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Effects of Ground Flaxseed on Growth Performance, Intestinal Morphology, and Meat Fortification with Fatty Acids in Finisher Male Broiler Chickens

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Abstract
The current study aimed to determine the optimum level of ground flaxseed needed to improve performance and enrich broiler meat with omega-3 fatty acids (n-3 FA). A total of 360 twenty-five-day-old male broilers (Ross 308) were reared on floor pens and fed diets supplemented with ground flaxseed during the finisher phase (d 25-45). The chickens were assigned to four treatments of six replicates as follows: 1) corn-soybean meal (CSM) based diet as control; 2) CSM supplemented by 3% ground flaxseed (flax3); 3) CSM supplemented by 6% ground flaxseed (flax6); 4) CSM supplemented by 9% ground flaxseed (flax9). Results showed that flax6 increased ($P < 0.05$) daily gain (ADG) and feed intake (ADFI), while it did not affect feed conversion ratio (FCR). In addition, flax9 decreased ($P < 0.05$) ADG and ADFI, and impaired ($P < 0.05$) FCR compared to the control group at the end of the experiment (d 45). Feeding flax6 increased ($P < 0.05$) polyunsaturated fatty acids (PUFA) and n-3 FA in breast and thigh muscles and lowered ($P < 0.05$) abdominal fat pad weight compared to the control group. Results revealed that feeding birds with ground flax6 improves growth performance, reducing fat depots and enriching meat with n-3 PUFA without negatively affecting gut morphology.

Introduction

Several studies have shown that the levels of polyunsaturated fatty acids (PUFA), particularly omega-3 (n-3) fatty acids, in human diets are below the levels recommended by nutritionists (Nutrition and Toxicity, 2004; WHO, 2003) to prevent different human disorders such as cardiovascular disease (Gibbs *et al*., 2010). Various strategies for increasing the n-3 FA content in the human diet have been proposed. Different oilseeds containing various levels of n-3 FA could be added to poultry diets in order to enrich poultry eggs and meat with n-3 FA (Moghadam and Cherian, 2017; Konieczka *et al*., 2017). For example, full-fat flaxseed (linseed) is an oilseed that contains a significant amount of oil (34%) (Slominski *et al*., 2006), with approximately 5% α- linolenic acid (ALA, 18:3 n-3) (Jia *et al*., 2010). These characteristics would make flaxseed an attractive ingredient for the production of n-3 enriched poultry eggs and meat. Indeed, previous studies showed that adding oilseeds rich in n-3 PUFA, such as flaxseed to poultry diets decreased FA deposition in the abdominal cavity and skin (Ferrini *et al*., 2008; Jia *et al*., 2010) and increased the n-3 FA content of the edible portions (Apperson and Cherian, 2017). However, the results are inconsistent with regard to the effects of flaxseed on broiler productive performance. While some researchers (Lee *et al*., 1991, 1995) demonstrated that flaxseed improved energy utilization and performance in broiler chickens, Ortiz *et al*. (2001) and Alzueta *et al*. (2003) reported that flaxseed could suppress growth and meat production in broiler chickens due to containing different levels of anti-nutritional factors. Therefore, various methods such as soaking, pelleting, extruding and adding exogenous enzymes to diets, etc., have been suggested to decrease the anti-nutritional factors. For example, Jia and Slominski (2010) demonstrated that grinding flaxseed may destroy anti-nutritional factors and improve nutrient availability resulting in better growth performance and health conditions. Thus, the present study aimed to determine the optimal level of ground flaxseed required to simultaneously improve chicken growth and gut morphology as well as enrich broiler meat with n-3

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PUFA. In addition, previous studies had reported that feeding flaxseed-supplemented diets for a period of 14 to 21 days (depending on flaxseed level) was sufficient to increase the concentration of n-3 PUFA in poultry meat products, and longer time did not affect FA concentrations (Apperson and Cherian, 2017; Jia *et al.*, 2010), ground flaxseed was supplemented in the diet of chickens from 25 to 45 days of age in the current study.

Materials and Methods

All experiment procedures were approved by the Institutional Animal Care and Use Committee at Ferdowsi University of Mashhad (Protocol No. 1/41749).

Birds and experimental design

Four hundred day-old Ross 308 male chicks were obtained from a local hatchery, placed in the floor pens covered with wood shavings, and fed on Ross 308 standard diet until d 24. On d 25, 360 chickens in a completely randomized design were weighed and assigned to four treatments with six replicates (15 birds per replicate). Experimental diets comprise **1**) a corn-soybean meal (CSM) based diet as control; **2**) CSM supplemented by 3% ground flaxseed (**flax3**); **3**) CSM supplemented by 6% ground flaxseed (**flax6**); and **4**) CSM supplemented by 9% ground flaxseed (**flax9**). Flaxseed was provided by a local company that imports these seeds directly from Canada (Saleh Kashmar Company). The flaxseed (Table 1) was milled using a hammer mill (Asiab Co., Tehran, Iran) and mixed with experiment diets at the levels as mentioned earlier. All diets were in mash form and formulated to be isonitrogenous and isocaloric and to meet or exceed the minimum requirements of Ross 308 (Table 2).

Table 1. Chemical composition of ground flaxseed

Component	**Value** (%)
Dry matter	93.10
Metabolizable energy (kcal/kg)	3759.47
Crude protein	21.81
Ether extract	38.42
Crude fiber	6.10
Calcium	0.25
LA[1]	32.44
ALA	33.40
ARA	0.00
EPA	0.00
DPA	0.00
DHA	0.00
total SFA	12.84
total MUFA	21.33
total PUFA	65.80
total n-3	33.42
total n-6	32.41
n-6/ n-3	0.97
Total lipids	15.71

[1] **LA**: linoleic acid ($C_{18:2(n-6)}$); **ALA**: α-linolenic acid ($C_{18:3(n-3)}$); **ARA**: arachidonic acid ($C_{20:4(n-6)}$); **EPA**: eicosapentaenoic acid ($C_{20:5(n-3)}$); **DPA**: docosapentaenoic acid ($C_{22:5\ (n-3)}$); **DHA**: docosahexaenoic acid ($C_{22:6(n-3)}$); **total SFA**: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; **total MUFA:** total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; **total PUFA**: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4\ (n-6)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-3**: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-6:** total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4\ (n-6)}$.

The diets were analyzed for dry matter (DM), crude protein (CP), and gross energy (GE). DM content was determined according to the AOAC (Chemists, 1990; method 925.09) by oven drying 10 g of the sample at 100°C overnight. The total nitrogen content of diets was determined using the combustion method (Chemists, 1990; method 990.03), and CP was calculated as nitrogen × 6.25. An adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL) calibrated with benzoic acid was used to measure GE. The temperature was set at 32°C for the first three days, then gradually reduced to a constant 21°C by d 25 through the end of the experiment. Feed and water were provided ad libitum throughout the study period. The lighting program was 23L:1D for the first five days, then gradually decreased to 18L:6D on d 10, similar to the end of the experiment.

Table 2. Composition of experimental diets during 25 to 45 days of age

Ingredients	Ground flaxseed level (%)			
	0.00	3.00	6.00	9.00
Corn	65.15	64.25	63.35	61.65
Soybean meal [%44.1 CP]	24.50	22.40	19.80	18.00
Soybean Oil	4.00	4.00	4.00	4.00
Ground flaxseed	0.00	3.00	6.00	9.00
Corn gluten	2.50	2.50	3.00	3.50
Dicalcium phosphate	1.60	1.60	1.60	1.60
Limestone	0.90	0.90	0.90	0.90
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.19	0.18
L-Lysine	0.40	0.40	0.41	0.42
Vitamin Premix[1]	0.25	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25	0.25
Calculated analysis (%)				
Metabolizable energy (kcal/kg)	3200	3200	3200	3200
Crude protein	18.00	18.00	18.00	18.00
Lysine	1.15	1.15	1.13	1.10
Methionine	0.48	0.48	0.48	0.48
Methionine + Cystine	0.85	0.80	0.79	0.78
Threonine	0.78	0.77	0.75	0.75
Available Phosphorus	0.39	0.39	0.38	0.38
Calcium	0.78	0.77	0.79	0.78
Sodium	0.16	0.16	0.16	0.16
Fatty acids (%)				
LA[3]	46.82	43003	41.89	40.15
ALA	2.28	5.41	12.45	16.27
ARA	0.00	0.00	0.00	0.00
EPA	0.00	0.00	0.00	0.00
DPA	0.00	0.00	0.00	0.00
DHA	0.00	0.00	0.00	0.00
total SFA	19.01	19.13	18.12	17.03
total MUFA	31.88	28.75	27.27	26.54
total PUFA	49.10	48.45	54.92	56.43
total n-3	2.28	5.41	13.14	16.27
total n-6	46.82	43.03	41.78	40.15
n-6/ n-3	20.54	7.95	3.18	2.47
Total lipids	5.75	6.16	6.41	6.55

[1] Vitamin premix per kilogram contained retinol, 2.7 mg; cholecalciferol, 50 mcg; tocopherols, 188 mcg; phylloquinone, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamin, 0.012 mg, pantothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; choline chloride, 250 mg; antioxidant, 100 mg.

[2] Mineral premix per kilogram contained iron, 40 mg; zinc, 84.7 mg; manganese, 100 mg; copper, 10 mg; iodine, 0.9 mg; selenium, 0.2 mg.

[3] **LA**: linoleic acid ($C_{18:2(n-6)}$); **ALA**: α-linolenic acid ($C_{18:3(n-3)}$); **ARA**: arachidonic acid ($C_{20:4(n-6)}$); **EPA**: eicosapentaenoic acid ($C_{20:5(n-3)}$); **DPA**: docosapentaenoic acid ($C_{22:5\ (n-3)}$); **DHA**: docosahexaenoic acid ($C_{22:6(n-3)}$); **total SFA**: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; **total MUFA:** total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; **total PUFA**: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4\ (n-6)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-3**: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-6:** total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4\ (n-6)}$.

Growth performance

On d 34 and 45, the body weight and feed intake of chickens per pen were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). Mortality, if any, was recorded daily per pen, and performance traits were adjusted accordingly.

Sample collection

On sampling days (i.e., d 34 and 45), two birds from each pen (12 birds/treatment) were randomly euthanized by cervical dislocation, the viscera were excised, and the relative weight of the carcass (with skin), muscles (breast and thigh, without skin), internal organs (liver and heart), and abdominal fat pad (AFP) were measured. The intestine was carefully separated from the whole viscera and meticulously cleaned of adherent materials. The jejunal segment (from the end of the duodenal loop to Meckel's diverticulum) was immediately separated and gently emptied for further morphological analysis. After evisceration, samples from the breast (left pectoralis major), thigh (left biceps femoris) muscles, AFP (including fat around the gizzard), and skin (a mixture of breast and thigh skins) were removed, weighed, vacuum-packaged in plastic bags, and stored at -20ºC for further analysis.

Intestinal morphology

The methodology and equipment previously described (Daneshmand *et al*., 2017) were used to prepare samples for jejunal morphology. Briefly, about 2 cm of emptied jejunal tissue was fixed in formaldehyde phosphate buffer and embedded in paraffin. The paraffin block was sliced to a thickness of 5 µm by a microtome (Leica HI1210, Leica Microsystems Ltd., Wetzlar, Germany). Then, samples were placed on a slide and dehydrated on a hotplate (Leica ASP300S, Leica Microsystems Ltd., Wetzlar, Germany). Next, the dehydrated samples were stained with hematoxylin and eosin (Leica Autostain BRXL, Leica Microsystems Ltd., Wetzlar, Germany) and examined under a microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan). Nine slides were prepared (per jejunal per bird), and ten villi were measured per slide (90 villi/bird). The average of villi measurements was reported as a mean for each bird (12 birds/treatment). Villus width (VW), villus height (VH), crypt depth (CD), and the ratio of VH to CD (VH/CD) were measured.

Fatty acids analysis

For FA analysis, total lipids were extracted from experimental diets (Table 1), ground flaxseed (Table 2), minced breast and thigh tissues, and AFP using a mixture of chloroform and methanol (2:1, vol: vol) as described previously (Folch *et al*., 1957). Fatty acids were esterified based on previously described methodology and equipment (Cherian *et al*., 2002). Fatty acid methyl ester analysis was conducted on a 7890 A gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) equipped with a flame ionization detector FID and capillary column, 25m×0.25mm×0.22µm (BPX70, Trajan Scientific and Medical, Victoria, Australia). Each sample was injected with helium as a carrier gas onto the column. The column, detector, and injector temperatures were 180, 235, and 220°C, respectively. Fatty acid methyl esters were identified by comparison of their retention times with standards and reported as g/100 g of sample. All analyses were performed in triplicate.

Statistical analysis

The statistical analysis of all data was performed using the GLM procedure in SAS 9.1 software (SAS Institute, Cary, NC, USA). Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of increasing flaxseed levels on all variables. Significant differences among data means were compared using Tukey's test, and $P < 0.05$ was considered statistically significant.

Results

Growth performance

On day 34, broilers provided with the flax6 diet showed a quadratic increase ($P < 0.05$) in ADG and ADFI, and birds fed flax9 showed a quadratic decrease ($P < 0.05$) in these variables compared to the control group (Table 3). On day 45, flax6 quadratically increased ($P < 0.05$) ADG and ADFI, whereas flax9 showed a quadratic decrease ($P < 0.05$) in ADFI as compared to the control birds. The overall performance (from d 25 to 45) showed that flax6 quadratically increased ($P < 0.05$) ADG and ADFI while feeding flax9 to broilers resulted in a quadratic decrease ($P < 0.05$) in ADG and an increase ($P < 0.05$) in FCR. Birds fed flax3 had growth performance similar to that of the control group in each phase.

Intestinal morphology

Table 4 summarizes the effects of ground flaxseed levels on the morphological analysis of the jejunum. On day 45, chickens fed flax9 showed a quadratic reduction in villi height (P = 0.021) and width (P = 0.039) compared to the control group, while birds receiving flax3 and flax6 demonstrated no significant changes in villi morphology at the whole experimental phase.

Relative weights

Feeding flax6 and flax9 quadratically decreased ($P < 0.05$) the relative weight of AFP in comparison to the control group at both d 34 and 45 (Table 5). Adding various levels of ground flaxseed resulted in no significant effects on carcass yield, internal organs (heart and liver), and breast and thigh muscles relative weights at 34 and 45 days of age.

Fatty acids profile

The effects of ground flaxseed levels on the FA profile of breast and thigh muscle (without skin), skin, and AFP are shown in Tables 6, 7, 8, and 9, respectively. On day 34, flax6 quadratically increased ($P < 0.05$) ALA, DHA, PUFA, and total n-3 FA, and a quadratic decrease ($P < 0.05$) in the n-6/n-3 ratio in the breast muscle compared to the control group. In the thigh muscle, the inclusion of flax6 in the broiler diets resulted in a quadratic increase ($P < 0.05$) in ALA, EPA, and total n-3 FA, and a quadratic reduction ($P < 0.05$) in the n-6/n-3 ratio as compared to the control group. In the skin, the concentrations of EPA, DHA, and total PUFA were quadratically higher ($P < 0.05$), and the n-6/n-3 was quadratically lower ($P < 0.05$) in birds fed flax6 compared to those fed the control diet. On d 45, birds fed flax6 showed quadratically higher ($P < 0.05$) concentrations of ALA, EPA, and total PUFA and quadratically lower ($P < 0.05$) value of the n-6/n-3 ratio in the AFP compared to the control group. Birds fed flax3 had a quadratically higher ($P < 0.05$) concentration of ALA in the thigh muscle and AFP, and quadratically lower (P < 0.05) n-6/n-3 ratio in all muscles and tissues compared to the control birds. Feeding flax9 resulted in a quadratic reduction (P < 0.05) in the n-6/n-3 ratio of the thigh muscle, skin, and AFP compared to those birds that received the control diet. Flax9 did not affect the fatty acid profile of different muscles and tissues in comparison to the control group.

Table 3. Effect of various levels of flaxseed on growth performance of broilers

Period[day]	**Dietary treatments[1]**				***SEM*[3]**	**P-Value**		
	Control	**Flax3**	**Flax6**	**Flax9**		**ANOVA**	**L[4]**	**Q[4]**
d 25-34								
ADG[2] (g/d)	55.7^{b}	60.1^{ab}	64.3^{a}	48.0^{c}	1.51	0.001	0.085	0.001
ADFI (g/d)	82.0^{b}	86.1^{ab}	91.2^{a}	75.9^{c}	1.45	0.001	0.056	0.001
FCR	1.47^{ab}	1.44^{ab}	1.42^{b}	1.58^{a}	0.023	0.044	0.097	0.025
d 35-45								
ADG (g/d)	79.7^{bc}	87.8^{ab}	94.9^{a}	72.7^{c}	2.36	0.006	0.312	0.001
ADFI (g/d)	147.3^{b}	158.7^{ab}	161.9^{a}	146.7^{c}	2.08	0.004	0.919	0.004
FCR	1.85^{ab}	1.82^{ab}	1.71^{b}	2.02^{a}	0.039	0.031	0.209	0.019
d 25-45 (Overall)								
ADG (g/d)	67.8^{b}	73.0^{ab}	79.6^{a}	60.4^{c}	1.77	0.001	0.122	0.001
ADFI (g/d)	114.7^{b}	120.5^{ab}	126.6^{a}	111.3^{b}	1.58	0.001	0.423	0.001
FCR	1.69^{b}	1.65^{b}	1.59^{b}	1.84^{a}	0.027	0.001	0. 301	0.001

[a-c] Values within a row with different letters differ significantly (P < 0.05).
[1]Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.
[2]ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; g/d/b: grams per bird per day.
[3]*SEM*: standard errors of means.
[4] L, linear; Q, quadratic.

Table 4. Effects of various levels of flaxseed on villi morphology (μm) of jejunum in broiler chickens

Villi morphology	**Control[1]**	**Flax3**	**Flax6**	**Flax9**	***SEM*[2]**	**P-Value**		
						ANOVA	**L[3]**	**Q[4]**
Day 35								
villus height	1131	1137	1143	1096	39.7	0.859	0.165	0.091
villus width	306^{ab}	312^{ab}	319^{a}	242^{b}	18.3	0.026	0.247	0.411
crypt depth	306	279	273	286	11.2	0.312	0.302	0.084
villus height/crypt depth	3.7	4.1	4.2	3.8	0.18	0.241	0.071	0.103
Day 45								
villus height	1518^{ab}	1547^{a}	1636^{a}	1374^{b}	61.9	0.035	0.094	0.021
villus width	294^{ab}	305^{ab}	330^{a}	238^{b}	24.1	0.014	0.340	0.039
crypt depth	284	287	281	283	14.8	0.842	0.093	0.117
villus height/crypt depth	5.3	5.4	5.8	4.8	0.39	0.327	0.223	0.079

[a-c] Values within a row with different letters differ significantly (P < 0.05).
[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.
[2] *SEM*: standard error of means (results are given as means (n = 6) for each treatment).
[3] L, linear; Q, quadratic.

Table 5. Effect of various levels of flaxseed on the relative weight of organs to live body weight (w/w, %) in broilers

Relative weight (%)	**Dietary treatments[1]**				***SEM*[2]**	**P-Value**		
	Control	**Flax3**	**Flax6**	**Flax9**		**ANOVA**	**L[3]**	**Q[3]**
Day 34								
Dressing carcass	70.33^{ab}	70.95^{ab}	72.16^{a}	69.81^{b}	0.296	0.018	0.844	0.006
Thigh	14.97	15.04	16.19	14.80	0.222	0.096	0.674	0.096
Breast	26.91^{ab}	26.08^{b}	28.29^{a}	25.47^{b}	0.344	0.009	0.861	0.003
Abdominal fat	0.89^{a}	0.78^{ab}	0.62^{b}	0.57^{b}	0.042	0.011	0.655	0.001
Liver	2.05	2.00	1.92	2.06	0.041	0.667	0.922	0.305
Heart	0.52	0.49	0.51	0.53	0.018	0.859	0.715	0.478
Day 45								
Dressing carcass	69.17	70.43	71.36	68.59	0.402	0.057	0.784	0.010
Thigh	15.76	16.27	16.71	15.19	0.236	0.113	0.524	0.029
Breast	27.84^{ab}	27.30^{b}	28.71^{a}	26.93^{b}	0.419	0.002	0.525	0.004
Abdominal fat	0.79^{a}	0.72^{ab}	0.55^{b}	0.53^{b}	0.033	0.003	0.593	0.004
Liver	1.91	1.76	1.59	2.01	0.067	0.135	0.797	0.364
Heart	0.43	0.47	0.43	0.44	0.011	0.562	0.814	0.495

[a-b] Values within a row with different letters differ significantly (P < 0.05).
[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.
[2]*SEM*: standard errors of means (results are given as means (n = 6) for each treatment).
[3] L, linear; Q, quadratic.

Table 6. Effect of various levels of flaxseed on breast muscle fatty acids in broilers

Fatty acid level in organ's fat (%)	Dietary treatments[1]				SEM[3]	P-Value		
	Control	Flax3	Flax6	Flax9		ANOVA	L[4]	Q[4]
Day 34								
LA[2]	20.47	20.56	20.33	20.63	0.524	0.998	0.966	0.935
ALA	0.97[c]	1.73[bc]	2.84[a]	2.01[bc]	0.330	0.001	0.413	0.002
ARA	3.08[a]	2.78[ab]	2.27[b]	2.79[ab]	0.111	0.043	0.092	0.036
EPA	0.291	0.285	0.307	0.302	0.041	0.244	0.214	0.107
DPA	0.318	0.326	0.319	0.329	0.065	0.716	0.121	0.134
DHA	0.160	0.277	0.299	0.298	0.039	0.616	0.073	0.117
total SFA	33.03	34.80	36.89	32.16	0.722	0.066	0.920	0.218
total MUFA	40.55[a]	37.66[ab]	33.99[b]	37.49[ab]	0.845	0.020	0.081	0.020
total PUFA	26.41	28.99	30.24	27.71	0.810	0.369	0.534	0.118
total n-3	2.01[c]	4.59[ab]	5.73[a]	3.11[bc]	0.456	0.030	0.239	0.006
total n-6	24.40	24.40	24.51	24.60	0.531	0.673	0.411	0.846
n-6/ n-3	12.14[a]	5.31[bc]	4.28[c]	7.90[b]	0.969	0.001	0.313	0.020
Day 45								
LA	21.37	21.82	22.01	21.90	0.358	0.946	0.636	0.740
ALA	1.73[b]	3.67[ab]	4.13[a]	2.02[b]	0.365	0.013	0.529	0.002
ARA	3.46	3.72	2.07	2.44	0.305	0.156	0.079	0.915
EPA	0.123	0.305	0.335	0.234	0.034	0.104	0.063	0.488
DPA	0.448	0.703	0.813	0.658	0.062	0.217	0.070	0.671
DHA	0.174[c]	0.368[b]	0.425[a]	0.190[c]	0.041	0.013	0.278	0.041
total SFA	37.09	34.06	32.06	33.28	0.799	0.127	0.065	0.150
total MUFA	33.71	35.01	37.82	35.58	1.118	0.686	0.456	0.481
total PUFA	29.19[b]	32.28[ab]	34.83[a]	33.82[ab]	0.729	0.027	0.508	0.008
total n-3	2.94[b]	5.20[ab]	5.93[a]	4.63[ab]	0.433	0.029	0.239	0.006
total n-6	26.24	27.08	28.90	29.19	0.640	0.673	0.411	0.846
n-6/ n-3	8.92[a]	5.21[bc]	4.87[c]	6.30[b]	1.200	0.002	0.313	0.020

[a-c] Values within a row with different letters differ significantly ($P < 0.05$).

[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.

[2] LA: linoleic acid ($C_{18:2(n-6)}$); ALA: α-linolenic acid ($C_{18:3(n-3)}$); ARA: arachidonic acid ($C_{20:4(n-6)}$); EPA: eicosapentaenoic acid ($C_{20:5(n-3)}$); DPA: docosapentaenoic acid ($C_{22:5(n-3)}$); DHA: docosahexaenoic acid ($C_{22:6(n-3)}$); total SFA: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; total MUFA: total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; total PUFA: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3 (n-3)}$, $C_{20:3 (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4 (n-6)}$, $C_{22:5 (n-3)}$, and $C_{22:6(n-3)}$; total n-3: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3 (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5 (n-3)}$, and $C_{22:6(n-3)}$; total n-6: total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3 (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4 (n-6)}$.

[3] *SEM*: standard errors of means (results are given as means (n = 6) for each treatment).

[4] L, linear; Q, quadratic.

Table 7. Effect of various levels of flaxseed on thigh muscle fatty acids in broilers

Fatty acid level in organ's fat (%)	Dietary treatments[1]				*SEM*[3]	P-Value		
	Control	Flax3	Flax6	Flax9		ANOVA	L[4]	Q[4]
Day 34								
LA[2]	21.17	23.26	22.92	21.45	0.928	0.859	0.957	0.418
ALA	2.79	3.51	3.37	3.92	0.485	0.907	0.235	0.131
ARA	1.34	1.22	1.203	1.08	0.081	0.786	0.347	0.972
EPA	0.059	0.035	0.137	0.021	0.020	0.173	0.949	0.230
DPA	0.136	0.219	0.321	0.223	0.029	0.178	0.158	0.121
DHA	0.036	0.051	0.049	0.062	0.022	0.089	0.108	0.086
total SFA	31.13	31.53	32.41	29.73	0.463	0.236	0.406	0.105
total MUFA	44.02	40.40	38.86	42.25	1.441	0.675	0.633	0.288
total PUFA	24.65	29.07	29.41	25.77	1.226	0.739	0.419	0.481
total n-3	1.53^{c}	4.09^{ab}	4.92^{a}	2.87^{bc}	0.422	0.001	0.507	0.009
total n-6	23.12	24.98	24.49	22.90	1.014	0.495	0.372	0.392
n-6/ n-3	15.11^{a}	6.10^{c}	4.98^{c}	7.98^{b}	1.213	0.001	0.305	0.007
Day 45								
LA	24.44	23.01	18.10	22.05	1.483	0.531	0.401	0.404
ALA	1.10^{c}	3.60^{ab}	4.16^{a}	2.39^{bc}	0.385	0.001	0.528	0.004
ARA	1.20	1.16	0.81	1.01	0.084	0.391	0.255	0.492
EPA	0.029^{b}	0.070^{ab}	0.106^{a}	0.071^{ab}	0.011	0.036	0.054	0.044
DPA	0.153	0.254	0.200	0.266	0.025	0.386	0.224	0.730
DHA	0.112	0.092	0.122	0.106	0.015	0.126	0.117	0.226
total SFA	32.06	28.86	22.91	29.94	1.774	0.331	0.441	0.170
total MUFA	38.41	42.29	31.55	41.96	2.509	0.448	0.997	0.535
total PUFA	29.34	28.68	26.72	27.30	1.898	0.696	0.424	0.411
total n-3	3.19^{b}	4.07^{ab}	4.51^{a}	3.85^{b}	0.531	0.027	0.507	0.032
total n-6	26.15	24.61	22.21	23.45	1.567	0.495	0.372	0.392
n-6/ n-3	8.20^{a}	6.05^{b}	4.92^{b}	6.09^{b}	1.293	0.001	0.001	0.007

$^{a-c}$ Values within a row with different letters differ significantly ($P < 0.05$).

[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.

[2] **LA**: linoleic acid ($C_{18:2(n-6)}$); **ALA**: α -linolenic acid ($C_{18:3(n-3)}$); **ARA**: arachidonic acid ($C_{20:4(n-6)}$); **EPA**: eicosapentaenoic acid ($C_{20:5(n-3)}$); **DPA**: docosapentaenoic acid ($C_{22:5\ (n-3)}$); **DHA**: docosahexaenoic acid ($C_{22:6(n-3)}$); **total SFA**: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; **total MUFA:** total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; **total PUFA**: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4\ (n-6)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-3**: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-6:** total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4\ (n-6)}$.

[3]*SEM*: standard errors of means (results are given as means (n = 6) for each treatment).

[4] L, linear; Q, quadratic.

Discussion

The present study aimed to investigate the level of ground flaxseed fed to chickens in order to simultaneously improve broiler growth performance and enrich broiler meat with n-3 FA. Increasing the level of flaxseed from 0 to 6% quadratically improved growth in broilers, while the addition of 9% flaxseed reduced performance attributes. Although there are some inconsistencies among the results of previous studies evaluating the effects of flaxseed in broiler diets, most of the research indicated that high levels of flaxseed (> 6%) significantly decreased body weight and FI and also impaired FCR in birds (Anjum et al., 2013; Jankowski et al., 2015; Madhusudhan et al., 1986). These results align with those of the present study regarding flax9. It has been well-documented that flaxseed has anti-nutritional factors such as mucilage, trypsin inhibitors, and hydrocyanic acid (Alzueta et al., 2003), with the latter being responsible for the production of goitrogenic thiocyanates that are capable of causing growth retardation in birds (Madhusudhan et al., 1986). On the other hand, another study (Carragher et al., 2015) reported that the inclusion of 2.5% flaxseed in the broilers' diet for six weeks improved the growth performance, which is inconsistent with the present results. Moreover, the results of previous studies revealed that the form of flaxseed might affect growth performance in broilers. For example, Madhusudhan et al. (1986) showed that the inclusion of 6.5% raw flaxseed in the broilers' diet caused a significant growth depression, while 13% water-boiled flaxseed had no adverse effects on performance, and broilers showed the same growth as that of the control group. Thus, it could be inferred that the grinding of flaxseed in the current study might reduce the levels of anti-nutritional factors and consequently prevent the resultant negative effects in flax3 and flax6. In addition, the processing was not efficient in neutralizing the harmful effects of anti-nutritional content in the highest inclusion rate of flax9, which is in agreement with previous reports (Jia and Slominski, 2010; Slominski et al., 2006).

The results of the current study revealed that while flax9 destructed villi morphology compared to two other levels, various levels of ground flaxseed had no adverse effects on villi morphology compared to the control group, which may be due to the effects of grinding on the destruction of the anti-nutritional factors (Jia and Slominski, 2010). In another study (Apperson and Cherian, 2017), two levels of flaxseed (10% and 15%) with or without enzymes were added to chicken diets. The results showed that the inclusion of flaxseed without enzyme could significantly damage villi morphology. In contrast, adding enzymes may suppress the adverse effects of anti-nutritional factors on villi structure.

Table 8. Effect of various levels of flaxseed on skin fatty acids in broilers

Fatty acid level in organ's fat (%)	Dietary treatments[1]				*SEM*[3]	P-Value		
	Control	Flax3	Flax6	Flax9		ANOVA	L[4]	Q[4]
Day 34								
LA[2]	20.76	22.89	21.97	22.42	1.954	0.331	0.207	0.191
ALA	2.11	2.61	3.79	2.88	0.429	0.158	0.084	0.142
ARA	0.456	0.446	0.388	0.270	0.049	0.584	0.453	0.269
EPA	0.016[b]	0.044[ab]	0.081[a]	0.027[b]	0.010	0.014	0.170	0.013
DPA	0.004	0.057	0.076	0.005	0.012	0.063	0.810	0.119
DHA	0.005	0.006	0.061	0.004	0.009	0.091	0.494	0.101
total SFA	36.34	31.15	29.72	40.48	2.530	0.469	0.288	0.314
total MUFA	45.94	42.23	43.48	40.30	1.483	0.658	0.300	0.936
total PUFA	23.60[b]	27.20[ab]	29.18[a]	29.39[a]	1.025	0.006	0.098	0.009
total n-3	2.12	3.42	4.43	4.17	0.440	0.143	0.196	0.322
total n-6	21.48	23.78	24.75	25.22	1.504	0.318	0.314	0.508
n-6/ n-3	10.13[a]	6.95[b]	5.59[c]	6.05[bc]	1.579	0.001	0.025	0.295
Day 45								
LA	25.82	25.13	25.83	21.35	1.277	0.613	0.322	0.501
ALA	2.77	3.76	5.18	4.90	0.378	0.059	0.157	0.297
ARA	0.445	0.483	0.406	0.340	0.035	0.574	0.260	0.496
EPA	0.014[b]	0.056[ab]	0.079[a]	0.066[ab]	0.009	0.042	0.078	0.017
DPA	0.033	0.030	0.026	0.059	0.012	0.810	0.547	0.509
DHA	0.027[b]	0.030[b]	0.042[a]	0.032[ab]	0.002	0.008	0.311	0.027
total SFA	35.73	32.74	32.91	29.44	1.261	0.418	0.132	0.926
total MUFA	34.57	37.25	35.04	43.37	1.978	0.417	0.205	0.492
total PUFA	28.18[b]	30.59[ab]	31.83[a]	32.44[a]	1.605	0.036	0.342	0.027
total n-3	2.49	3.61	4.24	4.43	0.394	0.074	0.196	0.322
total n-6	25.69	26.98	27.59	28.01	1.326	0.613	0.314	0.508
n-6/ n-3	10.32[a]	7.47[b]	6.51[c]	6.32[c]	1.182	0.007	0.295	0.025

[a-c] Values within a row with different letters differ significantly ($P < 0.05$).

[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.

[2] **LA**: linoleic acid ($C_{18:2(n-6)}$); **ALA**: α-linolenic acid ($C_{18:3(n-3)}$); **ARA**: arachidonic acid ($C_{20:4(n-6)}$); **EPA**: eicosapentaenoic acid ($C_{20:5(n-3)}$); **DPA**: docosapentaenoic acid ($C_{22:5\ (n-3)}$); **DHA**: docosahexaenoic acid ($C_{22:6(n-3)}$); **total SFA**: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; **total MUFA:** total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; **total PUFA**: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4\ (n-6)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-3**: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-6:** total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4\ (n-6)}$.

[3] *SEM*: standard errors of means (results are given as means (n = 6) for each treatment).

[4] L, linear; Q, quadratic.

Table 9. Effect of various levels of flaxseed on abdominal fat pad fatty acids in broilers

Fatty acid level in organ's fat (%)	Dietary treatments[1]				*SEM*[3]	P-Value		
	Control	Flax3	Flax6	Flax9		ANOVA	L[4]	Q[4]
Day 45								
LA[2]	21.54	21.24	24.87	23.29	1.298	0.792	0.506	0.829
ALA	1.12^{c}	3.77^{ab}	21.24^{a}	2.40^{bc}	0.484	0.002	0.062	0.007
ARA	0.258	0.143	0.161	0.187	0.019	0.163	0.235	0.070
EPA	0.026^{b}	0.035^{ab}	0.068^{a}	0.058^{ab}	0.006	0.039	0.334	0.014
DPA	0.019	0.025	0.058	0.037	0.006	0.118	0.101	0.232
DHA	0.054	0.048	0.057	0.049	0.005	0.416	0.082	0.062
total SFA	31.14	26.13	28.53	31.34	1.349	0.536	0.815	0.198
total MUFA	45.71	49.69	41.42	39.74	2.581	0.585	0.310	0.613
total PUFA	23.14^{b}	26.29^{ab}	29.52^{a}	27.29^{ab}	2.033	0.055	0.171	0.025
total n-3	1.23^{c}	4.65^{ab}	5.14^{a}	2.53^{bc}	0.524	0.002	0.089	0.003
total n-6	21.91	21.64	24.38	24.76	1.316	0.780	0.492	0.821
n-6/ n-3	17.81^{a}	4.65^{c}	4.74^{c}	9.79^{b}	1.585	0.001	0.071	0.001

[a-c] Values within a row with different letters differ significantly ($P < 0.05$).

[1] Treatments description as follows: control, corn-soybean meal-based diet without flaxseed; **Flax3**, control diet supplemented with 3% flaxseed; **Flax6**, control diet supplemented with 6% flaxseed; **Flax9**, control diet supplemented with 9% flaxseed.

[2]**LA**: linoleic acid ($C_{18:2(n-6)}$); **ALA**: α-linolenic acid ($C_{18:3(n-3)}$); **ARA**: arachidonic acid ($C_{20:4(n-6)}$); **EPA**: eicosapentaenoic acid ($C_{20:5(n-3)}$); **DPA**: docosapentaenoic acid ($C_{22:5\ (n-3)}$); **DHA**: docosahexaenoic acid ($C_{22:6(n-3)}$); **total SFA**: total saturated fatty acids includes C12:0, C14:0, C16:0, C17:0, and C18:0; **total MUFA:** total monounsaturated fatty acids includes $C_{16:1}$ and $C_{18:1(n-9)}$; **total PUFA**: total polyunsaturated fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, $C_{20:5(n-3)}$, $C_{22:4\ (n-6)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-3**: total omega-3 fatty acids includes $C_{18:3(n-3)}$, $C_{20:3\ (n-3)}$, $C_{20:5(n-3)}$, $C_{22:5\ (n-3)}$, and $C_{22:6(n-3)}$; **total n-6:** total omega-6 fatty acids includes $C_{18:2(n-6)}$, $C_{18:3(n-6)}$, $C_{20:3\ (n-6)}$, $C_{20:4(n-6)}$, and $C_{22:4\ (n-6)}$.

[3]*SEM*: standard errors of means (results are given as means (n = 6) for each treatment).

[4] L, linear; Q, quadratic.

Adding various levels of ground flaxseed had no effects on carcass yield and the relative weight of internal organs and muscles, while flax6 and flax9 decreased the relative weight of AFP. The results of previous studies demonstrated that the inclusion of flaxseed in the diet had no significant effects on carcass yields, meat portions, or the tissues of turkeys (Jankowski *et al*., 2015) which is in agreement with the results of the current study. It has been well-documented that dietary lipids may influence the amount of fat deposits in the body of birds (Hermier, 1997; Gonzalez-Ortiz *et al*., 2013). In addition, previous studies (Ferrini *et al*., 2008; Gonzalez-Ortiz *et al*., 2013) revealed that diets rich in PUFA, as compared to MUFA and SFA-rich diets, could lower fat deposition in AFP, which is in agreement with the present findings. Although there is no fully understood mechanism to describe the lowering effects of PUFA-rich diets on AFP, this result could be attributed to the lipid metabolism enzymes. It has been reported that PUFA increases oxygen consumption and reduces the rate of oxygen consumption relative to the rate of carbon dioxide production (Newman *et al*., 2002). In addition, a PUFA-rich diet may inhibit hepatic fatty acid synthase mRNA expression and enhance carnitine palmitoyltransferase I (CPT1) and 3-hydroxyacyl-CoA dehydrogenase (3HACD) mRNA expression in broiler chickens (Sanz *et al*., 2000). These findings confirm that PUFA may decrease AFP depots by activating β-oxidation by regulating different enzymes and pathways rather than by diminishing fatty acid biosynthesis (Ferrini *et al*., 2010).

Flax6 increased the accumulation of ALA in both breast and thigh muscles in a similar proportion, which is in agreement with previous findings (Cortinas *et al*., 2004; Jankowski *et al*., 2015). In addition, flax6 increased the concentrations of DHA in the breast muscle and EPA in the thigh muscle, which may be explained by a series of elongation and desaturation pathways in poultry that converted ALA to the longer chain EPA and DHA (Jankowski *et al*., 2015). The latter differences in dominant long-chain FA in the breast and thigh may be related to the different contents of phospholipid in these tissues or to the roles of FA in different tissues (Cortinas *et al*., 2004). Flax6 thus increased PUFA and total n-3 FA and also decreased the n-6/n-3 ratio in both breast and thigh muscles in broilers, which is the purpose of n-3 FA enrichment of poultry meat (Jankowski *et al*., 2015). In agreement with the present results, Jankowski *et al*. (2015) reported that ground flaxseed (10%) was included in the diet-enriched turkey meat with n-3 FA by enhancing PUFA content and reducing the n-6/n-3 ratio without any negative impact on the physicochemical attributes of the meat.

Higher concentrations of ALA, EPA, PUFA, and n-3 FA and lower n-6/n-3 ratio were observed in the AFP of birds fed flax6. In agreement with our current observations, Ferrini *et al*. (2008) reported that feeding broilers with 10% different PUFA sources could alter the profile of FA in the AFP and skin. The alteration of the skin FA profile to PUFA is not desirable due to PUFA sensitivity to oxidation, which may harm the tearing resistance of the skin.

The present results suggest that feeding 6% ground flaxseed (flax6) in the finisher period (d 25–45) can quadratically improve growth performance, reduce the relative weight of AFP, and enrich broiler meat with PUFA and n-3 FA without causing adverse effects on jejunal villi morphology. However, our results also showed that grinding could not prevent the harmful effects of a higher dose of flaxseed (9%), and this level impaired most measured parameters. Therefore, it would be more practical for the next step to apply processing methods together, such as grinding and adding enzymes or grinding and soaking with higher levels of flaxseed to overcome the adverse effects of anti-nutritional factors. Finally, when an experiment or even a commercial farm aims to fortify meat with PUFA and n-3 FA by adding oily seeds (like flaxseed) to a diet, the skin PUFA should be taken into consideration to prevent a decrease in breaking strength in the slaughterhouse.

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