Introduction

False flax (Camelina, FF), which is also known as "gold of pleasure," is part of the Brassicaceae family a natural plant from Northern Europe and Middle Asia (Zubr 1997). Among the species of this plant, Camelina sativa L. adapts very well to cool temperate semi-arid climates. Also, due to a short growth season, it has developed the ability to grow efficiently in unfavorable soil and climatic conditions such as dry soil, low rainfall, and frost. As such, agronomic and breeding studies have been conducted on the plant's seed yield, especially in Germany, Canada, France, Australia, and Chile (Putnam et al 1993, Waraich et al 2013).

The oil of false flax is used as a biodiesel resource, and unsaturated fatty acids constitute more than 90% of the fatty acids (Zubr 1997, Zubr and Matthaus 2002). The meal obtained after oil extraction from false flax seeds has 35-40% crude protein, 4600-4800 kcal/kg of gross energy, 6-12% fat with α-linolenic acid constituting up to 30% total fatty acids, 6-7% ash, 41% neutral detergent fiber, 5% minerals, and a minor amount of vitamins and other substances. The protein within the meal is characterized by amino acids such as arginine, cysteine, glycine, lysine, methionine, and threonine (Zubr 1997, Cherian et al 2009, Cherian 2012). A substantial amount (29%) of the fatty acid composition in the meal is α-linolenic acid (18:3 n-3) (an omega-3 fatty acid). Linoleic acid (18:2 n-6) constitutes 23%. The total mono-unsaturated fat acids are 32%, constituting oleic and eicosenoic acids. Saturated fatty acids in the meal include palmitic acid (16:0, 9%) and stearic acid (18:0, 2.5%) (Cherian et al 2009). The high protein level, amino acid composition, energy, n-3 fatty acid content, and n-6 fatty acid content of the false flax meal (FFM) indicate that it can be incorporated in poultry feed (Zubr 1997, Ryhanen et al 2007, Cherian et al 2009, Aziza et al 2010).

The oxidative stability of food lipid is highly related to the diet and unsaturated fatty acids in the egg and serum (Cherian et al 2009, Bulbul et al 2012). Natural products are commonly used to prevent lipid oxidation in eggs and therefore they improved the egg quality and shelf life (Botsoglou et al 2013, Bulbul et al 2014). Additionally, new vegetable resources which are rich in omega-3 fatty acids have also shown potential for antioxidative activity in poultry (Abramovic et al 2007). Studies conducted on laying hens have evaluated the effects of using various levels of FFM on laying performance, egg quality, and egg fatty acid composition (Pilgeram et al 2007, Cherian et al 2009, Kakani et al 2012). However, no available scientific data has been found about the utilization of FFM in the diets of laying quails. The aim of this research was to determine the effects of dietary FFM in laying quails on performance, egg quality traits, serum oxidant-antioxidant balance, and malondialdehyde (MDA) levels of the egg yolk.

Materials and Methods

Animals, diets and experimental design

This study was carried out at the Animal Research Center of Afyon Kocatepe University, Turkey, following ethical committee approval (AKÜHADYEK-304-13). Totally of 240 (160 females and 80 males) eight-week-old Japanese quails *(Coturnix coturnix japonica)* were used in this study. The quails were randomly allocated into one control group and four treatment groups, each containing 48 quails. Each group was divided into four replicates as subgroups, comprising 12 (8 females and 4 males) quails each. They were placed into cages kept inside a windowed poultry house with a light regimen of 16 hours of light and 8 hours of dark. Feed and water were provided ad libitum. The experiment was completed in 8 weeks.

The FFM and other raw feed materials were obtained from a commercial company (Tınaztepe Feed Factory, Afyonkarahisar). The chemical composition of the FFM is presented in Table 1. Quails were fed a corn-soybean meal-based diet with FFM supplemented at 0% (control), 5% (FFM5), 10% (FFM10), 15% (FFM15), and 20% (FFM20). The diets were formulated to be isocaloric and isonitrogenic to meet the nutrient requirements recommended by the National Research Council (1994) for laying quails. Their composition is shown in Table 2.

Traits measured

The nutrient composition of the FFM and diets was determined according to the AOAC (2000). The FFM was also analyzed to determine the neutral detergent fiber and acid detergent fiber content, as described by Van Soest et al (1991). The metabolizable energy (ME) levels of the FFM and diets were estimated using the following equation devised by Carpenter and Clegg (Leeson and Summers 2001): ME, kcal/kg = 53 + 38 [(crude protein, %) + (2.25 x crude fat, %) + (1.1 x starch, %) + (sugar, %)].

Total lipids were extracted from false flax *(Camelina sativa L.)* meal by using n-hexane (Anwar et al 2008). Fatty acid methyl esters were prepared from lipid extracts, and the analysis of fatty acid composition was performed with an Agilent 7820A gas chromatograph (Agilent Technologies Inc., Palo Alto, CA) equipped with an autosampler, flame-ionization detector, and fused-silica capillary column, 60 m × 0.25 mm × 0.2 µm film thickness. Each sample was injected onto the column with helium as a carrier gas, programmed for increased oven temperatures. Peak areas and fatty acid percentages were calculated using Agilent Chem Station software. Fatty acid methyl esters were identified by comparison with retention times of authentic standards and were expressed as percentages of total fatty acid methyl esters.

Table 1. Chemical composition of false flax meal.

Variable	%
Dry matter	95.81
Crude protein	36.88
Crude fat	6.44
Crude fiber	17.4
Crude ash	5.97
Nitrogen free extract	29.12
Acid detergent fiber	24.7
Neutral detergent fiber	45.5
Metabolizable energy (MJ/kg)	9.1
Fatty acids (%)	
Palmitic acid (16:0)	7.73
Myristic acid (14:0)	0.26
Stearic acid (18:0)	2.76
Oleic acid (18:1)	12.8
Linoleic acid (18:2 n-6)	23.47
α-Linolenic acid (18:3 n-3)	36.11
Arachidic acid (20:0)	0.99
Eicosenoic acid (20:1)	8.85
Behenic acid (22:0)	2.18
Erucic acid (22:1)	2.31
Lignoseric acid (24:0)	2.55

Quails were weighed individually at the beginning and end of the experiment. Mortality was recorded when it occurred. Eggs were collected daily, and egg production was calculated based on percent production for egg numbers. Eggs were individually weighed two times per week. Feed intake was identified biweekly as the group average. Feed efficiency was calculated on the same days as the amount of feed consumed for per kilogram of egg.

Twelve eggs from each group (3 eggs from each replicate) were collected to determine the internal and external quality traits of the eggs once every four weeks. The eggs were examined for weight (g), length (mm), width (mm), egg shell thickness (mm), albumen index (%), yolk index (%), Haugh unit (HU), egg shape index, and yolk color index. Egg width, egg length, yolk width, albumen length, and albumen width were measured by caliper (Mitutoyo Digimatic Caliper, CDN-P20PMX, Japan) to the nearest 0.01 mm. The albumen and yolk heights were measured by micrometer to the nearest 0.01 mm. Egg shape, yolk, and albumen indexes were calculated from these measures (Card and Nesheim 1972). The HU was calculated with the formula developed by Haugh (1937). Measurements of the thickness of dried shells with the membrane were obtained from two sides in the equatorial region, as well as on the blunt and pointed edges with a micrometer to the nearest 0.01 mm (Card and Nesheim 1972). The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the 1961 "Roch Improved Yolk Color Fan." The formulas used in the measurement of egg traits were as follows:

Shape index (%) = [egg width (mm)/egg length (mm)] x 100

Yolk index (%) = [yolk height (mm)/yolk width (mm)] x 100

Albumen index (%) = albumen height (mm)/[(average albumen length (mm) + width (mm)/2) x 100]

HU = 100 log [albumen height (mm) + 7.57 - 1.7 x egg weight$^{0.37}$ (g)]

At the end of the experiment, 8 animals from each group (2 animals from each replicate) were slaughtered, and blood samples were kept in opaque heparin-free tubes at +4°C for 24 hours. Right after that, serums were obtained and placed in a centrifuge for 15 minutes at 3.000 rpm. The serums were put into opaque eppendorf tubes and stored at -18°C in order to determine the serum MDA and antioxidant activity (AOA) levels. Serum MDA levels were determined using the double-boiling method for MDA resulting from free radicals, as reported by Draper and Hadley (1990). AOA was determined colorimetrically in serum through a modified method from Koracevic et al (2001).

At the end of the experiment, 32 eggs from each group (8 eggs from each replicate) were stored in a refrigerator at +4°C. On the 1st and 15th day of storage, 8 yolks were weighed and placed into opaque glass tubes. Then, those yolks were kept at -18°C on the days mentioned in order to determine the MDA level. Yolk MDA levels on the 1st and 15th days of storage were measured by a modification of the spectrophotometric method presented by Kanner and Rosenthal (1992) using ELISA. Samples weighing 0.2 g were derived from sample yolks for thiobarbituric acid reactive substances (TBARS) analysis and put into 10 mL test tubes with 1.8 mL of 3.86% perchloric acid per tube. This homogenized mixture was filtered through filter paper, and 0.5 mL of the filtrate was stirred with 1 mL of 20 mL TBA solution and left in a boiling water bath for 30 minutes. The absorbance was read at 532 nm on a spectrophotometer.

Statistical analyses

Data from treatment means were analyzed using the General Linear Models procedure of SPSS 13.0 for Windows. The effect of FFM at different levels on laying performance, egg traits, serum MDA and AOA levels, and egg yolk MDA levels were subjected to ANOVA procedures appropriate for a completely randomized design. All replicates were the experimental unit for all analysis. When differences ($P<0.05$) among means were found, means were separated using the Tukey test. The

Table 2. Ingredients and chemical composition of the diets (%).

	Treatment groups1				
Ingredients	Control	FFM5	FFM10	FFM15	FFM20
Corn	49.8	50.7	49.72	48.1	46.8
Wheat	7.5	5.6	5.4	5.7	5.6
Full fat soybean	7.1	5	4	3	3
Soybean meal (48%)	24.8	22.7	19.7	16.7	13
False flax meal	0	5	10	15	20
Meat and bone meal (38%)	2	2	2	2	2
Vegetable oil	2	2.3	2.6	3	3.2
Limestone	5.1	5.1	5	5	5
Salt	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.8	0.7	0.7	0.7	0.6
NaHCO3	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.1	0.1	0.08	0	0
Vitamin-mineral premix1	0.35	0.35	0.35	0.35	0.35
Chemical composition (analyzed)					
Crude protein (%)	20.17	20.25	20.28	20.19	20.27
Metabolizable energy [2] (MJ/kg)	12.23	12.18	12.22	12.26	12.19
Calcium (%)	2.49	2.48	2.48	2.49	2.49
Total phosphorus (%)	0.35	0.34	0.35	0.36	0.36

[1]Composition per 2.5 kg: 12.000.000 IU vitamin A, 2.400.000 IU vitamin D3, 30 g vitamin E, 2.5 g vitamin K3, 2.5 g vitamin B1, 6 g vitamin B2, 4 g vitamin B6, 20 mg vitamin B12, 25 g niacin, 8 g calcium-D-panthotenate, 1 g folic acid, 50 g vitamin C, 50 mg D-biotin, 400 g choline chloride, 1.5 g canthaxanthin, 80 g Mn, 60 g Zn, 60 g Fe, 5 g Cu, 1 g I, 0.5 g Co, 0.15 g Se. [2]Metabolizable energy content of diets was estimated according to Leeson and Summers (2001).

effects of increasing dietary concentrations of supplemental FFM were partitioned into linear and nonlinear components using orthogonal polynomial contrasts.

Results

The chemical composition of FFM used in layer feeding is presented in Table 1. FFM contains high amounts of crude protein (36.88%), ME (9.1 MJ/kg), crude fat (6.44%), crude fiber (17.4%), and fibrous fractions such as neutral detergent fiber (45.5%) and acid detergent fiber (24.7%). The major fatty acids of FFM were α-linolenic acid (36.11%), linoleic acid (23.47%), and oleic acid (12.8%). Total monounsaturated fatty acid constituted 23.96%, while total polyunsaturated fatty acid constituted 59.58%. Total saturated fatty acids constituted 16.47%.

Supplementation of FFM to the diets of laying quails had a linear effect on final body weight and feed intake. The final body weight decreased in the groups supplemented with 15% and 20% FFM compared with the control group ($P<0.05$). Feed intake decreased in all experimental groups and the lowest feed intakes were in the FFM15 and FFM20 groups ($P<0.01$). Egg production decreased in the FFM15 and FFM20 groups compared with the control group ($P<0.01$). Initial body weight, egg weight, and feed efficiency were not affected by FFM levels in the diets ($P>0.05$, Table 3). Egg yolk color index increased in the FFM20 group in the 4th week ($P<0.001$) and in all experimental groups in the 8th week ($P<0.01$). At the 4th and 8th weeks of the experiment no changes in the other egg quality traits were found in terms of the shape index, shell thickness, albumen index, yolk index, and Haugh unit ($P>0.05$, Table 4).

FFM dietary supplementation had a linear effect on MDA and AOA levels in serum, as well as on egg yolk MDA levels. While the serum MDA level decreased ($P<0.05$) in the FFM10, FFM15, and FFM20 groups, the serum AOA level increased ($P<0.01$) in all experimental groups compared with the control group (Table 5). The egg yolk MDA level decreased in all experimental groups compared with the control group on the 1st ($P<0.05$) and 15th ($P<0.001$) days of storage. The lowest yolk MDA level was in the FFM15 and FFM20 groups on the 15th day (Table 6).

Discussion

The high protein and energy content, as well as rich n-3 and n-6 fatty acid levels of the FFM made it a potentially suitable source of plant protein and essential fatty acid source in

Table 3. Effect of false flax meal on performance of laying quails.

	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
Initial body weight (g)	187.85	189.95	187.34	188.24	188.53	1.02	0.958	0.964	0.992
Final body weight (g)	201.11^{a}	199.67^{a}	195.98^{abc}	193.20^{bc}	191.96^{bc}	1.27	0.025	0.01	0.870
Feed intake (g/day)	38.05^{a}	36.33^{b}	35.74^{b}	34.20^{c}	34.33^{c}	0.375	0.001	0.001	0.715
Egg weight (g)	11.50	11.53	11.27	11.18	11.17	0.071	0.088	0.057	0.478
Egg production (%)	86.79^{a}	85.40^{ab}	85.45^{ab}	83.70^{bc}	82.29^{c}	0.459	0.008	0.000	0.755
Feed efficiency (kg feed/kg egg)	3.81	3.69	3.74	3.65	3.73	0.026	0.422	0.299	0.625

^{a,b,c}:Different letters in the same line indicate significant (n=10).

laying quail diets as shown in Table 1. These results are in agreement with the results of some studies that analyzed composition of FFM (Cherian et al 2009, Aziza et al 2010). The chemical composition of the experimental diets indicates that the diets were well balanced (Table 2).

The effect of FFM on laying performance is shown in Table 3. The final body weight decreased in the groups supplemented with 15% and 20% FFM (P<0.05). While feed intake decreased in all experimental groups, the lowest feed intakes were in the FFM15 and FFM20 groups (P<0.01). There has been no previous study assessing the effect of FFM on body weight of laying quails and hens. However, some studies have reported that the supplementation of FFM to broiler diets at levels of 10% (Ryhanen et al 2007), 15% and 20% (Frame et al 2007), and 5% and 10% (Pekel et al 2009) have adverse effects on body weight. However, FFM supplementation has been reported that it is not change the body weight for turkey hens at 10% (Frame et al 2008) and at 5% and 10% for broilers (Aziza et al 2010). It has also been reported that feed intake numerically declined in laying hens with FFM supplementation at levels of 5%, 10%, and 15% (Cherian et al 2009). Some studies have claimed that FFM supplementation in the diet decreased feed intake in broilers (Ryhanen et al 2007, Pekel et al 2009) and turkeys (Frame et al 2007). In the present study, lower feed intake in FFM15 and FFM20 groups can be attributed to several nutritional factors. Firstly, poultry generally choose shiny and brightly coloured feed and are affected by the physical structure and particle size of diets (Ferket and Gernat 2006). In the current study, FFM diets are darker and thinner than the diet containing soybean meal. The second reason for the negative effect of FFM on feed intake may be related to secondary plant metabolites such as glucosinolates, phytic acid, condensed tannins, and sinapine, which have antinutritive effects of FFM (Matthaus 1997, Schuster and Friedt 1998, Matthaus and Zubr 2000, Thacker and Widyaratne 2012). In addition, the higher neutral detergent fibre content of the meal decreases the digestibility of diet and subsequent performance of bird (Erener et al 2009). FFM's richness in fibrous fractions such as NDF and ADF is considered as the third reason. In this study, the major cause of decreased body weight could be attributed to reduce in feed intake.

It was determined that egg production decreased in the FFM15 and FFM20 groups compared with the control group (P<0.01). The literature revealed various assertions about egg production. Cherian et al (2009) reported that 15% dietary FFM supplementation reduced egg production in laying hens. On the other hand, some studies have reported that the supplementation of FFM at 5%, 10% (Cherian et al 2009, Kakani et al 2012), and 15% (Pilgeram et al 2007) to laying hens does not change egg production. In this study, the decline in egg production in the groups receiving the highest level of FFM can be related to the antinutritive compound of FFM or decreasing feed intake and body weight within these groups.

It was determined that egg weight had no differences between the groups (P>0.05). Similarly, Cherian et al (2009) observed that FFM added to laying hens' diets at levels of 5%, 10%, and 15% does not change the egg weight. The finding that FFM supplementation in quail diets does not change the egg weight in this study indicates that the diets in the groups may have had similar protein and energy contents.

Although egg production was reduced in laying quails fed diets with 15% and 20% FFM (P<0.01), no difference was discovered between the groups in terms of feed efficiency due to feed intake reduction in all experimental groups. Similarly, FFM supplementation has no effect on the feed efficiency at 10% in turkey hens (Frame et al 2008) and at 5% and 10% in laying hens (Kakani et al 2012).

It was observed that the external quality and internal quality traits of the egg such as the yolk index, albumen index, and HU were not affected by FFM supplementation to the diets (P>0.05, Table 4). Cherian et al (2009) reported that 5%, 10%, and 15% FFM supplementations to diets did not change shell thickness or yolk and albumen weights. FFM supplementation had linear and nonlinear effects on egg yolk index. The egg yolk color index increased (P<0.001) in the FFM20 group in the fourth week of the experiment, while yolk color improved (P<0.01) in all experimental groups in the eighth week compared with the control group (Table 4). On the other hand, FFM supplementation to laying hen diets has been reported to decrease yolk color (Cherian et al 2009). The co-

Table 4. Effect of false flax meal on egg quality traits performance of laying quails.

	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
Egg shell thickness (mm/100)									
4th week	21.36±0.28	21.14±0.63	21.89±0.88	21.41±0.36	21.54±0.57	0.254	0.925	0.969	0.829
8th week	19.83±0.99	19.98±0.75	20.01±0.54	18.11±0.52	19.24±0.43	0.308	0.252	0.622	0.111
Yolk color index									
4th week	5.20±0.85^{b}	5.00±0.33^{b}	4.60±0.30^{b}	4.40±0.26^{b}	7.80±0.69^{a}	0.292	0.000	0.043	0.001
8th week	3.40±0.42^{b}	6.90±0.72^{a}	5.90±0.62^{a}	7.30±0.77^{a}	6.80±0.62^{a}	0.342	0.001	0.005	0.008
Shape index (%)									
4th week	77.95±1.41	78.69±0.59	77.43±0.89	77.73±0.77	76.22±1.25	0.465	0.549	0.506	0.288
8th week	78.09±0.76	78.73±1.18	76.22±1.39	77.22±1.40	80.58±1.24	0.564	0.144	0.430	0.101
Yolk index (%)									
4th week	55.93±1.57	59.48±1.28	56.27±1.97	58.43±1.39	55.75±1.83	0.744	0.440	0.574	0.387
8th week	46.82±0.99	47.76±1.41	47.63±1.09	51.12±1.80	48.33±1.46	0.630	0.245	0.787	0.089
Albumen index (%)									
4th week	13.12±0.66	11.95±0.73	13.99±1.35	13.40±1.11	12.84±0.64	0.402	0.624	0.528	0.525
8th week	8.32±0.70	9.88±0.73	8.58±0.43	8.88±0.85	9.67±1.02	0.346	0.569	0.497	0.314
Haugh unit									
4th week	72.49±2.47	72.10±3.19	79.76±4.02	76.63±3.97	72.77±2.07	1.452	0.440	0.325	0.431
8th week	57.75±2.73	64.09±2.64	59.05±2.09	62.49±2.82	63.54±2.93	1.195	0.386	0.328	0.191

^{a,b}: Different letters in the same line indicate statistically significant.

Table 5. Effect of false flax meal on serum MDA and AOA levels of laying quails.

	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
MDA (nmol/L)	4.85^{a}	4.22^{ab}	3.95^{b}	3.59^{b}	3.62^{b}	0.139	0.012	0.001	0.604
AOA (mmol/L)	7.37^{a}	9.09^{b}	8.88^{b}	9.41^{b}	9.59^{b}	0.199	0.001	0.000	0.150

^{a,b}: Different letters in the same line indicate statistically significant.

Table 6. Effect of false flax meal on egg yolk MDA level (mg MDA/kg sample) of laying quails.

	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
1st day	0.102^{a}	0.084^{b}	0.087^{b}	0.084^{b}	0.084^{b}	0.002	0.022	0.013	0.126
15th day	0.191^{a}	0.141^{b}	0.120^{bc}	0.103^{c}	0.098^{c}	0.006	0.000	0.000	0.162

^{a,b}: Different letters in the same line indicate statistically significant.

lor of the yolk depends on the storage of pigments called carotenoids in the yolk (Goodwin 1980). Moreover, it has been reported that with unsaturated fatty acids in the diets, antioxidants are more effective in improving yolk color (Aziza et al 2010). In the study, the improvement of pigmentation in egg yolk color in noted groups may be associated with substantially high n-3 and n-6 fatty acid contents as well as adequate amount of fat-soluble pigments such as carotenoids in the levels of FFM used.

In the present study, serum MDA level decreased (P<0.05) in the FFM10, FFM15, and FFM20 groups, while the serum AOA level increased (P<0.01) in all experimental groups compared with the control group (Table 5). It was also determined that depending on the storage, the egg yolk MDA levels decreased in all groups supplemented with FFM on the 1st (P<0.05) and 15th (P<0.001) days. The decrease on the 15th day was more severe in the FFM15 and FFM20 groups (Table 6). The decrease of MDA concentrations, which is a product of lipid peroxidation, suggests that lipid peroxidation decreased with dietary FFM supplementation. FFM is a good resource of long-chain fatty acids for both serum and the egg (Zubr 1997). As shown in Table 1, FFM is rich in α-linolenic acid, linoleic acid, and oleic acid. Even though it was not assessed in this study, dietary FFM supplementation was reported by several studies to increase the level of unsa-

turated fatty acids in the egg (Rokka et al 2002, Cherian et al 2009, Kakani et al 2012). In addition to omega-3 fatty acid, FFM includes other bioactive compounds with antioxidant properties such as tocopherols and phenolic compounds (Matthaus 2002, Salminen et al 2006). In this study, it has been determined that FFM supplementation to quail diets which are rich in polyunsaturated fatty acids prevents lipid oxidation in the egg. Therefore, it was determined that the lipid oxidation in the eggs of quails fed on FFM-supplemented diets decreased, and FFM had an influence on storage time.

Conclusions

It may be concluded that FFM supplementation to laying quail diets do not affect egg weight, feed efficiency, and some egg traits. The FFM may improve egg yolk color and prevented lipid peroxidation in serum and eggs. Based on these results, it is stated that up to 10% FFM can be used as an alternative protein source in laying quail diets.

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