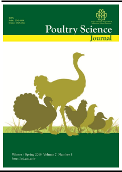

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Effect of Rolled or Extruded Flaxseeds in Finisher Diet on Pellet Quality, Performance, and n-3 Fatty Acids in Breast and Thigh Muscles of Broiler Chickens

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Abstract
An experiment was conducted to compare the effect of corn-soybean meal finisher (29-42d) diets containing flaxseeds (rolled or extruded) on pellet quality, performance, n-3 fatty acids (FA) and oxidative stability of meat in broiler chickens. Seven pelleted diets were provided in a 3×2 factorial arrangement with three flaxseed levels (5, 10, and 15%) and two processing methods (rolled or extruded) and a zero flaxseed diet (control). Birds fed diet containing 15% flaxseed had significantly lower weight gain and higher feed conversion ratio compared to those fed diets contained 0, 5 and/or 10% flaxseed. The type of flaxseed processing did not influence (P > 0.05) fatty acid profile and the MDA content in muscles. Chickens fed the control diet had relatively high levels of saturated and monounsaturated fatty acids and low levels of polyunsaturated fatty acids. Feeding diets contained flaxseed markedly reduced the levels of saturated fatty acids, monounsaturated fatty acids and increased polyunsaturated fatty acids, particularly the n-3 fatty acids in meat. It is concluded that the addition of 15% flaxseeds to finisher diet can increase n-3 fatty acids and lipid peroxidation in meat, while reducing growth performance of broiler chickens. However, feeding finisher diet containing 10% flaxseed compared to 5 or 15% flaxseed is of more practical to achieve an acceptable level of Omega-3 fatty acids in breast and thigh muscles without compromising the performance of broiler chickens.

Introduction

Flaxseed (*Linumusitatissimum* L.), is an oil seed that contains high levels of protein (22-26%), oil (41-43%) and NSP (Jia *et al*., 2009), and is an excellent source of n-3 fatty acids, particularly linolenic acid (52-58%) (Bhatty, 1993). Because of the positive effects in prevention and treatment of cardiovascular diseases, the n-3 fatty acids are of interest to both animal and human health (Cunnane *et al*., 1995) and this is why the poultry industry is trying to enrich the egg and meat with n-3 FA. Recent researches showed that diets containing flaxseed can modify the omega-3 fatty acid contents of meat in broiler chickens (Lopez-Ferrer *et al*., 2001), and enriched meat may serve as a source of omega-3 fatty acids for humans (Ajuyah *et al*., 1991, 1993). However, the addition of flaxseed in broiler diets, especially for young chickens is limited, because of poor growth performance (Ajuyah *et al*., 1991; Lee *et al*., 1991) and lowering nutrient consumption (Lee *et al*., 1991; Rodrıguez *et al.*, 2001) due to the presence of different anti nutritional factors including mucilage, linatine, cyanogenic glycosides, trypsin inhibitors and phytic acid (Madhusudhan *et al*., 1986; Bhatty, 1993). Mucilage in flaxseed is an important water-soluble NSP, which increases the digesta viscosity in broiler chickens (Rodriguez *et al*., 2001; Alzueta *et al*., 2003), and hence reduces the digestion and absorption of nutrients, particularly the fat (Smits *et al*., 1997). It is also well known that oil droplets in the cells of flaxseed are covered with non-digestible polysaccharides.

Although, chickens do not have endogenous enzymes for these NSP, the digestion of oil droplets by endogenous lipases is reduced and resulting lower energy utilization from flaxseed (Lee *et al*., 1995). In poultry, it is possible that flaxseed be processed to destroy its anti-nutritional substances. The

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extrusion technology is a controlled temperature and pressure conditions in which an ingredient or a mixture of feedstuffs can be extruded (Wu *et al.*, 2008).The extrusion process can reduce some anti-nutritional factors (more than 93%) and improve nutrient consumption in animals (Anjum *et al.*, 2013).Therefore, this study was conducted to investigate the effect of different levels of rolled or extruded flaxseeds in finisher diet on pellet quality, performance, n-3 fatty acids and MDA contents of thigh and breast muscles in broiler chickens.

Materials and Methods

Birds and Housing

A total number of 600 day-old male broiler chicks (Ross 308 strain) with an average weight of 41±2 g obtained from a local commercial hatchery, placed in a poultry research house and commercially raised for 28 days. Four hundred ninety of birds were randomly selected and divided into 49 groups of similar body weight and allocated to 49 floor pens of 10 birds each on d 28. Each pen (1 m^2) was equipped with a manual hanging feeder and two nipple drinkers and covered with a clean wood shaving. The ventilation rate of 0.12 m/s, house temperature of 20±2 °C and light:Dark program at the rate of 20:4 h were maintained during the whole experimental period (29-42d). All the experimental procedures and birds treatments were reviewed and approved by the Animal Care Committee at the Ferdowsi University of Mashhad (Mashhad, Iran).

Diets

Corn-soybean starter (0-10d), Grower (11-24d) and finisher (24-28d) diets without flaxseed were fed to all birds. A 1000 Kg flaxseed from a single batch was obtained from a commercial supplier and divided into two equal parts. One part was rolled with a roller mill and the second part was extruded with an extruder (Yemmak Co. Turkey) at a temperature of 155°C for 20 seconds. Seven finisher diets including a control (with zero) and six others with either rolled or extruded flaxseeds at the rate of 5, 10, and 15 percent flaxseed were formulated to meet or exceed nutrient requirements of finisher broiler chickens as recommended by Ross 308 management guide (Aviagen, 2014). The mixed diets were pelleted using 4-mm pellet die openings (Table 1).

Table 1. Composition of the finisher diets (fed from 29-42d old broiler chickens)

Ingredients (%)	Control	5% Flaxseed	10 %Flaxseed	15 % Flaxseed
Corn	63.50	61.18	58.86	56.55
Soybean meal	30.50	28.45	26.41	24.33
Flaxseed (Rolled/Extruded)	00.00	5.00	10.00	15.00
Soybean oil	2.65	2.05	1.41	0.79
Dicalcium phosphate	1.46	1.45	1.45	1.46
Limestone	0.90	0.86	0.85	0.83
Salt	0.36	0.35	0.34	0.34
Mineral-vitamin premix[a]	0.2	0.2	0.2	0.2
DL-Met	0.26	0.25	0.25	0.25
L-Lys	0.12	0.15	0.17	0.19
L-Thr	0.05	0.06	0.06	0.06
Total	100	100	100	100
Calculated nutrients				
Metabolisable energy (Kcal/kg)	3050	3050	3050	3050
Crude protein (%)	18.5	18.5	18.5	18.5
Crude fiber (%)	3.72	3.82	3.93	4.03
Crude fat (%)	5.24	6.22	7.20	8.18
Calcium (%)	0.75	0.75	0.75	0.75
Available phosphorus (%)	0.375	0.375	0.375	0.375
Sodium (%)	0.17	0.17	0.17	0.17
Methionine (%)	0.40	0.40	0.40	0.40
Methionine + cysteine (%)	0.76	0.76	0.76	0.76
Arginine (%)	1.04	1.04	1.04	1.04
Lysine (%)	0.97	0.97	0.97	0.97
Threonine (%)	0.65	0.65	0.65	0.65
Tryptophan (%)	0.15	0.15	0.15	0.15

Analyzed nutrients (%)	control	rolled	extruded	Rolled	extruded	rolled	extruded
Dry matter	88.8	88.7	88.8	88.8	88.6	88.3	88.8
Crude Protein	18.55	18.62	18.74	18.57	19.1	18.57	18.96
Crude fat	5.85	5.91	6.30	7.51	7.83	8.12	8.65
Ash	4.9	4,85	4.88	4.73	4.92	4.64	6.72

[a]Supplied per kilogram of diet: antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin, 35 mg; pyridoxine, 10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α- tocopheryl acetate, 60 mg; choline chloride, 638 mg; Co, 0.3 mg; Cu, 3.0 mg; Fe, 25 mg; I, 1 mg; Mn, 125 mg; Mo, 0.5 mg; Se, 200 mg; Zn, 60 mg.

Pellet Quality

Five replications of each pelleted diet were collected at the end of productions line to measure the pellet durability index (PDI) by a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) as described by Abdollahi *et al*. (2010).

Performance

Birds' performance including live weight on d42 and weight gain, feed intake, and feed conversion ratio were calculated during the 29 to 42 days of age. Feed per unit gain values were corrected for the mortalities occurred during the course of the experiment.

Viscosity

One bird from each replicate pen was randomly selected, weighed and killed by cervical dislocation on d42. The content of jejunum (from the end of the duodenum to Meckel's diverticulum) was collected, immediately 1.5 g of that centrifuged at 5443g for 10 min, and viscosity of the supernatant was determined at 40°C using the Brookfield digital viscometer (model DVII+ LV, Brookfield Engineering Laboratories, Stoughton, MA).

Meat Quality and Fatty Acids Analysis

The weight of carcass and cuts including thigh and breast of the same birds euthanized by cervical dislocation on d42 were weighed, percentages of carcass and cuts were calculated as a % of live weight and carcass weight, receptively. About 50g from each sample (whole meat from the left side of breast or thigh of each replicate) was weighed and stored in refrigerator for 24h (Betti *et al*., 2009b) to estimate the percentage of cooking and free water losses. The whole meat from the right side of breast or thigh of each replicate bird excised and minced 3 times to have a uniform mix. About 10g of minced thigh and/or breast meats were collected at random and kept at 4°C for 24 hours in order to determine the MDA content. About 4g of each thigh and breast minced sample was randomly collected and stored at –20 °C to determine fatty acids composition according to Chartrin *et al*. (2005).

Laboratory Analysis

Diets and minced breast and thigh meat moisture, fat, crude protein, and ash were determined according to the AOAC standard procedure (AOAC, 2005). The cooking loss of meat was determined by wrapping of a meat sample (50 g with a thickness of 1.5 cm) under vacuum in a plastic bag and cooked in a water bath for 60 min at 80°C. Then, the meat was dried and weighed following washing with cold water, (Honikel, 1998). In order to determine the percentage of free water in meat, based on the method described by Grau *et al*. (2001), and modified by Pohja and Niinivaara (1957), 0.3 g of ground meat was placed on weighed Whatman No.1 paper-filter and exposed to two kg pressure between two glass plates for 5 minutes. After this time, the meat sample was completely separated from the filter paper and the filter paper was weighed and recorded. The percentage of free water in meat was calculated by weight fraction of filter paper before and after pressure, divided by weight of meat.

The MDA as a second production of oxidation was measured by the Thiobarbituric acid (TBA) index as described by Cunnane *et al*. (1994). Shortly, one g of minced meat was transferred into the 25 ml test tube and was mixed with 2.5 mL of 0.8% BHT in hexane and 4 mL of TCA 5% in water, and vortexed for 30 seconds. The lower phase was then filtered and 1.4 mL of that was mixed with 0.7 mL of 5% TCA solution and 1.5 mL of 0.8% TBA in 1 mM NaOH. The resulting mixture was incubated for 30 min in the 70°C water bath and cooled in ice for 15 min, then 2 mL of butanol was added to extract the lipid oxidation compounds. After centrifugation, the butanol phase was read at 535 nm using a spectrophotometer (UV-visible S2100, Scinco, Korea) against a butanol blank. Absorbance values were converted in μg equivalent MDA per g of meat to a calibration curve using 1,1,3,3-tetramethoxypropane as MDA precursor. Finally, the amount of TBA as mg MDA in kg meat was reported (Agarwal and Chase, 2002).

The composition of fatty acids content of rolled and extruded flaxseed, experimental diets and the minced breast and thigh meats were determined using gas liquid chromatography (Unicam 4600, USA). Briefly, each minced sample was weighed into a test tube with solvent (chloroform: methanol=2:1, v:v) and homogenized. Ten mL solvent of chloroform: methanol was added to the homogenized solvent and kept for 24 h. The homogenate solvent was filtered into a graduated cylinder and 5 mL of 0.88% NaCl solution was added. Then, the filtrate was mixed well and kept for 12 h. The contents were kept until the aqueous and organic layers clearly separated. The upper layer was discarded and evaporated (Folch *et al*., 1957). The fatty acids of lipids were freed by saponification and then methylated by methanol. A gas chromatograph equipped with thermal sensor (Unicam 4600, USA) was used to separate and quantify the methyl esters of fatty acids. The concentration of fatty acids (mg g^{-1} sample) was determined by Nuchromprogram (Metcalf, 1966).

Statistical Analysis

The analysis of variance was performed using the GLM procedure of SAS software based on a 3 × 2 (three flaxseed levels 5, 10, and 15%) and (two processing methods rolled and extruded) factorial design (SAS, 2004). The significant difference between treatments were calculated by the LSD's test ($P < 0.05$). Orthogonal contrasts were used to compare the mean response variables of birds fed control vs extruded/rolled flaxseed diets. Moreover, the Linear and Quadratic contrasts were used to find the trend of changes in different parameters with increasing the level of rolled/extruded flaxseed in finisher diets.

Results

Pellet Quality

Effect of different levels of rolled and extruded flaxseed in diets on PDI) measured in various suspension times are shown in Table 2. PDI was affected by the different levels of flaxseed in diet. The percentage of highest PDI was observed when 10 and 15% of either rolled or extruded flaxseed was included in diet ($P < 0.05$). There was not a significant difference in PDI of diets containing either rolled or extruded flaxseed. However, the orthogonal contrasts, indicated that durability was linearly enhanced with increasing percentage of flaxseed in finisher corn-soy diet ($P < 0.05$).

Table 2. Effect of different levels of rolled and extruded flaxseed in diets on pelleted durability index (PDI).

Treatment	PDI (%)
Control (no flaxseed)	0.75^b
5% Rolled flaxseed	0.75^b
5% extrude flaxseed	0.75^b
10% Rolled flaxseed	0.77^a
10% extrude flaxseed	0.76^a
15% Rolled flaxseed	0.77^a
15% extrude flaxseed	0.77^a
SEM	0.191
Contrasts:	p-Value
Linear	0.001
Quadratic	0.011

The means with uncommon letter are significantly different ($P < 0.05$).

Table 3. Effect of flaxseed level and processing methods (rolled or extruded) in diet on the performance in broiler chickens during 29 to 42 days of age

Main effects	Body weight (g/bird)	Body weight gain (g/bird/d)	Feed intake (g/bird/d)	Feed conversion ratio (g/g)
Control (no flaxseed)	2484.70	91.53	167.48	1.82
Flaxseed levels (% of diet)				
5	2489.36^a	92.09^a	167.67	1.82^b
10	2489.06^a	91.52^a	168.73	1.84^b
15	2422.72^b	87.22^b	169.71	1.95^a
SEM	0.162	0.119	0.154	0.020
Flaxseed processing method				
Rolled	2464.71	90.33	168.40	1.86
Extruded	2467.38	90.23	169.00	1.87
SEM	0.133	0.970	0.126	0.020
Interaction (between level and processing method)				
5% × rolled	2482.42	91.60	166.28	1.81
5% × extrude	2496.28	92.59	169.07	1.82
10% × rolled	2496.71	92.62	170.35	1.84
10% × extrude	2475.39	90.43	167.10	1.84
15% × rolled	2415.00	86.78	168.57	1.94
15% ×extrude	2430.44	87.67	170.85	1.96
SEM	0.230	0.168	0.218	0.030
Effects		**p-Value**		
Flaxseed levels (%ration)	0.009	0.012	0.650	0.001
Flaxseed processing method	0.883	0.939	0.736	0.668
Level × processing method	0.668	0.566	0.319	0.998
Contrasts:				
Control vs 5% rolled	0.940	0.977	0.687	0.877
Control vs 5% extruded	0.705	0.638	0.593	0.951
Control vs 10% rolled	0.694	0.628	0.335	0.735
Control vs 10% extruded	0.761	0.621	0.898	0.601
Control vs 15% rolled	0.027	0.038	0.713	0.012
Control vs 15% extruded	0.081	0.089	0.259	0.004
Linear	0.014	0.017	0.488	0.001
Quadratic	0.035	0.045	0.453	0.074

The means of each column with uncommon letter are significantly different ($P < 0.05$)

Performance

The effect of level and processing methods (rolled or extruded) of flaxseed in finisher diet on the performance during 29 to 42 days of age are shown in Table 3. The processing method of flaxseed and its interaction with flaxseed level in finisher diet did not significantly (P > 0.05) affect performance criteria in broiler chickens. But flaxseed level in finisher diet significantly influenced live weight on d 42, weight gain and feed conversion ratio of broiler chickens during 29 to 42 of age. The inclusion of 5 or 10 % flaxseed in diet did not have a significant effect on live weight, daily weight gain, feed intake, and feed conversion ratio compared to those fed control diet (Table 3, orthogonal contrasts). However, the increment of flaxseed in finisher diet reduced final body weight, weight gain and increased FCR linearly, but did not affect feed intake. The comparison of control diet with diet containing different levels of flaxseed showed that only chickens fed finisher diet with 15% flaxseed had significantly lower body weight, weight gain and higher FCR than those fed control diet.

Carcass and Cuts Yield

The relative weight of carcass to live weight (≈ 69%) and the relative weight of breast (≈ 26.2%), thigh (≈ 19.2%), neck (≈ 7%), and wing (≈ 5.5%) to carcass weight were not influenced (P > 0.05) by the dietary level and processing method of flaxseed (detail of data analysis are not shown). The interaction between flaxseed level and processing method was not significant.

Meat Composition

The percentage of moisture, protein, fat, and ash in either breast or thigh muscles were not influenced by processing method and dietary level of flaxseed in finisher diet and were about 76, 22.3, 2.3, 1.5 and 77.5, 20.2, 4.5, 1.5, respectively (detail of data analysis are not shown).

Table 4: Effect of flaxseed level and processing method (rolled or extruded) on viscosity of jejunum contents, the MDA content of breast and thigh muscle measured during 29 to 42 d of age

Effects	Viscosity (Santi poise)	MDA (mg/kg meat)	
		breast	thigh
Control (no flaxseed)	1.41	0.116	0.188
The flaxseed levels (% diet)			
5	1.46^{b}	0.114	0.191^{b}
10	1.54^{b}	0.119	0.213^{a}
15	2.34^{a}	0.128	0.220^{a}
SEM	0.020	0.280	0.050
Flaxseed processing method			
Rolled	1.78	0.119	0.208
Extruded	1.78	0.122	0.208
SEM	0.010	0.080	0.040
Interaction (between level and processing method)			
5% × rolled	1.47	0.114	0.191
5% × extruded	1.46	0.115	0.192
10% × rolled	1.53	0.116	0.212
10% × extruded	1.52	0.122	0.213
15% × rolled	2.35	0.127	0.219
15% × extruded	2.34	0.130	0.220
SEM	0.030	0.140	0.070
Effects		p-Value	
Flaxseed levels (%ration)	0.001	0.620	0.002
Flaxseed processing method	0.880	0.749	0.905
Level - processing method	0.998	0.994	0.998
Contrasts			
Control vs 5% rolled	0.251	0.923	0.764
Control vs 5% extruded	0.269	0.969	0.752
Control vs 10% rolled	0.016	0.997	0.045
Control vs 10% extruded	0.021	0.773	0.054
Control vs 15% rolled	0.001	0.559	0.014
Control vs 15% extruded	0.001	0.503	0.014
Linear	0.001	0.050	0.035
Quadratic	0.004	0.269	0.675

[a,b]Means within each column for each effect with uncommon superscript letter are significantly different ($P < 0.05$).

Viscosity and MDA Concentration

Effect of flaxseed level and processing methods (rolled and extrude) in finisher diet on the viscosity of jejunum contents (Santi poise) and the MDA content of breast and thigh muscles measured at 42 day of age are shown in Table 4. Types of flaxseed processing and its interaction with inclusion level in finisher diet did not influence ($P > 0.05$) the jejunum content viscosity and the MDA in thigh and/or breast meats. Digesta viscosity in birds fed finisher diet containing 15% flaxseed was significantly higher than those fed diets containing 5 or 10% flaxseed. Compared to control diet, digesta viscosity was similar to birds fed finisher diet contained 5% flaxseed, but was significantly lower that those fed diets contained 10 and/or 15% flaxseed. The regression analysis revealed a positive linear relationship between dietary flaxseed level and digesta viscosity ($P < 0{\cdot}001$).

The flaxseed level in finisher diet significantly ($P < 0.05$) enhanced MDA content in thigh muscle when chickens fed finisher diets containing 10 or 15% flaxseed compared to those fed diets containing 0 or 5 % flaxseed. But, these changes in the MDA content was not observed in the breast muscle. The regression analysis showed a significant positive linear relationship between the level of flaxseed in diet and MDA content of both thigh and breast meats (Table 4).

Fatty Acids Composition of Diets and Meat

Fatty acid composition of rolled or extruded flaxseeds and the finisher diets are shown in Table 5. The major fatty acids in the rolled or extruded flaxseeds was Linolenic acid (C18:3), whereas, oleic acid (C18:1) was dominant in the basal diet. Addition of flaxseed noticeably increased the percentage of Linolenic acid (C18:3) and thus, increased the n-3 to n-6 PUFA ratio from 0.07 (in the control diet) to 0.48 in the diet contained 15% flaxseed. The extrusion and/or rolling of the flaxseed did not affect the dietary composition of fatty acids; thus, diets with the same rate of extruded or rolled flaxseed had similar fatty acid composition profiles.

Table 5. Fatty acid composition of rolled or extruded flaxseeds and finisher diets.

Fatty acid	Flaxseeds		Finisher diets						
	Rolled	Extruded	Control	Rolled 5%	Extruded 5 %	Rolled 10%	Extruded 10 %	Rolled 15%	Extrude d 15%
C14:0	0.14	0.15	1.04	1.03	1.04	1.02	1.00	0.94	0.93
C16:0	5.69	5.71	22.87	22.14	22.12	21.11	21.20	20.68	20.70
C16:1	0.29	0.28	8.45	8.56	8.65	8.56	8.58	8.45	8.48
C18:0	4.11	4.10	7.32	6.78	6.72	6.54	6.52	6.11	6.11
C18:1	22.6	22.7	46.60	47.25	47.20	47.16	47.14	46.86	46.85
C18:2	29.7	29.9	11.67	11.58	11.56	11.54	11.52	11.40	11.35
C18:3	37.1	37.2	0.86	2.22	2.34	4.10	4.04	5.50	5.48
C20:4	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
SFA	9.94	9.96	31.23	29.95	29.88	28.67	28.72	27.73	27.74
MUFA	22.89	22.98	55.05	55.81	55.85	55.72	55.72	55.31	55.33
PUFA	66.8	67.1	12.53	13.80	13.90	14.98	15.03	16.98	16.83
n-3	37.1	37.2	0.86	2.22	2.34	4.10	4.04	5.50	5.48
n-6	29.7	29.9	11.67	11.58	11.56	11.54	11.52	11.40	11.35
n-3/n-6	1.24	1.24	0.07	0.19	0.20	0.35	0.35	0.48	0.48

C18:1= Oleic acid; C18:2= Linoleic acid; C18:3= Linolenic acid; n-3= Omega-3 FA; n-6= Omega-6 FA; SFA= Saturated fatty acid; MUFA= Monounsaturated fatty acid; PUFA= Polyunsaturated fatty acid; n3/n6: the ratio of n-3 to n-6 PUFA. SFA=C14:0+C16:0+C18:0; MUFA=C16:1+C18:1; PUFA=C18:2+C18:3+C204 and Nd=not detected

The effect of flaxseed processing method (rolled or extruded) and flaxseed level in the finisher diet on fatty acid composition (%) in breast and thigh muscles measured at 42d of age are shown in Table 6 and 7, respectively. In general, the meat in chickens fed the non-added flaxseed control diet had quite high amount of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and low composition of polyunsaturated fatty acids (PUFA). Feeding diet containing rolled/extruded flaxseed markedly reduced the levels of SFA (C16:0 and C18:0 in thigh muscle) and MUFA (C16:1 in thigh muscle and C18:1 in breast muscle) and markedly increased the levels of PUFA, particularly of the linolenic acid in both thigh and breast muscles. The percentage of PUFA, n-3 and n-3/n-6 ratio in breast and thigh muscles were linearly increased with increasing the percentage of flaxseed in finisher diet. Processing methods of flaxseed did not have a significant effect on fatty acid composition of breast and/or thigh muscles (Tables 6 and 7).

Table 6. Effect of flaxseed level and processing method (rolled or extruded) in finisher diet on fatty acid composition (%) in breast muscles measured at 42d of age (mg g^{-1} of meat).

Main effects	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6
Control (no flaxseed)	0.20	2.50	1.02	1.02	5.57	1.91	0.14	0.13	3.72	6.59	2.18	0.14	2.04	0.06
Flaxseed levels (% of diet)														
5	0.18	2.49^{a}	1.02^{a}	0.94	5.48	1.86	0.13^{b}	0.20	3.61	6.50	2.52	0.13^{b}	2.07	0.06^{c}
10	0.16	2.42^{ab}	0.99^{ab}	0.96	5.19	1.87	0.22^{b}	0.20	3.54	6.03	2.30	0.22^{b}	2.07	0.10^{b}
15	0.15	2.36^{b}	0.84^{b}	0.93	4.32	1.91	0.33^{a}	0.28	3.44	5.32	2.20	0.33^{a}	2.19	0.15^{a}
SEM	0.010	0.015	0.020	0.011	0.051	0.014	0.015	0.026	0.019	0.052	0.029	0.003	0.027	0.010
Flaxseed processing method														
Rolled	0.17	2.42	1.00	0.94	5.23	1.85	0.22	0.23	3.52	6.25	2.38	0.22	2.15	0.10
Extruded	0.16	2.43	0.91	0.95	4.77	1.91	0.23	0.22	3.55	5.66	2.30	0.23	2.07	0.11
SEM	0.010	0.024	0.040	0.013	0.268	0.021	0.024	0.068	0.038	0.275	0.085	0.024	0.073	0.010
Effects					**p-Value**									
Flaxseed levels (%ration)	0.470	0.036	0.046	0.626	0.202	0.618	0.002	0.850	0.233	0.230	0.333	0.002	0.758	0.001
processing method	0.773	0.729	0.051	0.607	0.387	0.214	0.709	0.283	0.755	0.290	0.679	0.709	0.583	0.392
Level × processing method	0.978	0.775	0.59	0.482	0.380	0.439	0.990	0.090	0.990	0.457	0.362	0.990	0.278	0.684
Contrasts:														
Control vs 5% rolled	0.836	0.950	0.917	0.091	0.868	0.574	0.863	0.737	0.756	0.882	0.671	0.863	0.662	0.938
Control vs 5% extruded	0.680	0.757	1.00	0.164	0.962	0.402	0.182	0.300	0.651	0.658	0.401	0.182	0.527	0.207
Control vs 10% rolled	0.538	0.711	1.00	0.416	0.646	0.421	0.037	0.100	0.444	0.571	0.105	0.061	0.244	0.052
Control vs 10% extruded	0.414	0.075	0.002	0.164	0.672	0.484	0.051	0.701	0.831	0.963	0.549	0.057	0.502	0.075
Control vs 15% rolled	0.311	0.037	0.017	0.024	0.065	0.887	0.007	0.773	0.671	0.422	0.461	0.003	0.720	0.014
Control vs 15% extruded	0.311	0.044	0.030	0.022	0.030	0.331	0.002	0.321	0.492	0.033	0.222	0.007	0.968	0.001
Linear	0.326	0.617	0.415	0.124	0.071	0.667	0.001	0.280	0.067	0.067	0.008	0.001	0.093	0.49
Quadratic	0.870	1.00	0.364	1.00	0.069	0.199	0.141	0.089	0.933	0.564	0.490	0.647	0.240	0.113

[a…c] Means within each column for each effect with uncommon superscript letter are significantly different ($P < 0.05$).

Table 7. Effect of flaxseed level and processing method (rolled or extruded) in finisher diet on fatty acid composition (%) in thigh muscles measured at 42 d of age (mg g^{-1} of meat).

Main effects	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	SFA	MUFA	PUFA	n-3	n-6	n-3/n-6
Control (no flaxseed)	0.50	13.50	6.80	2.90	23.52	6.10	0.10	0.17	16.90	30.32	6.37	0.10	6.27	0.015
Flaxseed levels (% of diet)														
5	0.48	12.11^{a}	6.62^{a}	2.72^{a}	23.46^{a}	6.06^{a}	0.13^{c}	0.20	15.31^{a}	30.09^{a}	6.39^{b}	0.13^{c}	6.26^{a}	0.020^{c}
10	0.47	11.05^{b}	6.34^{b}	2.41^{b}	23.28^{b}	5.96^{b}	0.22^{b}	0.24	13.94^{b}	29.62^{b}	6.42^{ab}	0.22^{b}	6.19^{b}	0.035^{b}
15	0.44	10.72^{c}	6.13^{b}	2.24^{b}	23.26^{b}	5.88^{b}	0.33^{a}	0.25	13.41^{c}	29.39^{b}	6.46^{a}	0.33^{a}	6.13^{b}	0.053^{a}
SEM	0.012	0.037	0.024	0.024	0.017	0.015	0.014	0.010	0.045	0.028	0.021	0.023	0.015	0.008
Flaxseed processing method														
Rolled	0.46	11.29	6.34	2.44	23.30	5.95	0.23	0.24	14.24	29.64	6.11	0.91	6.19	0.14
Extruded	0.47	11.30	6.39	2.47	23.36	5.98	0.23	0.23	14.20	29.75	6.15	0.93	6.22	0.15
SEM	0.015	0.144	0.062	0.062	0.029	0.023	0.020	0.010	0.203	0.083	0.045	0.053	0.025	0.008
Effects					**p-Value**									
Flaxseed levels (%ration)	0.927	0.001	0.002	0.004	0.004	0.002	0.001	0.853	0.001	0.004	0.013	0.014	0.001	0.001
Flaxseed processing method	0.649	0.869	0.577	0.765	0.188	0.378	0.587	0.857	0.792	0.291	0.530	0.548	0.604	0.650
Level × processing method	0.966	0.806	0.959	0.996	0.627	0.969	0.974	0.774	0.967	0.807	0.984	0.886	0.961	0.842
Contrasts														
Control vs 5% rolled	0.764	0.045	0.061	0.051	0.329	0.085	0.051	0.802	0.061	0.001	0.078	0.001	0.162	0.001
Control vs 5% extruded	0.880	0.051	0.055	0.057	0.573	0.160	0.049	0.458	0.051	0.001	0.035	0.001	0.359	0.001
Control vs 10% rolled	0.653	0.023	0.002	0.025	0.007	0.006	0.001	0.618	0.001	0.001	0.005	0.001	0.024	0.001
Control vs 10% extruded	0.764	0.011	0.002	0.036	0.021	0.016	0.001	0.867	0.001	0.001	0.002	0.001	0.041	0.001
Control vs 15% rolled	0.456	0.005	0.001	0.001	0.001	0.001	0.001	0.326	0.001	0.001	0.007	0.001	0.008	0.001
Control vs 15% extruded	0.373	0.001	0.001	0.001	0.003	0.001	0.001	0.618	0.001	0.001	0.005	0.001	0.008	0.001
Linear	0.439	0.001	0.002	0.001	0.005	0.006	0.001	0.150	0.001	0.001	0.001	0.001	0.459	0.001
Quadratic	1.00	0.065	0.134	0.416	0.360	0.562	0.063	0.791	0.071	0.063	0.081	0.064	0.777	0.370

[a…c] Means within each column for each effect with uncommon superscript letter are significantly different ($P < 0.05$).

Discussion

The pellet quality is important in broiler performance and affected by the diet formulation (Abdollahi *et al*., 2010). Pellet quality in the present study was significantly improved as the percentage of flaxseed increased in diet. However, the performance characteristics were adversely depressed when chickens fed diet containing 15% flaxseed. Flaxseed is an ingredient with high amounts of soluble fiber (Jia *et al*., 2009), that may encapsulate feed particles and thereby improve pellet quality (Corzo and Loar, 2011). The mucilage of flaxseed makes a large portion of the soluble fiber in the seed. The presents study revealed that the viscosity of jejunum contents was much higher in chickens received the flaxseed diets compared to those fed the control diet. Similarly viscosity increased with the addition of flaxseed to diet as reported by Rodriguez *et al*., (2010).The mechanism that water soluble fiber (β-glucans, arabinoxylan and/or pectins) interfere with nutrient digestibility is by reducing the normal passage of digesta through the gastrointestinal tract and contact of the endogenous enzymes to their substrates (Longstaff and McNab, 1991; Bedford, 1995). In addition, it is shown that the increase in digesta viscosity in jejunum causes an increased in microbial proliferation in intestine and thus higher competition with the host animal for nutrients (Bedford, 1995; Choct *et al*., 1996). It was reported that the increased viscosity affects fat digestion more than that of other available nutrients because of the size of chylomicrons (Rodriguez *et al*., 2010). Thus, the lower efficiency of nutrient utilization produced by the inclusion of flaxseed in diets may be related to fat and fatty acids than for nitrogen and amino acids (Edney *et al*., 1989).

The decrease in performance with the inclusion of flaxseed at the rate of more than 100 g/kg in finisher diet in our study is inconsistent with previous reports (Lee *et al*., 1991; Ajuyah et al., 1993) in which the ground flaxseed was incorporated into a broiler diet from 1 to 42 d (Bond *et al*., 1997). As it is shown in our experiment, some reports revealed that the performance of finisher broilers was less affected by anti-nutrients in flaxseed (Bhatty, 1993) compared with the starter and grower broilers in other report (Rodriguez *et al*., 2010). The decrease observed in performance fed finisher diets containing 15% flaxseed may attribute to lower efficiency of feed utilization such as protein digestibility and energy utilization (Rodriguez *et al*., 2010). Nam *et al*. (1998) reported that the processing of raw flaxseed like with heat detoxifies anti-nutrients and thereby improves performance. Extrusion increased protein and energy digestibility in raw flaxseed and improved feed conversion compared with non-extruded flaxseed (Thacker *et al*., 1994). However, in our study the performance parameters were similar in chickens fed finisher diets containing of either extruded or rolled flaxseed.

Similar to the present study, in the report of Kamran Azad *et al*. (2009) the relative weight of breast and thigh yield were unaffected by the inclusion of different levels of flaxseed (7.5 or 15%) in the finisher diet. In contrast, compared with the control diet, adding 15 and 20% raw flaxseed to diet, reduced live weight, carcass weight, carcass and abdominal fat and increased leg yield (Ajuyah et al., 1991. 1993). The significant changes in carcass cuts produced by the experimental diets may be of economic and commercial importance to broiler producers (Ajuyah *et al*., 1991).

The present study revealed that the presence of 5 to 15% of either rolled or extruded flaxseed in finisher diets did not influence the moisture, protein, fat, and ash content in the breast and thigh muscles. It was demonstrated that 5 or 7.5% flaxseed (ground or whole seed) did not affect the total lipid content in thigh tissues (Roth-Maier *et al*., 1998). Other reports showed that the addition of either flaxseed oil (Olomu and Baracos, 1991) or fish oil (Hulan *et al*., 1984) to iso-caloric and iso-nitrogenous diets did not change the lipid content in breast or thigh muscles. Similar to our study, Mridula et al. (2011) showed that in broilers, the dietary addition of up to 15% flaxseed meal did not have a significant effect on chemical composition of breast and thigh muscles. However, Ajuyah *et al*. (1990) reported that including either flaxseed or flaxseed meal at the rate of 10 or 20% significantly reduced the lipid content in breast and thigh muscles. It is also reported by several investigators that the percentage of lipid in breast is less than that in thigh muscles, irrespective of dietary composition (Hulan *et al*., 1984; Ajuyah *et al*., 1990). In agreement with the present study, Ajuyah *et al*. (1991) showed heat treatment of flaxseed had non-significant effect on lipid content of breast and/or thigh muscles.

Due to increased demand for higher quality meat by consumers, meat functional properties including cooking loss, free water loss and ultimate pH after slaughter have become a major poultry industry concern. The cooking and free water losses were compromised by the time of feeding flaxseed (Betti *et al*., 2009b). Similar to the present study, the level of flaxseed fed to the birds (5 to 20%) did not have a significant effect on cooking and free water losses of breast and thigh muscles (Betti *et al*., 2009b). The ultimate of meat pH in broilers fed flaxseed was reduced and this was due to the negative effect of flaxseed nutrients on muscle metabolism (Baeza *et al*., 2015). The relationship between pH and meat functional properties in broiler meat (cooking and free water losses) was also reported by Fletcher (1999). However, there is no evidence about the effects of anti-nutritional factors on meat quality.

Oxidation of lipid in meat is a major cause of quality deterioration and low storage life of meat. The MDA has been selected as the oxidation marker. The MDA contents of thigh muscle were strongly affected by the level of flaxseed feeding, but not in the breast muscle. The MDA contents were almost two times higher in thigh than in breast muscle of chickens (Table 4), this is probably due to the high lipid content of thigh than breast muscles (4.5% vs 2.5%). It was shown that the addition of vegetable oil and fish oils to the diet in order to enrich meat with n-3 fatty acids caused a higher thigh MDA contents than that in breast muscle (Betti *et al*., 2009a). Oil seeds inclusion particularly polyunsaturated fatty acids sources in broiler diets decreased the oxidative stability of raw meat. This result may relate to the polyunsaturated fatty acid content that is very susceptible to oxidation. The type of flaxseed processing did not affect the MDA content in either breast or thigh muscles.

It is well known that the degree of unsaturation has a positive correlation to the oxidative stability of lipids in meat, and meat with similar concentration of linolenic acid is more vulnerable to oxidation than that with linoleic acid (Rymer and Givens, 2005). It is hypothesized that the influence of feeding flaxseed on meat quality is through the increase in the formation of reactive oxygen species (ROS) in muscles that have high n-3 fatty acids deposition. The ROS can be produced from the unsaturated fatty acids reaction with some elements such as iron (Morrissey *et al*., 1998; Zmijewski *et al*., 2005), and the higher concentration of Thiobarbituric Acid Reaction (TBARS) in our study revealed a higher sensitivity to oxidation in enriched thigh muscle.

In our study, the inclusion of flaxseed (rolled or extruded) in finisher diet increased the linolenic acid (C18:3) and n-3/n-6 ratio in muscles and also these increasing trends to continue with increasing the level of flaxseed from 5 to 15 % in finisher diet. This positive relationship of meat enrichment with the rate of flaxseed in diet is shown by other investigators (Gonzalez-Esquerra and Leeson, 2000; Shen *et al*., 2005). It is shown that the inclusion of flaxseed in diet would increases the concentration of linolenic acid in broiler chicken meat because dietary fatty acids can directly be incorporated into tissue lipids, and thus the fatty acids in muscles become similar to those in diets (Salmon and O'neil, 1973; Shen *et al*., 2005). As a result of higher concentrations of α-linolenic acid and lower concentrations of arachidonic acid in the lipid of the muscles, addition of oil seeds to the diet significantly ($P < 0.01$) increased the n-3/n-6 ratio in breast and thigh muscles. The α-linolenic acid values tend to be higher in thigh than in breast and this may be due to the higher triglyceride deposited in the thigh muscle (Hulan *et al*., 1988). However, in the present study it is showed that extrusion of flaxseed compared to rolling did not have a marked effect on linolenic acid of thigh and/or breast meat.

Conclusion

This study demonstrates that growth performance of broiler chickens could negatively be affected by the rolled/extruded flaxseed in finisher diet, while the n-3 fatty acids and lipid peroxidation increased. It is concluded that to minimize anti-nutritional effects of flaxseed on performance and intestinal viscosity, broilers may be fed a finisher diet with 10% flaxseed-rather than 5 or 15% to obtain an acceptable carcass weight, meat quality, n-3 fatty acids and MDA content in meat.

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