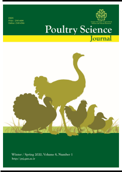

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Effects of One Week Feeding Finisher Diets Containing Rolled and Extruded Flaxseed on Performance, Lipid Peroxidation and Omega-3 Fatty Acids in Breast and Thigh Meat of Broiler Chickens

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Abstract

The purpose of this experiment was to investigate the effect of one-week feeding of flaxseeds (rolled/extruded) on performance, n-3 fatty acids and oxidative stability of meat in broiler chickens. Seven pelleted diets (36-42d) were provided in a 3×2 factorial arrangement with three flaxseed levels (5, 10, and 15%) and two processing methods (rolled/extruded) and a zero flaxseed control diet. Before the main trial, a total collection method experiment showed that the replacement of 10 and 15% of rolled/extruded flaxseeds in broiler diets caused a marked reduction in the apparent metabolizable energy (AME_n) as compared to those fed basal or 5% flaxseed replaced diets. In the main trial; weight gain was significantly reduced when flaxseed was supplemented at the level of 15%. Lipid peroxidation in thigh meat of birds fed diets contained zero or 5% flaxseed were lower than those fed diet with 10 or 15% flaxseed. The inclusion of flaxseed in the last week of feeding finisher diet linearly reduced the concentrations of saturated fatty acids and monounsaturated fatty acids, in the thigh and breast meat, respectively, whereas, the n-3 and n-3:n-6 ratio linearly increased in both thigh and breast meat. Processing methods of flaxseed did not affect the bird's performance and fatty acid profiles in meat. It is concluded that the replacement of diets with 5% flaxseed did not affect the AME_n of diet. Feeding diets containing flaxseed for one week before marketing can reduce saturated FA and increase n-3 and n-3: n-6 ratio in meat, but the adverse effect on performance appeared when diets contained 15% flaxseed.

Introduction

Poultry meat production has become very important in the last few decades (FAO, 2010). Broiler meat is a major source of high-quality proteins for humans. But, saturated and unsaturated fatty acid (FA), low content of n-3 FA and a poor n-3 to n-6 fatty acids ratio in poultry meat are undesirable to consumers (Delgado-Pando *et al.*, 2010). Fatty acid profile in broiler meat may be improved through diets rich in n-3 (Bou *et al.*, 2009). Flaxseed lipid contains about 73% polyunsaturated fatty acid content, 53% linolenic acid and may be used in poultry diet. Linolenic acid or n-3 FA has been reported to have a good effect on human health including a reduction in risks of atherosclerosis, inflammation and cardiovascular problems (Connor, 2000). On the other hand, flaxseed has several anti-nutritional substances which limit its inclusion in poultry diet. Alzueta *et al.* (2003) reported that flaxseed contains soluble fiber mucilage that increased intestinal digesta viscosity and reduced nutrient digestion. Processing is usually used to destroy the anti-nutritional factors in feedstuff. Maddock *et al* (2004) have been reported that rolled or ground of flaxseed were increased gain and gain efficiency compared to whole flaxseed in beef feedlot. The extrusion of flaxseed in controlled temperature and pressure conditions may have a beneficial effect to reduce solubility of soluble carbohydrates and improve the nutrients intake and digestion (Wu *et al.*, 2008; Anjum *et al.*, 2013) and thus improving weight gain

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and feed conversion ratio in broiler chickens. Recently, we found that feeding of finisher diets containing 5, 10, and 15 % of rolled or extruded flaxseed for two weeks increased the linolenic acid (C18:3) and n-3/n-6 ratio of muscles in broiler chickens (Zhaleh *et al*, 2019). Even though, the flaxseed is expensive compared to other plant protein feedstuffs, but its feeding for a shot term to broiler chickens may be economical. Therefore, we designed one more experiment to evaluate the inclusion level and processing methods of flaxseed in the last week of finisher diet on performance, meat quality and n-3 FA of thigh and breast meat in broiler chickens.

Materials and Methods

Birds and housing

A total number of 600, day-old male broiler chicks (Ross 308 strain) was purchased from a local commercial hatchery and raised commercially to 35 days of age before starting the experiment. Ninety-eight of them were selected randomly at 31 days of age to determining the energy values of diets while four hundred ninety of them were randomly divided into 49 groups of similar body weight and allocated to 49 floor pens of 10 birds each to measure the growth performance and meat quality. 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 of 20:4h were maintained during the experiment (36-42d). All the experimental procedures were approved by the Ferdowsi University of Mashhad Animal Care Committee, Mashhad, Iran.

Diets

Corn-soybean meal commercial starter (1-10 d), Grower (11-24 d) and finisher (25-35 d) diets without flaxseed were fed to all birds raised in a commercial house. 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 (85 °C, pressure 1.8 bar, 16-17% humidity for 20 seconds) and the second part was extruded with an extruder (155 °C, pressure 35.5 bar, 25-26% humidity for 20 seconds) (Yemmak Co. Turkey). Seven iso-caloric and iso-nitrogenous finisher diets including a control (with zero flaxseed) and six others with either rolled or extruded flaxseeds at the rate of 5, 10 and, 15 percent were formulated to meet all nutrient requirements of finisher broiler chickens as recommended by Ross 308 management guide (composition of diets are published in our previous study by Zhaleh *et al*. (2019). The mixed diets were pelleted using 4-mm pellet die openings.

Apparent metabolizable energy (AME) determination

A corn-soybean meal basal diet was formulated in mash form and was replaced with either rolled/extruded flaxseeds at the rate of 5, 10 and 15 % to provide seven diets. The AME_n value of these diets was determined by a total collection method (Ortiz *et al*, 2001). A total of 98 thirty-one day-old male broiler chickens (Ross 308) (with almost similar weights) were randomly assigned into 49 cages of two birds each. Each diet was randomly fed to 7 groups of birds. Chickens had free access to tests and basal diets for 3 days (31-33 d). Then, birds were starved of food for 12 h. Feed intake was measured and also excreta from each cage were collected for the last three days. The birds were also starved for the last 12 h of the experiment and then excreta were collected. All samples of diets and excreta were dried and analyzed for gross energy by applying the adiabatic bomb calorimeter (Shimatzu model, Japan) and nitrogen (Kjeldahl method, AOAC, 2000). The AME (Sibbald, 1989) and AME_n (Hill and Anderson, 1958) values of diets were calculated by following the equations:

$$AME = \frac{(F_i \times GE_f) - (E \times GE_e)}{F_i}$$

$$AME_n = \frac{[(F_i \times GE_f) - (E \times GE_e)] - (NR \times K)}{F_i}$$

$$\mathrm{NR} = (\mathrm{F_i} \times \mathrm{N_f}) - (\mathrm{E} \times \mathrm{N_e})$$

Where Fi is feed intake (g), GEf: gross energy of feed sample (kcal/kg), E: excreta (g), GEe: gross energy of excreta sample (kcal/kg)., NR: nitrogen retention (g), K: nitrogen retention corrected coefficient (8.37 kcal/ g for each g N). Nf: feed nitrogen (%), Ne: fecal nitrogen (%).

Performance

Bird performance including average body weight on 42d and weight gain, feed intake and feed conversion ratio were calculated for one week before marketing (36 to 42 days). Feed per unit gain values were corrected for the mortalities occurred during the course of the experiment.

Viscosity

One bird from each 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 and immediately 1.5 gr of that centrifuged at 9,000 g for 10 min. The 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

Sample preparation

The weight of carcass and cuts including thigh and breast of the same birds slaughtered on d 42 were weighed and the percentages of carcass and cuts were calculated as a % of live weight and carcass weight, respectively. The whole meat from the left side of the breast or thigh of each replicate bird excised and about 50 g from each sample was weighed and stored in refrigerator for 24h (Betti *et al*., 2009) to determine the percentage of cooking and free water losses. The whole meat from the right side of the breast or thigh of each replicate bird excised and minced 3 times to have a uniform mix. About 10g sample of minced thigh or breast muscles were collected at random and kept at 4 ºC for 24 hours to determine the MDA content. About 4 g of each thigh and breast minced sample was randomly collected and stored at –20 ºC to determine fatty acids composition according to Chatrin *et al*. (2005).

Laboratory analysis

Minced breast and thigh muscles moisture, crude fat, crude protein, and ash were determined according to the AOAC standard procedures (AOAC, 2000). The cooking loss of meat was determined by wrapping of meat (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). To determine the percentage of free water in meat, according to the method of Grau *et al*. (2001), as modified by Pohja and Niinivaara (1957), 0.3g of ground meat (weighed accurately to 0.001g) placed on weighed Whatman No.1 paper-filter, and exposed to 2 kg pressure between 2 glass plates for a period of five 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 the weight fraction of filter paper before and after pressure, divided by the weight of the meat.

The MDA as a second production of oxidation was measured by the Thiobarbituric acid (TBA) index as described by Botsoglou *et al*. (1994). Briefly, one g of minced meat was transferred into a 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 NaOH 1 mM. 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-tetra methoxy propane as MDA precursor. The standard curve equation was y= 132287x +18751 and R_2= 0.999. 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 /extruded flaxseeds of the minced breast and thigh muscles were determined using gas-liquid chromatography (Unicam 4600, USA). Briefly, 4 g of minced sample was weighed into a test tube with 5 mL solvent (chloroform: methanol=2:1, vol.vol.) 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 separated. The upper layer was discarded and evaporated and the organic layer remained (Folch *et al*., 1957). The fatty acids of lipids were freed by saponification (NaOH) and then methylated by methanol. The methyl esters of fatty acids were separated and quantified by gas chromatograph equipped with a thermal sensor (Unicam 4600, USA). The relative concentration of fatty acids was calculated by Nuchrom program automatically (Metcalfe *et al*., 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 treatment means were calculated by the LSD's test ($P < 0.05$). Orthogonal contrasts were used to compare mean response variables in control vs rolled/extruded. Moreover, the Linear and Quadratic contrasts were used to find the trend of changes in different measured responses in birds to the increasing level of flaxseed in finisher diets.

Results

Apparent metabolizable energy (AME)

The effect of replacing various levels of flaxseed (rolled/extruded) in basal diet on AME_n of diets is shown in Table 1. The AME_n values in diets was significantly decreased as the rate of flaxseed replacement in the basal diet was increased except 5% replacement ($P < 0.05$). Also, the replacement of extruded flaxseed in basal diet numerically improved AME_n values of diet as compared to that of rolled flaxseed ($P > 0.05$).

Table 1. Effect of replacing various levels of flaxseed and processing methods (rolled/ extruded) in basal diet on AME_n of diets (Kcal/kg)

		AME_n (Kcal/kg) of diets
Control (no flaxseed)		3003.9
The flaxseed levels (% diet)		
5		2991.9[a]
10		2878.2[b]
15		2496.4[c]
SEM		0.172
Flaxseed processing method		
rolled		2758.8[b]
extruded		2818.9[a]
SEM		0.166
		P-value
	Flaxseed levels (% diet)	0.035
	Flaxseed processing method	0.044
	Level × processing method	0.063
Contrasts:		
	Control vs rolled 5%	0.178
	Control vs extruded 5%	0.133
	Control vs rolled 10%	0.040
	Control vs extruded 10%	0.038
	Control vs rolled 15%	0.021
	Control vs extruded 15%	0.020
	Linear	0.033
	Quadratic	0.088

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

Performance

The effect of level and processing methods (rolled/ extruded) of flaxseed in the diet on live weight at 42 d, average daily gain, feed intake and feed conversion ratio during 36 to 42 days of age are shown in Table 2. Birds fed diet contained 5 or 10% flaxseed had similar total weight and weight gain compared to the control group but both criteria were significantly reduced when flaxseed was supplemented at the rate of 15%. The processing method of flaxseed (rolled/extruded) did not affect the bird's performance. The interaction effect of the level and processing method of flaxseed was not significant for weight gain. The increment of flaxseed in the last week of feeding finisher diet linearly reduced final body weight. The level, processing method and their interaction on feed intake were not significant. Feed conversion ratio was significantly higher in birds fed diet contained 15% flaxseed compared to those in control or other treatments. But there was not a significant difference between FCR of birds fed finisher diet contained 5 or 10% flaxseed compared to those fed control diet. Processing methods of flaxseed and the interaction of the level and processing method did not have a significant effect on FCR.

Carcass and cuts yield

There was not a significant difference between the relative weight of carcass (≈69.23; P=0.860), breast (≈25.98; P=0.946), thigh (≈18.71; P=0.648), neck (≈6.85; P=0.119), and wings (≈5.80; P=0.922) of birds fed finisher diet for one week with different level of flaxseed compared to those fed control diet. The level of processing method of flaxseed or their interaction did not affect the relative weight of carcass, breast, thigh, neck, and wings (Data are not shown).

Meat composition

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

Cooking loss and water loss

There was not a significant difference between cooking losses of breast (%21.58; P= 0.982) and thigh muscles (%27.23; P= 0.270) and water losses of breast (%27.22; P= 0.596) and thigh muscles (%32.64; P= 0.953) of birds fed with different level of flaxseed compared to those fed control diet. Processing method and the interaction effect of level × processing method of flaxseed did not have a significant effect on cooking losses of breast (P=0.954; P=0.950, respectively) and thigh (P=0.269; P=0.638, respectively) muscles and water losses of breast (P=0.871; P=0.991, respectively) and thigh (P=0.343; P=0.738, respectively) muscles. (Data are not shown).

Table 2. Effect of flaxseed level and processing methods (rolled/ extruded) on body weight at 42 d and daily weight gain, feed intake and feed conversion ratio in feeding diets for one week (36-42d) in broiler chickens

	Body weight	weight gain	Feed intake	FCR
	g	g/b/d	g/ b/d	
Control (no flaxseed)	2447.44	109.14	193.73	1.78
The flaxseed levels (% diet)				
5	2458.07[a]	111.15[a]	195.61	1.76[b]
10	2453.15[a]	109.13[a]	194.30	1.78[b]
15	2390.54[b]	99.95[b]	187.33	1.89[a]
SEM	0.155	0.191	0.170	0.03
Flaxseed processing method				
rolled	2437.44	107.36	192.96	1.80
extruded	2430.40	106.13	191.86	1.82
SEM	0.176	0.137	0.159	0.02
	P-value			
Flaxseed levels (% diet)	0.045	0.023	0.155	0.025
Flaxseed processing method	0.770	0.718	0.766	0.697
Level × processing method	0.23	0.164	0.136	0.345
Contrasts:				
Control vs rolled 5%	0.432	0.359	0.619	0.379
Control vs extruded 5%	0.789	0.847	0.902	0.772
Control vs rolled 10%	0.602	0.466	0.536	0.553
Control vs extruded 10%	0.424	0.467	0.421	0.669
Control vs rolled 15%	0.319	0.250	0.797	0.145
Control vs extruded 15%	0.078	0.054	0.069	0.052
Linear	0.035	0.011	0.081	0.010
Quadratic	0.287	0.332	0.484	0.339

The means of each column with an uncommon letter are significantly different ($P < 0.05$). Bird's means body weight was 1680 g at 35 days of age.

Table 3. Effect of flaxseed level and processing method (rolled/ extruded) on the jejunal digesta viscosity, the MDA content of breast and thigh muscles (mg/kg meat) in feeding diets for one week (36-42d) in broiler chickens

		MDA	
	Viscosity	Thigh	Breast
Control (no flaxseed)	1.41	0.188	0.116
The flaxseed levels (% diet)			
5	1.46[b]	0.186[b]	0.109
10	1.50[b]	0.191[a]	0.112
15	2.33[a]	0.210[a]	0.119
SEM	0.02	0.02	0.13
Flaxseed processing method			
rolled	1.75	0.195	0.112
extruded	1.77	0.197	0.114
SEM	0.01	0.01	0.10
	P-value		
Flaxseed levels (%diet)	0.001	0.046	0.879
Flaxseed processing method	0.599	0.901	0.896
Level × processing method	0.998	0.997	0.998
Contrasts:			
Control vs rolled 5%	0.348	0.908	0.940
Control vs extruded 5%	0.493	0.996	0.798
Control vs rolled 10%	0.042	0.052	0.946
Control vs extruded 10%	0.046	0.054	0.821
Control vs rolled 15%	0.001	0.042	0.862
Control vs extruded 15%	0.001	0.041	0.862
Linear	0.001	0.045	0.224
Quadratic	0.001	0.916	0.668

The means of each column for every effect with uncommon letters are significantly different ($P < 0.05$).

Table 4. Effect of flaxseed level and processing method (rolled/ extruded) on fatty acid composition (%) in breast muscles measured in feeding diets for one week (36-42d) in broiler chickens (mg/ g of meat)

Main effects	C14	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)	0.19	2.45	1.02	1.05	5.56	1.92	0.15	0.12	3.69	6.58	2.19	0.15	2.04	0.07
Flaxseed levels (%diet)														
5	0.19	2.42	1.01	0.92	5.46	1.87	0.16[b]	0.19[b]	3.53	6.47	2.22[b]	0.16[b]	2.06	0.07[b]
10	0.17	2.40	1.05	0.95	5.35	1.88	0.20[b]	0.20[b]	3.52	6.40	2.28[b]	0.20[b]	2.08	0.09[b]
15	0.16	2.37	1.02	0.89	4.95	1.90	0.36[a]	0.30[a]	3.42	5.97	2.56[a]	0.36[a]	2.20	0.16[a]
SEM	0.01	0.012	0.02	0.012	0.030	0.014	0.02	0.004	0.017	0.031	0.023	0.02	0.014	0.014
Flaxseed processing method														
Rolled	0.17	2.29	1.02	0.93	5.21	1.68	0.93	0.053	3.39	6.23	2.67	0.93	1.73	0.53
Extruded	0.17	2.31	1.03	0.94	5.26	1.69	0.95	0.060	3.43	6.29	2.71	0.95	1.75	0.54
SEM	0.006	0.008	0.013	0.008	0.020	0.009	0.013	0.002	0.011	0.020	0.015	0.013	0.009	0.009
							P-value							
Flaxseed levels (%diet)	0.076	0.505	0.923	0.29	0.258	0.780	0.001	0.043	0.192	0.330	0.007	0.001	0.775	0.001
processing method	0.098	0.561	0.919	0.531	0.790	0.835	0.606	0.182	0.566	0.770	0.652	0.606	0.753	0.560
Level × processing method	0.917	0.769	0.989	0.697	0.986	0.790	0.898	0.618	0.985	0.976	0.969	0.898	0.859	0.637
Contrasts:														
Control vs 5% rolled	0.537	0.433	0.990	0.278	0.195	0.133	0.088	0.095	0.110	0.228	0.068	0.088	0.273	0.085
Control vs 5% extruded	0.226	0.528	0.703	0.221	0.261	0.343	0.080	0.085	0.486	0.100	0.077	0.080	0.577	0.096
Control vs 10% rolled	0.226	0.277	0.949	0.195	0.055	0.240	0.078	0.052	0.245	0.322	0.059	0.078	0.437	0.071
Control vs 10% extruded	0.756	0.349	0.898	0.188	0.059	0.288	0.085	0.072	0.183	0.255	0.081	0.085	0.822	0.085
Control vs 15% rolled	0.358	0.874	0.750	0.014	0.016	0.474	0.001	0.001	0.041	0.047	0.001	0.001	0.577	0.001
Control vs 15% extruded	0.226	0.433	0.848	0.024	0.028	0.288	0.001	0.002	0.024	0.025	0.001	0.003	0.655	0.001
Linear	0.058	0.899	0.928	0.367	0.109	0.612	0.003	0.023	0.319	0.069	0.030	0.001	0.637	0.001
Quadratic	0.265	0.226	0.679	0.051	0.823	0.922	0.954	0.626	0.696	0.753	0.894	0.954	0.858	0.626

The means of each column for every effect with uncommon letters are significantly different ($P < 0.05$).

Table 5. Effect of flaxseed level and processing method (rolled/ extruded) on fatty acid composition (%) in thigh muscle measure in feeding diets for one week (36-42d) in broiler chickens (mg/ g of meat)

Main effects	C14	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)	0.49	13.51	6.81	2.89	23.49	6.12	0.09	0.19	16.89	30.30	6.40	0.09	6.31	0.014
Flaxseed levels (%diet)														
5	0.48	12.15^{a}	6.62^{a}	2.74^{a}	23.45	6.08^{a}	0.12^{b}	0.20	15.10^{a}	30.07^{a}	6.39	0.12^{b}	6.28^{a}	0.019^{b}
10	0.46	11.07^{a}	6.30^{b}	2.59^{a}	23.30	5.96^{a}	0.19^{b}	0.22	14.12^{ab}	29.60^{b}	6.37	0.19^{b}	6.18^{a}	0.030^{b}
15	0.40	10.32^{b}	6.12^{c}	2.21^{c}	23.23	5.86^{b}	0.30^{a}	0.27	12.93^{b}	29.35^{b}	6.43	0.30^{a}	6.13^{b}	0.048^{a}
SEM	0.008	0.074	0.073	0.022	0.017	0.017	0.016	0.009	0.045	0.037	0.021	0.022	0.035	0.013
Flaxseed processing method														
Rolled	0.44	10.33	6.34	2.42	23.29	5.92	0.21	0.24	13.20	29.64	6.38	0.21	6.17	0.03
Extruded	0.45	11.36	6.35	2.41	23.32	5.96	0.24	0.26	14.23	29.67	6.46	0.24	6.22	0.04
SEM	0.005	0.049	0.048	0.014	0.011	0.011	0.010	0.006	0.030	0.025	0.014	0.014	0.023	0.009
								P-value						
Flaxseed levels (%diet)	0.235	0.039	0.001	0.003	0.183	0.001	0.004	0.326	0.044	0.001	0.862	0.004	0.027	0.003
processing method	0.195	0.330	0.789	0.858	0.703	0.429	0.526	0.237	0.346	0.739	0.376	0.526	0.345	0.541
Level × processing method	0.367	0.431	0.612	0.711	0.960	0.603	0.917	0.672	0.442	0.933	0.799	0.917	0.743	0.924
Contrasts:														
Control vs 5% rolled	0.616	0.406	0.354	0.291	0.167	0.241	0.168	0.954	0.299	0.061	0.163	0.168	0.354	0.074
Control vs 5% extruded	0.548	0.437	0.450	0.256	0.156	0.203	0.190	0.213	0.300	0.061	0.240	0.190	0.497	0.096
Control vs 10% rolled	0.146	0.153	0.256	0.152	0.051	0.083	0.098	0.345	0.076	0.081	0.097	0.098	0.151	0.085
Control vs 10% extruded	0.372	0.160	0.242	0.135	0.058	0.096	0.089	0.356	0.084	0.091	0.147	0.089	0.145	0.062
Control vs 15% rolled	0.008	0.083	0.041	0.021	0.007	0.003	0.017	0.213	0.041	0.001	0.047	0.017	0.051	0.024
Control vs 15% extruded	0.015	0.067	0.035	0.035	0.003	0.003	0.025	0.184	0.046	0.001	0.025	0.025	0.050	0.038
Linear	0.067	0.040	0.001	0.001	0.071	0.034	0.001	0.153	0.001	0.041	0.071	0.001	0.023	0.025
Quadratic	0.032	0.762	0.172	0.608	0.969	0.158	0.653	0.712	0.051	0.725	0.063	0.653	0.315	0.351

The means of each column for every effect with uncommon letters are significantly different ($P < 0.05$).

Viscosity and MDA concentration

Effect of flaxseed level and processing methods (rolled and extrude) in the last week of finisher diet on the viscosity of jejunum contents (Santi poise) measured at 42 days of age are shown in Table 3. The jejunal digesta viscosity of birds fed a finisher diet containing 15% flaxseed was significantly higher than those fed diet contained zero, 5 or 10% flaxseed. The processing method of flaxseed did not significantly affect jejunal digesta viscosity. The interaction effect of level × processing methods of flaxseed in the diet on jejunal digesta viscosity was not significant. There was a positive linear relationship between dietary flaxseed levels and digesta viscosity ($P < 0{\cdot}001$). The effect of level and processing method (rolled/ extruded) of flaxseed in finisher diet on the MDA content of breast and thigh muscles measured at 42 d of age are shown in Table 3. TBA index in the meat of birds fed diet contained 10 or 15% flaxseed were significantly higher than those fed diets contained zero or 5% flaxseed. The processing method of flaxseed did not affect lipid peroxidation. The interaction effect of the level × processing method was not significant for the TBA index.

Fatty acids composition in breast and thigh muscles

The effect of flaxseed level and processing method (rolled/ extruded) in the finisher diet on fatty acid composition (%) in breast and thigh muscles measured at 42 d of age are shown in Tables 4 and 5, respectively. Birds fed the control diet had relatively high levels of saturated (SFA) and monounsaturated fatty acids (MUFA) and relatively low levels of PUFA. The percentage of n-3 and n-3/n-6 ratio in breast and thigh muscles was significantly higher in birds fed diet contained 15% flaxseed than other treatments. Feeding diets contained flaxseed (rolled or extruded) significantly reduced the levels of SFA (C16:0 and C18:0 in thigh muscle) and MUFA (C16:1 in thigh muscle) and significantly increased the levels of PUFA, particularly of the linolenic acid (18:3) in both thigh and breast muscles. Processing methods did not have any effect on the fatty acid composition of muscles. The 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, whereas the percentage of PUFA, was linearly increased in thigh muscle, but on change in breast muscle was observed (Tables 4 and 5).

Discussion

The AME_n values of diets were decreased as the rate of rolled and extruded flaxseeds replacement increase in diet except 5 % replacement. Inconsistent with this finding, Ortiz *et al.* (2001) demonstrated that the highest of AME_n value of diet was obtained when the replacement of linseed was minimal. Also, it was found that AME_n of diet and digesta viscosity values were considerably decreased and increased, respectively followed by each increment up to 16 % linseed replacement in broiler diets (Rodriguez *et al*, 2001). The current study indicated that the replacement of flaxseed in corn-soy basal diet resulted in a significant decrease in AME_n which was explained that the increase in flaxseed replacement in the diet may increase the amount of mucilage and anti-nutrients (including linatine and cyanogenic glycosides) in the lumen (Conn, 1969; Klosterman, 1974; Madhusudhan *et al*, 1986; Bhatty, 1995). These results documented that rolled and/or extruded flaxseeds have adverse effects on dietary components and reduced dietary AME_n for broiler chickens.

In the present study, Feeding a finisher diet containing 15% flaxseed compared to those fed diets contained up to 10% flaxseed significantly reduced growth performance during 36-42 days of age. Body weight, weight gain, and FCR of birds fed diets containing 5 or 10% flaxseed were similar to those fed control diet for one week before marketing. Similarly, Zhaleh *et al*, (2019) reported that body weight, weight gain, and FCR did not change as comparedto those fed control diet for two weeks before marketing the broiler chickens. However, the linear relationship in both cases revealed that a gradual decrease in weight gain and an increase in FCR of chickens occurred regardless of feeding for either one or two weeks before marketing. The feed intake in both cases (present trial vs. Zhaleh *et al* 2019) was not changed as the level of flaxseed (rolled/extruded) increased up to 15% in diet

In contrast, Mridula *et al*. (2015) in a 42d trial observed that with increasing levels of flaxseed in diet from 2.5% to 5, 7.5, and 10% decreased weight gain of broiler chickens at 42d. They also reported that broilers fed the control and 2.5% flaxseed diets had significantly higher weight gain compared to those fed diets containing 5, 7.5, and 10% flaxseed. Lee *et al*. (1991) in 42 days reported that broilers fed diet contained 15% flaxseed had less body weight (4.9-6.7 %) than those fed diet supplemented with canola (10%). Less weight gain and poor FCR were observed in birds fed diet contained flaxseed compared to that fed canola or extruded full fat soybean-based diet were observed by Shen *et al.* (2005). Rahimi *et al.* (2011) also observed the better weight gain and FCR with the control diets than diet contained flaxseed (7.5–15%). Most of the studies have shown that flaxseed supplemented diet caused a reduction in the growth performance of broiler chickens and this decline will increase with increment of flaxseed levels. The negative effect of flaxseed on performance may be due to the existence of antinutritional factors such as mucilage, cyanogenic glycosides and trypsin inhibitors (Alzueta *et al.*, 2003). Linatin in mucilage by decreasing the amount of endogenous enzymes released from the pancreas reduces the digestibility of diet (Klosterman *et al*., 1967). Also, the non-starch polysaccharides in mucilage enhance intestinal viscosity and cause a decline in nutrient availability (Chotinsky, 2015) and thus, adversely affect the productivity in poultry. In this study, the processing method of flaxseed (rolled/extruded) did not have any effect on growth performance.

Similarly, Alzueta *et al*. (2003) reported that there was not a significant difference between birds in terms of feed intake when diets were supplemented with flaxseed compared to control diet. Inconsistent result was reported by Gonzalez-Esquerra and Leeson (2000) who observed higher feed intake in birds fed flaxseed supplemented diet (10%) with different levels of fish oil compared to those fed control diet. Probably, these results may be due to a reduction in apparent metabolizable energy (AME) and compensation on increased feed consumption. Zuidhof *et al*. (2009) and Anjum *et al*. (2013) reported that the bird's feed intake increased with an increase in the level of flaxseed. Hayat *et al*. (2009) observed that feed intake was lower in birds fed diet contained flaxseed than those fed control diet. This reduction is likely due to antinutritional factors of flaxseed which have a negative effect on palatability. Due to the effect of oil and antinutritional factors of flaxseed (even after the extrusion process) on feed intake, various results that reported on the effect of flaxseed on feed intake may be due to the difference in the amount of oil-containing, antinutritional factors in flaxseed and different experimental diet.

FCR, the ratio between weight gain/feed intake, represents the efficiency of birds to convert feed into body weight. In our study, FCR increased with increasing in the flaxseed levels in diet. Maximum FCR related to the level of 15% of flaxseed (rolled/extruded) which was likely due to a reduction in bird's weight gain in this group. Similarly, Nguyen *et al*. (2003) reported that a birds fed diet contained a high level of flaxseed had high FCR which was likely due to low digestibility and high digesta viscosity in the jejunum.

Different levels of flaxseed feeding to birds (rolled/extruded) did not have a significant effect on the relative weight of carcass and internal organs (breast, thigh, neck, and wing) compared to control diet. Similarly, Arshami *et al*. (2010) did not observe significant results for the percent of breast weight between flaxseed treatment (5-10%) and the control group. Pekel *et al*. (2009) reported none significant effect of flaxseed supplementation on carcass and breast weight. The addition of Flaxseed into the diet of broiler chickens did not affect meat quality factors including meat cooking loss or water holding capacity. Similar to our results, different levels of flaxseed in diet did not influence the protein, fat and ash content of thigh meat (Mridula *et al*., 2015). Roth-Maier *et al*. (1998) observed none significant effect of 5 or 7.5% flaxseed in the diet (ground/ whole seed) on total lipid content in thigh meat samples. Olomu and Baracos (1991) reported that the lipid content in either breast or thigh did not alter by adding up to 4.5% flaxseed oil. Similarly, when 16% flaxseed was substituted for corn, soybean meal and sunflower oil in the control diet, the jejunal digesta viscosity was increased for more than 70-fold compared to birds fed the control diet.

In this study, broilers fed diets supplemented with 5, 10, and 15 % rolled/ extruded flaxseeds for one week before marketing showed a significantly increased in jejunal digesta viscosity and MDA content of thigh muscle which are inconsistent with our previous study when birds fed the same diets for two weeks (Zhaleh *et al*, 2019). Also, the use of linseed with no mucilage instead of regular linseed caused a large decrease in the digesta viscosity, but still significantly higher than control diet because of its residual mucilage content (13 g mucilage/kg) (Alzueta *et al*., 2003). The mucilage, a water-soluble polysaccharide in the hull of flaxseed, is composed of an acidic fraction and galacturonic acid (Fedeniuk and Biliaderis, 1994). The flaxseed mucilage caused high water-holding capacity and viscosity. Fengler and Marquardt (1988) reported that viscosities more than 250 SP reduce the rate of diffusion of solutes and the movement of digesta through the digestive tract. These results may support the hypothesis that the increase in digesta viscosity associated with linseed is the main factor in lowering the broiler chickens performance.

Lipid peroxidation is the most important factor in meat quality and the TBA index is a practical method to determine the amount of peroxidation. In our results, the TBA index in birds fed the diet supplemented with 5% flaxseed or control diet were lower than those fed diet contained 10 or 15% of flaxseed. Similarly, Anjum *et al*. (2013) reported that the control group had lower TBA value compared with the birds fed diet contained 15% extruded flaxseed. The TBA index increased with increasing levels of extruded flaxseed in the diet (Betti *et al*., 2009). The high concentration of polyunsaturated fatty acids in broiler meat caused high susceptibility to oxidative reactions (Luna *et al*., 2010). Similar results were reported by Betti *et al*. (2009) who showed that the TBA index increased in birds fed a higher level of flaxseed.

The processing method of the flaxseed did not affect the fatty acids composition of diets; thus, at every level of flaxseed, the fatty acid composition was similar for the diets containing either rolled or extruded flaxseed (data are published in our previous study by Zhaleh *et al*, 2019). In the current study, the inclusion of flaxseed at the level of

15% (rolled or extruded) in the finisher diet increased the linolenic acid (C18:3) and n-3/n-6 ratio in muscles. Similar to our finding, the n-3 content increased significantly in the meat when the level of flaxseed was increased in the finisher diet except diet containing 2.5% flaxseed (Mridula *et al*., 2015). The composition of fatty acids in muscles depends on the dosage and duration of feeding flaxseed diet (Ajuyah *et al*., 1993; Shen *et al*., 2005; Betti *et al*., 2009; Rahimi *et al*., 2011). It was expected that the feeding of flaxseed would elevate the level of linolenic acid in meat because it is clear that dietary fatty acids are incorporated into tissue lipids, and hence the tissue fatty acids reflect those of the diets (Shen *et al*., 2005). Our previous investigation documented that, inclusion of 10 and 15 % flaxseed in broiler diets could increase n-3 FA concentrations of breast and thigh muscles in comparison to those fed control diet for two weeks before marketing (Zhaleh *et al*, 2019). As a result of higher concentrations of α-linolenic acid (18:3) and lower concentrations of Arachidonic acid in the lipid of the muscles, the addition of oilseeds to the diet increases the n-3/n-6 ratio in muscles. The α-linolenic acid (18:3) deposition values tend to be higher in the thigh than in the breast. These results could be explained by the higher triglyceride fraction in the thigh in which α-linolenic acid (18:3) is normally deposited (Hulan *et al*., 1984). It is shown that an increase in α-linolenic acid, omega 3, and n-3/n-6 ratio in breast and/or thigh muscles can be reached by increasing the inclusion rate of flaxseed in the diet up to 15 % when birds are fed for one or two weeks before marketing (current study vs Zhaleh *et al*. 2019). The absolute values for α-linolenic acid, omega 3, and n-3/n-6 ratio in dark or white muscles of chickens fed either control diets or diets containing each level of flaxseed were similar in both experiments (current study vs Zhaleh *et al*. 2019). In the present study, it is showed that extruded compared to rolled flaxseed did not have a significant effect on any of the fatty acids in thigh and/or breast meat.

Conclusion

Our results revealed that feeding a finisher diet containing 10% flaxseed for one week before the marketing age did not have a negative effect on weight gain, digesta viscosity and lipid peroxidation in meat. Although increased flaxseed to 15% in finisher diet increased digesta viscosity and caused a decline in broiler chickens performance, but n-3 fatty acids in tissues were further improved. So, it seems that the cost-benefit value of using flaxseed in the finisher diet fed for one week depends on the goal of the producer. In conclusion, our research demonstrated that broilers feeding by rolled or extruded flaxseed in pelleted diets had similar effects on performance and fatty acids profiles of muscles in the last week of the experimental period.

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