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EFFECT OF FALSE FLAX MEAL ON CERTAIN GROWTH, SERUM AND MEAT PARAMETERS OF JAPANESE QUAILS

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ABSTRACT

False flax (*Camelina sativa L.*) is an oil seed crop, a member of Brassicaceae family which has got popularity in biofuel production. The meal of false flax seed has a good potential to be used as a cheap alternative protein source in livestock. False Flax Meal (FFM) has not been used and evaluated in quail diet earlier. The present study was conducted to determine the effects of FFM supplementation in quail diets on growth performance, some carcass characteristics, and lipid oxidation in meat. A total of 300 five-day-old Japanese quails (*Coturnix coturnix japonica*), including both sexes were divided into 5 groups, each consisting of 60 quails. They were kept in pens and fed a corn-soybean meal-based diet with FFM supplemented at 0% (Control), 5% (FFM5), 10% (FFM10), 15% (FFM15), and 20% (FFM20). The experimental period was lasted for 35 days. Results showed that there were no changes in body weight, body weight gain and feed intake as well as hot and cold carcass weights, relative weight of liver, heart, spleen, gizzard and proventriculus in all experimental groups with FFM supplementation ($P>0.05$). FCR was impaired in the FFM15 and FFM20 groups compared with the control group ($P<0.01$). Malondialdehyde levels of serum ($P<0.001$) and breast meat ($P<0.01$) decreased in the FFM10, FFM15 and FFM20 groups, whereas serum antioxidant activity level ($P<0.01$) increased in all experimental groups compared with the control group. In conclusion, it may be stated that dietary supplementation of FFM up to 10% prevents lipid oxidation without any adverse effect on performance and carcass characteristics in quails.

Key words: False flax meal, performance, carcass, lipid oxidation, quail.

INTRODUCTION

The protein requirement of quails in early age is high as nutrient utilization efficiency and growth rate is highest in young growing quails (NRC, 1994). Grains and oilseed meals are the major ingredients which constitute the main components of poultry diets (Senköylü, 2001). Soybean meal is mainly used as a protein source which puts high cost on production. In recent years, research has been directed towards finding certain alternative economical feed ingredients especially protein sources to replace expensive ones and increase performance (Cherian, 2012).

False Flax (FF) as an alternative oilseed crop, (*Camelina sativa L.*) belongs to the Brassicaceae family and can be grown in unfavorable climatic soil conditions (Zubr, 2003) and can be used in biodiesel fuel production (Budin *et al.* 1995). One of the major product of FF is False Flax Meal (FFM) which has 35-40% crude protein, 4600-4800 kcal/kg of gross energy, 6-12% fat, 6-7% ash, 41% neutral detergent fiber, 5% minerals, and a minor amount of vitamins and other substances; in terms of biological value this meal is similar to soybean meal (Cherian, 2012). False Falx meal (FFM) is rich in protein having crude protein similar to canola and rich in PUFAs, especially n-3 (omega-3) fatty acids (Putnam *et al*., 1993) and comparatively cheaper than other sources especially soybean. FFM also contains anti-nutrive compounds such as glucosinolates, tannins, phytic acid and sinapine (Matthaus and Zubr, 2000). Additionally some other bioactive compounds such as tocopherols and several phenolic compounds of *Camelina sativa L.* have also shown potential for antioxidative activity in poultry (Aziza *et al.*, 2010b).

Poultry meat is rich in PUFAs and low in antioxidant factors (Barroeta, 2007) which results in early deterioration of meat especially due to the addition of high amount of PUFAs, particularly when new vegetable resources for omega-3 fatty acids added to poultry diets (Abramovic *et al.*, 2007). Studies conducted on different bird species have evaluated the effects of various levels of FFM on performance (Thacker and Widyaratne, 2012) and meat oxidative stability (Aziza *et al*., 2010a,b). However, no available scientific data has been found about the utilization of FFM in the diets of quails. The aim of this research was to determine the effects of dietary FFM on growth performance, some carcass characteristics, serum oxidant-antioxidant balance, and malondialdehyde (MDA) levels of the breast meat in quails.

MATERIALS AND METHODS

Experimental Design and Diets: The present study was conducted at the Animal Research Center of Afyon Kocatepe University, Turkey, upon the approval of the ethical committee (AKÜHADYEK-304-13). A total of 300 five-day-old Japanese quail chicks (*Coturnix coturnix japonica*) of both sexes were used. The birds were divided into one control group and four treatment groups, each consisting of 60 quails. Each group was further sub-divided into five replicates consisting of 12 quails in each. Feed and water were offered *ad libitum* and daily 24 hours light was provided during the whole experiment. The experiment lasted for 35 days.

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 isocaloric and isonitogenous according to the nutrient requirements of quails as recommended by NRC (1994). The ingredient, chemical composition of diets and false flax chemical composition is shown in Table 1 and II respectively.

Parameters Analysed: Chemical composition of FFM and other diets were analysed according to the AOAC (2000). The FFM was also analyzed to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents, by Van Soest *et al.* (1991) method. The metabolizable energy (ME) values of the FFM and other diets were calculated by Carpenter and Clegg equation (Leeson and Summers, 2001):

ME kcal/kg = 53 + 38 [(crude protein %) + (2.25 x crude fat %) + (1.1 x starch %) + (1.05 x sugar %)].

Total lipids were extracted from false flax (*Camelina sativa L.*) meal by using n-hexane (Anwar *et al.*, 2008). Lipid extracts were used to prepare fatty acid methyl esters and fatty acid composition was analysed by Agilent 7820A gas chromatograph (Agilent Technologies Inc., Palo Alto, CA.) having autosampler, ionization detector and silica capillary column (60m* 0.25mm* 0.2µm). Helium gas was used as a carrier to inject samples. Fatty acid values (%) and peak areas were measured using Agilent Chem Satation Software. Fatty acid methyl esters were calculated by comparing with standards used in Agilent and were written as percentage of total fatty acid methyl esters.

Performance: At day 1st of experiment, chicks were weighed individualy. Weekly body weight, body weight gain, feed intake and feed conversion ratio was calculated.

Carcass Characteristics: At 35th day of age, 5 males and 5 females from each group (total 50 quails) were randomly selected and weighed. The quails were slaughtered. The inert organs were removed, and then defeathering was performed through a defeathering mechine. Hot carcass weights were determined after slaughter and cold carcass weight was determined after storage at +4^{o}C for 18 h. The liver, heart, spleen, gizzard and proventriculus were removed. Relative organ weights were calculated as organ weight (g) / body weight (g), which is equivalent to % of body weight.

Determination of Antioxidant Status: At the end of the study, 10 birds from each group (2 animals from each replicate) were slaughtered, and blood samples were kept in opaque heparin-free tubes at +4^{o}C for 24 hours. Serums were centrifuged at 3,000 rpm for 15 minutes. The serums were then put into opaque eppendorf tubes and stored at -18^{o}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). The antioxidant activity was determined colorimetrically in serum through a modified method by Koracevic *et al.* (2001).

At day 35 of experiment, 40 breast meat samples (8 meat samples from each group) were analyzed for thiobarbituric acid (TBA) activity. Breast meat samples which were stored at +4 ^{o}C, were analyzed for lipid oxidation by modified 2-TBA method which is based on red color observation that is emitted from the oxidation of unsaturated fatty acids with TBA after heating MDA and results were shown as the amount of 2-TBA reactive substances (mg MDA) (Ke *et al.*,1977). For the purpose of analysis 10 g of sample was put into blender and homogenized and then poured into Kjeldhal flask for distillation by adding 2.5 ml 4M HCL (Merck, Germany) and antifoam A (1ml). 5 ml TBA reagent was mixed with 5 ml distillate and incubated in water bath for 30 minutes at boiling temperature. The final solution obtained and blank solution was observed in spectrophotometer at 538 nm and values were expressed as mg MDA/kg sample (Yesilbag *et al.*, 2012).

Statistical analysis: All data were analyzed using the GLM procedures of the SPSS 13.0 for window version. For body weight, feed intake, and FCR data, replicates means served as the experimental unit for statistical analysis. Data of relative organ weights and length of gut, meat quality traits, serum MDA, AOA, and carcass MDA, blood parameters individually were considered as the experimental unit. In order to evaluate the data statistically, one-way analysis of variance (ANOVA) was performed in a completely randomized design: $\hat{\mathbf{Y}}_{ij} = \mu + \alpha_i + \mathbf{e}_{ij}$, where $\hat{\mathbf{Y}}_{ij}$ are observation values (body weight gain, feed consumption, feed to gain, carcass traits, serum parameters, etc), μ is the overall mean, α_i is the effect of the **i**th treatment (**i**: 1,2,3,4,5; 0 FFM, 5 FFM, 10 FFM, 15 FFM and 20 FFM) and $\mathbf{e}_{ij}$ = residual error. Tukey test was then used to separate these differences. The effects of increasing dietary concentrations of supplemental FFM were analysed as an orthogonal polynomial contrasts.

Linear and nonlinear effects were determined by orthogonal polynomial contrasts. For all tests, the level of significance was set at 0.05.

RESULTS

The chemical composition of diets and FFM are presented in Table 1 and 2, respectively. FFM contains high amounts of crude protein (36.88%), ME (2177 kcal/kg), crude fat (6.44%), crude fiber (17.4%), and fibrous fractions such as NDF (45.5%) and ADF (24.7%). FFM used in this study was rich in essential fatty acids and the determined composition showing high proportions of α-linolenic acid (36.11%) and linoleic acid (23.47%) and moderate proportions of oleic acid (12.8%), eicosenoic acid (8.85%) and palmitic acid (7.73%). Total mono-unsaturated fatty acid constituted 23.96%, while total PUFA constituted 59.58%. Total saturated fatty acids constituted 16.47%.

In the present study, FFM dietary supplementation had a linear effect on feed conversion ratio, MDA and AOA levels in serum, as well as on breast meat MDA levels. No significant differences were observed between the groups regarding body weight, body weight gain and feed intake ($P > 0.05$). It was determined that FCR increased ($P < 0.01$) in the FFM15 and FFM20 groups compared with the control and FFM5 groups (Table 3).

Hot and cold carcass weights and relative weight of liver, heart, spleen, gizzard and proventriculus were not affected by the FFM supplementation in the diets ($P> 0.05$; Table IV). Serum MDA level decreased ($P< 0.001$) in the FFM10, FFM15 and FFM20 groups, whereas serum AOA level increased ($P< 0.01$) in all experimental groups compared with the Control group (Table V). It was also determined that breast meat MDA level decreased ($P< 0.01$) in the FFM10, FFM15 and FFM20 groups compared with the Control group (Table V).

Table 1. Ingredients and chemical composition of the diets (%)

Ingredients	Treatment groups				
	Control	FFM5	FFM10	FFM15	FFM20
Corn	43.1	41.6	40.6	38.84	37.7
Wheat	3	3	3	3	3
Full fat soybean	12.6	10.6	9.4	8.7	7.5
Soybean meal (48%)	33	31	28	25	22
False flax meal	0	5	10	15	20
Meat and bone meal (38%)	2	2	2	2	2
Vegetable oil	4	4.5	4.7	5.2	5.6
Limestone	1	1	1	1	1
Salt	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	0.8	0.8	0.8	0.76	0.7
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25	0.25
Chemical Composition (Analyzed)					
Crude protein (%)	24.28	24.45	24.25	24.37	24.3
Metabolizable energy[2] (kcal/kg)	2934	2939	2915	2928	2926
Calcium (%)	0.85	0.85	0.86	0.88	0.89
Total phosphorus (%)	0.3	0.33	0.35	0.35	0.35

[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).

Table 2. Chemical composition of false flax meal

Nutrients	% in FFM	Fatty Acids	% in FFM
Dry matter	95.81	Palmitic acid (16:0)	7.73
Crude protein	36.88	Myristic acid (14:0)	0.26
Crude fat	6.44	Stearic acid (18:0)	2.76
Crude fiber	17.4	Oleic acid (18:1)	12.8
Crude ash	5.97	Linoleic acid (18:2 n-6)	23.47
Nitrogen free extract	33.31	α-Linolenic acid (18:3 n-3)	36.11
Acid detergent fiber	24.7	Arachidic acid (20:0)	0.99
Neutral detergent fiber	45.5	Eicosenoic acid (20:1)	8.85
Metabolizable energy (kcal/kg)	2177	Behenic acid (22:0)	2.18
		Erucic acid (22:1)	2.31
		Lignoseric acid (24:0)	2.55

Table 3. Effects of dietary false flax meal supplementation on growth performance of quails

Parameters	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
Initial body weight (g)	12.13	12.12	12.25	13.09	13.35	0.220	0.229	0.811	0.487
Final body weight (g)	192.18	194.83	193.24	195.53	189.87	1.22	0.645	0.520	0.245
Body weight gain (g/day)	180.05	182.70	180.99	182.43	176.52	1.23	0.846	0.740	0.916
Feed intake (g/day)	616.82	624.53	627.72	648.09	634.74	5.39	0.455	0.136	0.588
Feed conversion ratio (g feed/g)	3.42c	3.41c	3.46bc	3.55ab	3.59a	0.019	0.004	0.000	0.268

a, b, c: Different letters in the same line are statistically significant (P<0.05).

Table 4. Effects of dietary false flax meal supplementation on carcass (g) and relative organ (%) weights of quails

Parameters	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
Hot carcass weight	122.73	121.01	121.37	117.26	124.38	1.801	0.814	0.670	0.652
Cold carcas weight	116.79	117.91	116.02	111.69	118.87	1.785	0.781	0.901	0.699
Liver	2.56	2.25	2.34	2.33	2.18	0.075	0.597	0.220	0.794
Heart	0.918	0.837	0.847	0.891	0.871	0.017	0.621	0.763	0.310
Spleen	0.074	0.049	0.059	0.066	0.060	0.010	0.124	0.083	0.214
Gizzard	1.781	1.825	1.862	1.895	1.901	0.039	0.211	0.094	0.318
Proventriculus	0.440	0.412	0.419	0.463	0.479	0.012	0.381	0.187	0.571

Table 5. Effect of dietary false flax meal supplementation on serum MDA, serum AOA and breast meat MDA level in quails

Parameters	Control	FFM5	FFM10	FFM15	FFM20	SEM	P	Linear	Nonlinear
Serum MDA and AOA level									
MDA (nmol/L)	4.71a	4.28ab	3.95b	3.72b	3.66bc	0.082	0.000	0.000	0.489
AOA (mmol/L)	2.77b	3.34a	3.54a	3.77a	3.60a	0.098	0.009	0.002	0.256
Breast meat MDA level									
MDA (mg/kg sample)	1.210a	1.260ab	1.089b	1.071b	0.973b	0.027	0.003	0.000	0.397

a, b, c: Different letters in the same line are statistically significant (P<0.05).

DISCUSSION

The present study was conducted to investigate the effect of graded levels of FFM on growth performance, carcass characteristics, and lipid oxidation in meat of quails. Presently, there is no available information using FFM as protein replacement in the diet of quails. It is the first study on utilization of cultivated FFM as a protein replacement for soybean meal in the grower diets of quails.

Chemial Composition of FFM: The high protein and energy values and also higher contents of n-3 and n-6

fatty acids made it suitable plant protein and fatty acid source in quail diets. The nutrient composition of the FFM used in this study was in agreement with the values obtained in earlier experiments (Cherian *et al.,* 2009; Aziza *et al.,* 2010a).

Performance: The supplementation of FFM to the diets of quails had no differences on body weight, body weight gain and feed intake ($P > 0.05$). Similarly, it was reported that FFM supplementation has no effect on body weight of young turkey at 5% and 15% (Frame *et al.,* 2007) and on body weight gain at 5% and 10% of broilers (Aziza *et al*., 2010a). However, some studies have reported that the supplementation of FFM to broiler diets at different levels (Ryhanen *et al.,* 2007; Pekel *et al.,* 2009) have adverse effects on body weight. The body weight and body weight gain does not change in this study which might be due to no change in feed intake.

Some studies reported that the supplementation of false flax seed did not change feed intake in turkey (Frame *et al.,* 2007) and broiler (Aziza *et al.,* 2010a). However in other studies, FFM supplementation has been reported to decrease feed intake in broiler (Ryhanen *et al.,* 2007; Pekel *et al.,* 2009) and turkey; (Frame *et al.,* 2007). In present study FFM supplementation in quail diets does not change the feed intake which might be because of similar protein and energy contents in diets.

In this study, supplementation of FFM to diet at levels of 15% and 20% impaired feed conversion ratio. Similar results were found in other studies that FFM supplementation has negative effect on the feed conversion ratio at 10% (Ryhanen *et al.,* 2007) and 15% (Thacker and Widyaratne, 2012) in broilers and at 20% in turkeys (Frame *et al*., 2007). Some other studies reported that the supplementation of FFM at 2.5%, 5% and 10% (Aziza *et al*., 2010a) and 10% (Pekel *et al*., 2009) in broilers did not change feed conversion ratio. Because of the presence of plant secondary metabolites such as glucosinolates, phytic acid, condensed tannins, and sinapine which reduces the digestibility of nutrients by making complexes with them and high fiber content, FFM has negative effect on digestibility and nutrient utilization (Jorgensen *et al.,* 1996; Matthaus and Zubr, 2000; Russo and Reggiani, 2012). FFM in present study also contains high levels of fibrous fractions such as neutral detergent fiber and acid detergent fiber. In this study, the impaired feed conversion ratio in the groups receiving the highest level of FFM might have resulted from fibrous parts and anti-nutritional factors in the content of the meal as well as low utilizing ability of the birds.

Carcass Characteristics: It was observed that the carcass weights and relative weight of liver, heart, spleen, gizzard, and proventriculus were not affected by FFM supplementation to the diets ($P>0.05$). Similarly, Aziza *et al.* (2010a) observed that FFM added to broiler's diets at levels of 2.5%, 5%, and 10% did not change the carcass weights, relative weight of the organ such as heart, spleen, and gastrointestinal tract weight. However, some studies reported that the supplementation of FFM had increased relative liver weight at 2.5% (Aziza *et al*., 2010a), whereas hot carcass weight was decreased at 10% (Pekel *et al*., 2009).

Antioxidant Status: MDA levels of serum ($P< 0.001$) and breast meat ($P< 0.01$) decreased in the FFM10, FFM15 and FFM20 groups, whereas serum AOA level increased ($P<0.01$) in all experimental groups in this study. The decrease in AOA was more pronounced in the FFM15 and FFM20 groups. In this context, the decrease of MDA levels, which is the end product of lipid peroxidation, suggested that lipid oxidation decreased as linear with dietary FFM supplementation. FFM meal contains some antioxidant compounds (Aziza *et al*., 2010b) and some other bioactive compounds with antioxidant properties such as tocopherols and phenolics (Matthaus, 2002; Salminen *et al.,* 2006) which results in increased AOA and decreased MDA level in serum and breast meat.

Conclusion: It is concluded that FFM supplementation to quail diets did not affect body weight, body weight gain and feed intake as well as carcass characteristics, whereas the supplementation of FFM up to 10% to quail diets proved to be more effective on feed conversion ratio. The FFM prevented lipid oxidation in meat. Based on these results, it is stated that up to 10% FFM can be used as an alternative protein source in quail diets.

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