega-3 FA content of chicken eggs (Gonzalez-Esquerra and Leeson, 2001; Cherian, 2008a). In this regard, flax seeds, due to their high protein (>22%), fat (>40%), and α-linolenic acid content (>50%) and availability are the most common and attractive feed ingredient used in the table egg industry to produce omega-3 FA modified chicken eggs.

Several authors have reported both short- and long-term feeding trials using flax (5 to 30%) in laying hen diets (Novak and Scheideler, 2001; Bean and Leeson, 2003; Cherian, 2008a). The drawbacks of including higher levels (>8%) of flax have been associated with its various components affecting palatability, feed intake, egg production, egg quality and organoleptic aspects (Bhatty, 1995). Similarly, the role of flaxseed in increasing longer chain (>C_{20}) omega-3 FA content was limited beyond 10% addition. Cherian and Sim (1991), who fed 8% compared with 16% flax in a hen diet, reported no increase in the content of longer chain omega-3 FA in eggs. Dietary α-linolenic acid is metabolized to long chain omega-3 FA by Δ^6, Δ^5, and Δ^4 desaturases and elongases (Cooke, 1991). There is a competition between omega-3 and omega-6 FA for the enzymes, with a preference of omega-3 over omega-6 FA (Brenner, 1971). Activity of enzymes catalyzing the digestion and metabolism of PUFA of omega-3 and 6 series is controlled by a complex system. For example, saturated and *trans* fats inhibit Δ^6, Δ^5 pathways limiting long chain FA concentration (Das, 2006). Inclusion of vitamin E has been reported to modulate Δ^6 activity and increase long chain omega-3 FA concentration in liver tissue in rodent models (Özkan et al. 2005). Feeding fish oil along with tocopherols, (Cherian, et al., 1996) reported a significant increase in the content of EPA and DHA in chicken eggs indicating the role of vitamin E in modulating the desaturase and elongase enzyme pathway in laying hens.

Antioxidants like tocopheroles (natural) and butylated hydroxy toluene (BHT; synthetic) are included to poultry diets to prevent oxidative destruction of dietary fats. Synthetic antioxidants have been widely used as food or feed preservatives, due to their low cost and effectiveness. Lipid oxidation leading to off-flavor or "fishy" flavor and reduction in acceptance by panelists is a major concern with eggs from hens fed flax (Jiang et al., 1992; Gonzalez-Esquerra and Leeson, 2001; Cherian, 2002). For this reason, adding vitamin E (10 vs. 100 IU) to the hen's diet is a common practice for omega-3 FA-enriched egg production (Cherian, 2002; Rymer and Givens, 2005; Cherian, 2008a). In a previous study we have looked into feeding different full fat oilseeds to laying hens and found 10% flaxseed most optimum with regard to egg fatty acid profile and production performance. But we are unaware of studies evaluating the different types of antioxidants (natural vs. synthetic) and their levels on FA incorporation, hen production, egg quality and egg lipid components. Feeding two levels of vitamin E (27 vs. 50 IU), Scheideler and Froning (1996) reported an improvement in egg production during an 8 week feeding period. However, no effect of including 10 vs. 100 IU/kg vitamin E in layer diets on egg production or egg weight was reported by Gonzalez-Esquerra and Leeson (2001). The objective of this study was to evaluate the effects of feeding flax seed with two types of antioxidants [natural, α-tocopherols (Toc); synthetic, butylated hydroxy toluene (BHT)] at three levels (50, 100, 150 IU or mg/kg) on 8-wk production performance, egg quality characteristics, egg yolk cholesterol, egg vitamin E content, and FA profile.

5.3 MATERIALS AND METHODS

These experiments were reviewed by the Oregon State University Animal Care Committee to ensure adherence to Animal Care Guidelines.

Birds, diets and housing

A total of 120 ISA Brown layer pullets (ISA Babcock, Ithaca, NY) were reared in floor pens to18 wks of age. At 18 wk, 96 hens on the basis of uniform live weight were moved to laying cages in a room maintained at 20^{o}C, and a step-up lighting schedule was used. Hens received 15L: 9D from 18 to 21 wk of age and 16L: 8D from wk 21 to the end of experiment. Diets were provided as follows: commercial chick starter from 0 to 6 wk; grower diet from 16 to 23 wk; and the experimental layer diet from 24 to 32 wk. Hens were distributed randomly to the experimental diets. Two hens housed in one experimental cage (38 x 41 cm) were considered as 1 replicate. Six replications were made for each diet. The 8 experimental diets formulated according to standard specifications for ISA brown layers, were as follows: a corn-soybean based diet with no added antioxidants (control); flax = basal diet with 10% flaxseed and no added antioxidants; flax + 50 IU Toc diet = basal diet with 10% flaxseed plus 50 IU tocopherols; flax + 100 IU Toc diet = basal diet with 10% flaxseed plus 100 IU tocopherols; flax + 150 IU Toc diet = basal diet with 10% flaxseed plus 150 IU tocopherols; flax + 50 mg BHT diet = basal diet with 10% flaxseed plus 50 mg/kg BHT; flax + 100 mg BHT = Basal diet with 10% flax plus 100 mg/kg BHT; Flax +

150 mg BHT diet = basal diet with 10% flaxseed plus 150 mg/kg BHT. Flaxseed along with other ingredients was purchased from the local feed mill and used without grinding. Antioxidants used were chemical grade and were already available in the laboratory. The diets were mixed every 2 wk and were stored in a cold room (4^0C) in airtight containers. Water and feed were provided *ad libitum* to all the birds. The experimental diets were fed for 56 days.

Production parameters

Production performance (egg production, egg mass, feed intake, feed conversion) was measured from 24-32 wk of hen age. Daily egg production per replicate was recorded and at the end of each l week total number of eggs laid per bird per week was determined. Eggs laid per replicate were weighed daily and at the end of each week, average weight for that particular week was calculated. The data generated (number of eggs and egg weight) was used to calculate egg mass per bird per week (weekly egg number in replicate x average egg weight). Feed intake was measured on a weekly basis (total feed offered in a week minus feed refused at the end of week). Data on feed intake and egg mass were used to calculate feed conversion (feed intake/egg mass; g/g).

Determination of egg quality parameters, total lipids, fatty acids and cholesterol

Detail of procedures for determination of egg quality parameters like yolk index, Haugh unit, shell thickness and yolk Color, total lipids, fatty acids and cholesterol were are given in the previous chapter number 4 of this dissertation.

Tocopherol assay

Egg yolk α- Toc was analyzed by HPLC as per Podda et al. (1996). Approximately 1 gm of egg yolk was weighed and an equal amount of distilled water was added. The contents were mixed and 1.0 ml was transferred to a screw-capped tube and mixed with 0.1 ml of the internal standard (rac-5, 7-dimethyltocol), 2 ml 1% ascorbic acid in ethanol and 0.3 ml saturated KOH. The samples were incubated in a 70ºC water bath for 30 min and then cooled on ice. Following addition of 0.1 ml of 0.25 mg BHT/ml in ethanol, 1.0 ml (1% ascorbic acid in H_2O) and 2.5 ml hexane, the samples were centrifuged for 5 min at 1,500 g. The upper hexane layer was transferred to a clean tube, evaporated under a stream of nitrogen and the residue was dissolved in 0.2 ml ethanol. The samples were transferred to 0.5 ml tubes and centrifuged at 8,000 g for 5 min after which 0.15 ml of the supernatant was taken for assay. A Shimadzu LC-2010 HT HPLC system was used with a LC2010 AHT High Speed Autosampler (Shimadzu, Columbia, MD). A Supelguard^{TM} LC-18 guard column (Superguard TM LC-18, Supelco, Bellefonte, PA) and 97.5% methanol as a mobile phase at a 1.0 mL/min flow rate were used. Detection was performed with a Shimadzu RF-535 fluorescence detector at an excitation wavelength of 295 nm. A Shimadzu EZSTART 7.3 chromatography data system was used to integrate peak areas. Concentration of α-Toc was calculated by comparing α-Toc peaks with peak areas of the internal standard (rac-5,7-dimethyltocol (Matreya, Pleasant Gap, PA) and quantified using authentic standards (DL-alpha-tocopherol; MP Biomedical Solon, OH).

Statistical analysis

The experiment used a completely randomized design, and the experimental unit was a replicate consisting of two hens. Hen performance, egg characteristics, egg quality and lipid components were analyzed by 1-way ANOVA using GLM procedure of SAS (SAS version 9.2, SAS Institute, USA, 2001) with treatments as main effects and replicates within treatment as error term. The following model was used:

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where Y_{ij} = variable measured for the j_{th} replicate, μ = overall mean, τ_i = fixed effect of the i_{th} treatment and ε_{ij} = error component. Mean values along with pooled SEM are reported. Values were considered significant if $P \leq 0.05$. In case of significant differences, the Duncan multiple range test was employed to compare differences among means (Duncan, 1955).

5.4 RESULTS AND DISCUSSION

The ingredient content and FA composition of the diet are shown in Table 5.1. The laying hen diets were isocaloric and isonitrogenous and provided 2,751 kcal/kg of ME and 16.5% CP as per ISA guidelines for brown layers. α-linolenic acid constituted 3.0 and 32.6% and the omega-6 to omega-3 FA ratios of the diets were 19.0 and 1.1, for the control and flax diets, respectively. Oleic acid constituted 23.6 and 18.4%, while total saturated fatty acids were 16.5 and 12.7 for the control and flax-based diets, respectively.

Egg production and feed intake

The effects of flax seed and antioxidant supplementation on egg production, egg weight, egg mass, feed intake and feed conversion are presented in Table 5.2. Feeding flax-based diets with and without added antioxidants had no effect on egg production, egg weight, egg mass or feed conversion when compared to the control diet. However, a trend for decreased egg production was observed for the flax diet with no added antioxidants. Egg production of hens fed the other flax-based diets with added antioxidants at different levels fell within very narrow ranges. Although not significant, eggs from hens fed flaxseed at 10%+ 150 mg BHT were heavier as compared with eggs from hens fed the other treatments, whereas eggs from hens fed flaxseed + 50 mg/kg BHT produced eggs with lowest weight (Table 5.3). Egg production and egg weight were not significantly affected by dietary treatments; likewise egg mass (g/d) also exhibited non significant effect. These results are in agreement with the findings of Baucells, et al., (2000), Bean and Leeson (2003), and Mazalli et al. (2004) who reported non significant differences in egg production attributable to incorporation of flaxseed. However, feed intake was reduced in hens fed flaxseed ($P<0.05$; Table 5.2). More than a 3 g/d per hen decrease in feed intake was noticed on all diets except flax +150 mg BHT as compared with the control diet. A decrease in feed intake attributable to addition of flaxseed was also reported by Scheideler and Froning (1996) in White Leghorn hens and by Bean and Leeson, (2003) in brown egg layers. The reduction in feed intake could have been due to antinutritional factors in flaxseed affecting the palatability, in accordance with the results of Hulan et al. (1989), who reported that palatability problems affected feed intake in poultry. Feed conversion was calculated using data on feed intake and egg mass (feed intake/egg mass; g/g), with no statistical differences found among different experimental diets. However, the best feed conversion occurred in hens fed the diets containing 10 % flaxseed + 150 mg/kg BHT.

Egg characteristics

Egg quality aspects such as yolk weight, shell weight, albumen weight and height, HU, yolk color, and shell thickness were unaffected by feeding flaxseed (Table 5.3). A non-significant trend for lower yolk weight was observed in birds fed flaxseed without added antioxidants, whereas the highest yolk weight percentage was recorded in birds fed 10 % flaxseed + 50 mg BHT. Eggs from hens fed flax + 50 mg BHT had the lowest shell weight percentage as compared to those from all other dietary treatments. The highest albumen weight percentage was recorded for eggs from flax-fed hens and the lowest from eggs laid by hens fed the control diet ($P>0.05$). Haugh unit value based on the albumen height and egg weight is an acceptable measure of the quality and freshness of shell eggs. Eggs produced by layers fed the flax + 50 IU Toc diet had the maximum value while those from flax + 100 IU Toc had the minimum HU. However, albumen height and Haugh unit were statistically independent of dietary treatments. Yolk color of eggs on all experimental diets were consistent within a narrow range, having a maximum value on all 3 diets containing BHT. There was no statistical difference among the different diets for yolk color. Shell thickness was also independent of dietary treatments. However, eggs from hens fed the flax + 50 IU Toc diet had the thickest shell while those obtained from flax + 50 mg BHT had minimum shell thickness. Egg components and quality parameters were independent of dietary treatments, and the lack of effect attributable to flaxseed corroborates with the results of Novak and Scheideler (2001) and Bean and Leeson (2003), who demonstrated non-significant differences in shell weight, albumen height, and shell thickness of eggs from hens fed flaxseed.

Egg Fatty acids

The FA compositions of eggs are given in Table 5.4. Incorporating flaxseed and antioxidants into layer diets imparted significant changes in the fatty acid profile of eggs. Compared to control eggs, the lowest total saturated FA (SFA) values were found in eggs produced from hens fed the diet containing flaxseed + Toc. The major unsaturated FA was C18:1, which constituted approximately 90% of total monounsaturated FA (MUFA). Palmitoleic (16:1) and oleic acids (18:1) were lowest in the flax + 50 mg BHT group, leading to a significant reduction in total MUFA in eggs from hens fed Flax + 50 mg BHT. Birds that consumed flaxseed deposited higher α-linolenic, DPA and DHA resulting in significant increase in total omega-3 FA and a decrease in arachidonic acid (20:4 omega-6) and total omega-6 to omega-3 FA ratio when compared to control eggs ($P<0.05$). The significant decline in arachidonic acid and omega-6 to omega-3 FA ratio in eggs from hens fed the flax-based diets corroborate previously reported research on laying hens (Cherian and Sim 1991). Arachidonic acid is formed from linoleic acid through desaturation and elongation in the hen liver. The Δ^6-desaturase is critical enzyme in the synthesis of arachidonic acid and long-chain omega-3 FA from their 18-carbon parent fatty acids (Brenner, 1971). Omega-6 and omega-3 fatty acids compete for desaturation and elongation, where omega-3 FA is the favored substrate, causing a decreased omega-6 to omega-3 FA ratio in the eggs from hens fed flax. Among the eggs from hens fed flax-based diets, the concentration of arachidonic acid was lowest in the eggs from the flax + Toc and BHT supplemented group when compared to the flax (no antioxidant) group, suggesting a role of antioxidants in modulating the Δ^6-desaturase pathway in a favorable way. An exception occurred with flax +150 Toc, for which no difference in arachidonic acid was observed when compared to the flax group.

Egg lipids are produced in the hen liver. The Δ^9-, Δ^6-desaturase pathway is involved in the synthesis of FA (Cooke, 1991). Alterations in Δ^9-, Δ^6-desaturase in the liver through supplemented vitamin E have been reported in animal models (Özkan et al. 2005). Addition of Toc (0.07%) to the diet of white Leghorn hens fed fish oil has been shown to increase EPA and DHA in eggs (Cherian et al., 1996). Therefore, it seems that the level of antioxidant in the diet may has an effect on Δ^9-, Δ^6-desaturases in laying birds, resulting in alteration of egg saturated, MUFA, omega-6 and omega-3 PUFA content as observed in the current study. Total omega-6 FA were lowest in eggs from hens fed flax +50 IU Toc, flax +50 mg BHT, flax +100 mg BHT, and flax +150 mg BHT, leading to a reduction in the omega-6 to omega-3 FA ratio when compared to eggs from hens fed flax (no antioxidant; $P<0.05$). In the flax-based diets, BHT was more effective than Toc in incorporating long chain omega-3 FA (DPA, DHA) into eggs. Total omega-3 FA content was highest in eggs from the flax + 50 mg BHT group. To minimize lipid peroxidation, precautions such as feed mixing after every two weeks, and storage at 4°C in tightly closed containers were followed. Therefore, it is not known whether the effectiveness of BHT compared with Toc may have been due to their stability in the feed.

Total lipids, cholesterol and vitamin E

Total lipids were highest in eggs from the flax + 50 or 100 IU of Toc group, the flax + 100 mg of BHT group, or flax + 150 mg of BHT group (Table 5.5). Flaxseed and antioxidant supplementation had no effect on cholesterol content when expressed as milligrams per gram of yolk or on a milligram per egg basis. Cholesterol content of egg was independent of dietary treatments and no significant change was observed in cholesterol concentration because of either flaxseed or antioxidant supplementation. In agreement with our results, Ferrier et al. (1995) and Scheideler and Froning (1996) also found no effect of dietary flaxseed in the cholesterol content of eggs.

Addition of α-Toc in diets showed a linear increase in its concentration in eggs ($P<0.05$). Inclusion of Toc led to more than 4.5- to 12-fold increase in Toc of eggs from the groups fed flax-based diets. According to the results from the current study, consuming 2 large eggs from hens fed flax + 150 IU Toc could provide over 11 mg of Toc to the human diet. Manipulation of the diet of the hen to increase the vitamin E content of chicken eggs, thus providing a natural source of health-enhancing vitamins through diet, has been reported (Qi and Sim, 1998; Cherian, 2008b) and the linear increase in egg Toc due to manipulation of the hen's diet is in agreement with previous reported studies (Qi and Sim, 1998; Meluzzi et al., 2000). Located in the phospholipids-rich cellular membranes, Toc can function as an intracellular and intercellular antioxidant, thus neutralizing free radicals, preventing oxidation and preserving the membrane integrity and structural properties. In addition, there are many health benefits of Toc especially in certain diseases such as cancer, coronary heart disease and immune functions (Diplock, 1991; Traber, 1999). Inclusion of BHT in the hen diet had no effect on Toc content of the eggs.

Overall, in light of findings of the present study, it may be concluded that eggs enriched with omega-3 FA can be produced by minor diet modifications without affecting overall production and egg quality parameters. The production of omega-3 FA-and Toc-enriched eggs may give poultry farmers an opportunity to be part of an emerging industry which can increase marketability and economic returns by offering consumers an alternate way of obtaining these health-promoting nutrients through their diet.

5.5 CONCLUSIONS AND APPLICATIONS

Inclusion of flax seed at 10% did not result in any changes in egg production, egg quality, or egg cholesterol. However, addition of antioxidants enhanced the long chain omega-3 FA content of eggs. Supplementation with Toc increased the Toc content of eggs and consuming two large eggs from hens fed 10% flax + 150 Toc could provide 11 mg Toc to the human diet along with over 500 mg omega-3 FA.

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Table 5.1: Ingredient content and fatty acid composition of experimental diets (%)

Ingredients	Control	Experimental	Fatty acid	Control	Experimental
Flax seed	0	10	Palmitic (16:0)	13.9	10.4
Yellow corn	59.5	51	Stearic (18:0)	2.5	2.3
Soybean meal	21.25	17.60	Oleic (18:1)	23.6	18.4
Wheat middling	7.58	10.83	Linoleic (18:2)	56.9	36.3
Limestone	8.45	8.40	α-Linolenic (18:3)	3.0	32.6
Dicalcium phosphate	1.4	1.35			
Salt	0.35	0.35			
Layer premix*	0.35	0.35			
DL Methionine	0.12	0.12			
Oil	1	-			
Total	100	100			
Calculated composition					
ME (kcal/kg)	2751	2751			
CP (%)	16.5	16.5			
Calcium (%)	3.64	3.66			
Available P (%)	0.42	0.42			
Sodium (%)	0.16	0.17			
Lysine (%)	0.97	0.96			
Methionine (%)	0.42	0.42			
Meth+Cyst (%)	0.71	0.71			

*Layer premix composition: copper, 2 g/kg; iodine, 1.15 g/kg; iron, 1.8%; manganese, 2.5%; selenium, 0.12 g/kg; zinc, 2%; vitamin A, 3,828,000 IU/kg; vitamin D3, 968,000 ICU/kg; vitamin E, 2,640 IU/kg; menadione, 0.3 g/kg; vitamin B12, 4 mg/kg; choline, 110 g/kg; folic acid, 99 mg/kg; niacin, 13.2 g/kg; pantothenic acid, 2.2 g/kg; pyridoxine, 0.4 g/kg; riboflavin, 1.7 g/kg; thiamine, 0.61 g/kg.

Table 5.2: Effect of feeding flaxseed with different antioxidants on production parameters from week 24 through 32

Production parameters	Dietary Treatments[1]								Pooled SEM	P-value
	Control	Flax	Flax + 50 IU Toc	Flax + 100 IU Toc	Flax + 150 IU Toc	Flax + 50 mg BHT	Flax + 100 mg BHT	Flax + 150 mg BHT		
Egg production (%)	97.47	95.85	97.28	97.62	96.49	96.23	97.02	96.13	0.602	0.305
Egg mass (g/hen/day)	58.76	58.06	58.23	58.97	57.32	56.89	59.72	59.43	0.724	0.101
Feed intake (g/hen/day)	120.4[a]	116.1[b]	115.3[b]	116.8[b]	116.4[b]	117.0[b]	117.9[ab]	116.3[b]	0.894	0.011
Feed conversion[2]	2.05	2.00	1.98	1.98	2.03	2.05	1.98	1.96	0.027	0.098

[a-b]Means within a row with no common superscript differ (*P*<0.05). n=12

[1]Control = Corn-soybean meal basal diet with no flax, no added antioxidant; Flax = Basal diet with 10% flax and no added antioxidants; Flax + 50 IU Toc = Basal diet with 10% flax and 50 IU tocopherols; Flax + 100 IU Toc = Basal diet with 10% flax and 100 IU tocopherols; Flax + 150 IU Toc = Basal diet with 10% flax and 150 IU tocopherols; Flax + 50 mg BHT = Basal diet with 10% flax and 50 mg/kg BHT; Flax + 100 mg BHT = Basal diet with 10% flax and 100 mg/kg BHT; Flax + 150 mg BHT = Basal diet with 10% flax and 150 mg/kg BHT.

[2] Feed conversion = g feed/g egg mass

Table 5.3: Effect of feeding flaxseed with different antioxidants on egg quality

Egg Components	Dietary Treatments[1]								Pooled SEM	P-value
	Control	Flax	Flax + 50 IU Toc	Flax + 100 IU Toc	Flax + 150 IU Toc	Flax + 50 mg BHT	Flax + 100 mg BHT	Flax + 150 mg BHT		
Egg weight (g)	60.27	60.58	59.87	60.42	59.44	59.11	61.56	61.81	0.692	0.103
Yolk wt (%)	25.98	23.41	25.30	24.52	24.24	26.21	25.98	25.97	0.402	0.186
Shell weight (%)	10.39	10.06	10.26	9.89	10.36	9.13	9.77	9.68	0.368	0.173
Albumen weight (%)	63.63	66.53	64.45	65.59	65.40	64.61	64.30	64.36	0.807	0.276
Albumen height (mm)	7.50	7.50	7.87	7.27	7.47	7.08	7.48	7.18	0.273	0.601
Haugh unit value	87.10	85.89	88.83	84.63	86.88	85.47	86.19	84.84	1.464	0.535
Yolk color	7.83	7.17	7.17	7.50	7.67	8.17	8.17	8.17	0.375	0.279
Shell thickness (mm)	0.48	0.49	0.49	0.48	0.48	0.47	0.47	0.47	0.009	0.496

$^{a-b}$Means within a row with no common superscript differ ($P<0.05$). n=12.

[1]Control = Corn-soybean meal basal diet with no flax, no added antioxidant; Flax = Basal diet with 10% flax and no added antioxidants; Flax + 50 IU Toc = Basal diet with 10% flax and 50 IU tocopherols; Flax + 100 IU Toc = Basal diet with 10% flax and 100 IU tocopherols; Flax + 150 IU Toc = Basal diet with 10% flax and 150 IU tocopherols; Flax + 50 mg BHT = Basal diet with 10% flax and 50 mg/kg BHT; Flax + 100 mg BHT = Basal diet with 10% flax and 100 mg/kg BHT; Flax + 150 mg BHT = Basal diet with 10% flax and 150 mg/kg BHT.

Table 5.4: Effect of feeding flaxseed with different antioxidants on egg fatty acids

Fatty acid (%)	Dietary Treatments[1]								Pooled SEM	P-value
	Control	Flax	Flax + 50 IU Toc	Flax + 100 IU Toc	Flax + 150 IU Toc	Flax + 50 mg BHT	Flax + 100 mg BHT	Flax + 150 mg BHT		
14:0	0.04	0.17	0.00	0.12	0.07	0.04	0.12	0.07	0.04	0.1805
16:0	28.75^{a}	27.97^{abc}	27.03^{c}	27.32^{c}	27.24^{c}	27.53^{bc}	28.61^{ab}	27.32^{c}	0.38	0.0121
17:0	0.19	0.20	0.19	0.19	0.17	0.21	0.20	0.18	0.01	0.1874
18:0	11.91^{ab}	12.77^{a}	12.21^{ab}	11.59^{b}	11.89^{b}	12.22^{ab}	11.33^{b}	12.72^{a}	0.34	0.0558
Total SFA	40.89^{a}	41.10^{a}	39.42^{cd}	39.21^{d}	39.37^{d}	39.99^{bcd}	40.25^{abc}	40.30^{ab}	0.28	<0.0001
16:1	3.01^{bcd}	3.13^{abcd}	3.24^{abcd}	3.41^{ab}	3.30^{abc}	2.74^{d}	3.58^{a}	2.85^{cd}	0.17	0.0147
18:1	36.64^{ab}	34.55^{b}	37.05^{a}	35.76^{ab}	35.30^{ab}	34.89^{b}	37.08^{a}	35.83^{ab}	0.66	0.063
20:1	0.18^{a}	0.06^{b}	0.03^{b}	0.09^{b}	0.00^{b}	0.00^{b}	0.03^{b}	0.00^{b}	0.03	0.0033
Total MUFA	39.80^{ab}	37.74^{bc}	40.33^{a}	39.25^{abc}	38.60^{abc}	37.62^{c}	40.69^{a}	38.67^{abc}	0.67	0.0145
18:2 ω-6	11.54^{ab}	12.04^{ab}	11.10^{bc}	11.99^{ab}	12.77^{a}	11.27^{abc}	9.82^{c}	10.75^{bc}	0.50	0.008
18:3 ω-3	0.10^{d}	1.05^{c}	1.40^{abc}	1.56^{abc}	1.42^{abc}	2.05^{a}	1.27^{bc}	1.82^{ab}	0.22	<0.0001
20:4 ω-6	4.34^{a}	3.80^{b}	3.13^{c}	3.14^{c}	3.43^{bc}	3.03^{c}	3.06^{c}	3.16^{c}	0.16	<0.0001
20:5 ω-3	0.00	0.00	0.00	0.05	0.08	0.05	0.05	0.00	0.03	0.5765
22:4 ω-6	0.18	0.16	0.04	0.06	0.08	0.04	0.04	0.00	0.17	0.2254
22:5 ω-6	1.06^{a}	0.22^{b}	0.12^{b}	0.04^{b}	0.16^{b}	0.07^{b}	0.11^{b}	0.00^{b}	0.22	<0.0001
22:5 ω-3	0.00^{d}	0.14^{cd}	0.27^{bc}	0.28^{abc}	0.28^{abc}	0.50^{a}	0.39^{ab}	0.45^{ab}	0.07	0.0002
22:6 ω-3	2.07^{d}	3.75^{c}	4.28^{bc}	4.41^{bc}	3.83^{c}	5.37^{a}	4.32^{bc}	4.85^{ab}	0.21	<0.0001
Total ω-6	17.11^{a}	16.22^{ab}	14.39^{cd}	15.25^{bc}	16.43^{ab}	14.42^{cd}	13.03^{d}	13.91^{cd}	0.43	<0.0001
Total ω-3	2.17^{e}	4.93^{d}	5.94^{bcd}	6.30^{bc}	5.60^{cd}	7.97^{a}	6.03^{bcd}	7.12^{ab}	0.25	<0.0001
Σ ω-6:Σ ω-3	7.96^{a}	3.42^{b}	2.45^{cd}	2.46^{cd}	3.20^{bc}	1.85^{d}	2.22^{d}	1.97^{d}	0.25	<0.0001

[a-d]Means within a row with no common superscript differ ($P<0.05$). [1]Control = Corn-soybean meal basal diet with no flax, no added antioxidant; Flax = Basal diet with 10% flax and no added antioxidants; Flax + 50 IU Toc = Basal diet with 10% flax and 50 IU tocopherols; Flax + 100 IU Toc = Basal diet with 10% flax and 100 IU tocopherols; Flax + 150 IU Toc = Basal diet with 10% flax and 150 IU tocopherols; Flax + 50 mg BHT = Basal diet with 10% flax and 50 mg/kg BHT; Flax + 100 mg BHT = Basal diet with 10% flax and 100 mg/kg BHT; Flax + 150 mg BHT = Basal diet with 10% flax and 150 mg/kg BHT. SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids. n=12.

Table 5.5: Effect of feeding flaxseed with different antioxidants on total lipids, cholesterol and vitamin E

Lipid Components	Dietary Treatments[1]								Pooled SEM	*P*-value
	Control	Flax	Flax + 50 IU Toc	Flax + 100 IU Toc	Flax + 150 IU Toc	Flax + 50 mg BHT	Flax + 100 mg BHT	Flax + 150 mg BHT		
Total egg lipids	30.88[b]	30.78[b]	32.48[a]	31.93[ab]	30.77[b]	30.90[b]	31.86[ab]	31.96[ab]	0.47	0.0610
Cholesterol (mg/g yolk)	11.62	12.93	12.27	12.31	12.23	12.02	13.08	12.32	0.39	0.1436
Cholesterol (mg/egg)	174.45	185.15	182.39	185.66	172.66	179.96	204.53	187.91	7.62	0.2255
Vitamin E (µg/g yolk)	21.44[d]	33.36[d]	150.00[c]	251.01[b]	389.29[a]	19.25[d]	22.31[d]	24.34[d]	18.62	<0.0001
Vitamin E (mg/egg)	0.32[d]	0.47[d]	2.23[c]	3.81[b]	5.49[a]	0.28[d]	0.35[d]	0.37[d]	9.69	<0.0001

[a-b]Means within a row with no common superscript differ (P<0.05). n=12.

[1]Control = Corn-soybean meal basal diet with no flax, no added antioxidant; Flax = Basal diet with 10% flax and no added antioxidants; Flax + 50 IU Toc = Basal diet with 10% flax and 50 IU tocopherols; Flax + 100 IU Toc = Basal diet with 10% flax and 100 IU tocopherols; Flax + 150 IU Toc = Basal diet with 10% flax and 150 IU tocopherols; Flax + 50 mg BHT = Basal diet with 10% flax and 50 mg/kg BHT; Flax + 100 mg BHT = Basal diet with 10% flax and 100 mg/kg BHT; Flax + 150 mg BHT = Basal diet with 10% flax and 150 mg/kg BHT.