



Effect of Flaxseed Supplementation of Feed on Growth, Carcass Yield, Meat and Fatty Acids Profile of Rabbit Carcass

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ABSTRACT

The aim of this study was to quantify the effect of two concentrations and technological forms of flaxseed supplementation on growth performance, carcass yield and meat quality of rabbits. Sixty indigenous rabbits were divided randomly into five treatment groups. The control group was fed on standard concentrate basal diet, whereas in the treated groups, the basal diet was supplemented with low (3.5%; LCF) or high (7%; HCF) level of crushed flaxseed and low (1.5%; LFO) or high (3%; HFO) level of flaxseed oil until slaughtering (day 90). Carcass fat deposition was greater ($P < 0.05$) in the supplemented groups as compared to the control group. The contents of myristic acid and palmitic acid reduced ($P < 0.05$), while those of linoleic acid and linolenic acid increased ($P < 0.05$) in the carcass of all supplemented groups. Moreover, the supplemented groups had lower ($P < 0.05$) $n-6:n-3$ ratio than the control group. Among the supplemented groups, HFO supplemented group had higher ($P < 0.05$) contents of total polyunsaturated FA and lower ($P < 0.05$) $n-6:n-3$ ratio. This study provides the first dataset on carcass yield and quality, physicochemical characteristics, and FA profile of indigenous rabbits and shows that supplementation of flaxseed favorably modulates the FAs composition of rabbit meat, with no negative effect on their growth performance and meat physicochemical quality.

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Authors' Contribution

SK and NAK conceived and planned the study. KK and NA performed the experimental work and analyzed the data. KK wrote the manuscript and NAK polished it for publication.

Key words

Rabbit, Flaxseed, Fatty acids, Meat.

INTRODUCTION

Overall, Asia accounts for 48.8% of global rabbit meat production, and most of the rabbit meat is produced under small scale production systems, such as in Pakistan (FAOSTAT, 2012). The Northern areas of Pakistan hosts the largest rabbit population of the country, and these rabbits are reared under small-holder subsistence production system for meat, livelihood and as pet animals (Khan *et al.*, 2014). Rabbit production is still a new farming enterprise, and over the past decade, it has rapidly grown from family consumption to medium and large scale commercial production. Recent studies show that indigenous rabbits can produce meat more efficiently in the intensive farming system with improved feeding and management (Khan *et al.*, 2017a, b). With proper feeding rabbit meat can present excellent dietetic and nutritional properties (Hernandez *et al.*, 2008), such as lower cholesterol contents and a higher linolenic acid (C18:3 $n-3$) contents (Arino *et al.*, 2007), which can benefit long term human health by reducing the risks of obesity,

cardiovascular diseases and type-2 diabetes (Hu and Willett, 2002). This background provides an impetus to fully exploit the potential of rabbit for quality meat production.

Research in the past two decades has shown that next to fat content, the nutritional quality and healthiness of meat is mainly influenced by its fatty acids (FA) profile (Kanatt *et al.*, 2006). There is a growing consumer preference for animal products that are enriched with beneficial unsaturated FAs. Next to water and protein, fats are the major component of meat. The fat content of meat is highly variable in terms of quantity and quality. The fat content is mostly influenced by the genetics, age and nutrition of the animal, whereas the FA profile is mainly influenced by the diet of the animal (Hernandez *et al.*, 2008). The meat researchers aimed to produce meat with lower fat content having lower content of saturated FA (SFA) and higher content of unsaturated FA (UFA) to benefit long term human health. Like other monogastric animals, rabbits are able to incorporate dietary FAs into adipose tissue and intramuscular fats, thus making it possible to modulate the FAs profile of rabbits through the strategic use of unsaturated dietary fat sources such as flaxseed.

Flaxseed (*Linum usitatissimum* L.), also known as

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linseed, is receiving growing attention from the feed industry due to its oil content, which is about 40% on a dry matter (DM) basis (Peiretti *et al.*, 2007; Khan *et al.*, 2015a). Moreover, the oil contains a high proportion of linolenic acid (C18:3n-3; Khan *et al.*, 2014), and recent research indicates that rabbits receiving flaxseed enriched diets produces meat with a high proportion of mega-3 FAs (Kouba *et al.*, 2008). This has led to a renewed interest in feeding of flaxseed to livestock to favorably modulate the FAs composition of milk and meat. However, to the author's knowledge, no systematic research has been conducted to determine the effect of flaxseed supplementation on the FA profile of rabbit meat. Therefore, the present study was designed to quantify the growth performance, carcass yield, physicochemical quality and FA profile of rabbit meat in Northern Pakistan, and further evaluated the effect of supplementing low (3.5%; LCF) or high (7%; HCF) concentrations of crushed flaxseed, and low (1.5%; LFO) or high (7%; HFO) concentrations of flaxseed oil on the FA profile of rabbit meat.

MATERIALS AND METHODS

Rabbit selection and experimental design

In the present research study, a total of sixty local rabbits (58 ± 3 days old; weight, 565 ± 5.1 g) were selected from the rabbit herd at the Rabbit Research Center of the University of Agriculture Peshawar. The experimental rabbits were divided into five dietary groups according to randomized complete block design. The blocks were balanced for age and body weight. Each dietary group consisted of 12 rabbits. Five iso-nitrogenous and iso-caloric concentrates were formulated (Table I). The control diet contain commercial concentrate as a basal diet, whereas in the treated groups low (3.5%; LCF) or higher (7%; HCF) levels of crushed flaxseed, and low (1.5%; LFO) or high (3%; HFO) levels of flaxseed oil were included in the control diet, as a replacement of yellow corn and soybean meal. The basal diet was formulated according to NRC (1977) and was converted into a pellet form at the feed processing unit of Agriculture University Peshawar. The ingredient composition of the basal diet is given in Table I. The experimental trial continued for 5 weeks including a week of the adaptation period at the Rabbit Research Center of the University of Agriculture Peshawar, and the experimental procedure was approved by the ethical committee of the University for Procedures involving the care, live rabbit handling, welfare, and standard laboratory protocols.

Data collection

Body weight was recorded weekly and the average

daily gain was calculated. Daily feed intake and feed conversion ratio were computed by measuring individual feed intake and weight. At 90 days of age, all the rabbits were slaughtered by cutting the carotid arteries and jugular veins. The carcass procedure was carried out with the standard procedure of the World Rabbit Science Association as reported by Khan *et al.* (2016). The pH was measured 24 h postmortem in triplicate on samples taken from right hind legs with a digital pH meter (JENWAY 3505, UK). Meat physical quality was determined on meat samples from left hind legs according to standard procedure for percentage of released water (Grau and Fleischmann, 1957), and cooking loss (Boccard *et al.*, 1981). Chemical composition was measured on a meat sample from fore-legs according to the standard method of the Association of Official and Analytical Chemist (1990).

Table I.- Ingredients (%) composition of the experimental diets.

Ingredients	Control	Treated groups ¹			
		LCF	HCF	LFO	HFO
Alfalfa hay	27	28	30	30	34
Yellow corn	23.5	20	9.4	20	10
Barley grain	5.4	5.4	7.5	5.4	8.5
Wheat bran	27	27	33	27	31.4
Soybean meal	15	14	11	14	11
Crushed Flaxseed	0	3.5	7	0	0
Flaxseed oil	0	0	0	1.5	3
Di-Ca-Phosphate	1	1	1	1	1
DL-Methionine	0.1	0.1	0.1	0.1	0.1
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Vit and Min. Premix ²	0.5	0.5	0.5	0.5	0.5

¹LCF, low (3.5%) or HCF, high (7%) concentration of crushed flaxseed. LFO, low (1.5%) or HFO, high (3%) concentration of flaxseed oil.

²Premix provided per kg of diet: Biotin, 33 mg; Choline chloride, 200 g; Co, 16.6 mg; Fe, 12.5 g; Folic acid, 0.83g; Mg, 66.7 g; Mn, 5 g; Pantothenic acid, 3.33 g; Se, 16.6 mg; Vit. A, 2000 IU; Vit. D, 150 IU; Vit. E, 8.33 g; Vit. K, 0.33 g; Vit. B1, 0.33 g; Vit. B2, 1.0 g; Vit. B6, 0.33g; Vit. B5, 8.33 g; Vit. B12, 1.7 mg; Zn, 11.7 g.

Chemical analysis

The sample of each diet was ground at 1-mm particle size and analyzed for the contents of DM (method 930.15), ether extract (EE, method 920.39), crude protein (CP; method 984.13; using a Kjeltac™ 2400 auto analyzer; Foss Analytical A/S, Hillerød, Denmark) and acid detergent fiber (ADF; method 973.18) according to the standard procedures of AOAC (1990). The NDF content was determined according to the procedure of Van Soest *et al.* (1991), with some modification in the use sodium sulphite and heat-stable α -amylase for the correction of residual starch and protein as described by Habib *et al.* (2016).

Table II.- Chemical and fatty acid (g per 100 g of all acids determined) composition of experimental diets.

Chemical Analysis ²	Control	Treated groups ¹ (%)			
		LCF	HCF	LFO	HFO
Dry matter	89.3	90.1	90.2	90.3	90.5
Crude protein	15.3	15.5	15.6	15.7	15.8
Ether extract	3.34	4.32	5.34	5.40	5.51
Neutral detergent fiber	34.2	35.6	36.7	36.5	36.6
Acid detergent fiber	16.8	17.6	17.9	18.6	18.9
Fatty acid, (g/100 g)					
C14:0	0.53	0.37	0.35	0.25	0.18
C16:1	0.22	0.56	0.63	0.63	0.76
C16:0	16.3	12.5	12.3	12.5	11.6
C18:0	2.43	2.30	2.23	2.14	2.00
C18:1c	20.4	27.5	28.5	30.0	30.5
C18:1n9T	0.32	0.54	0.61	0.62	0.67
C20:0	0.57	0.46	0.47	0.47	0.44
C22:0	0.49	0.41	0.41	0.41	0.32
C22:1	0.12	0.31	0.32	0.31	0.32
C18:2n6	32.6	34.2	34.8	35.6	36.5
C20:5n3	0.00	0.57	0.62	0.54	0.67
C18:3n3	6.75	15.0	15.3	15.2	15.6
C22:6n3	0.00	1.04	1.05	1.03	1.06
ΣSFA ³	20.3	16.0	15.8	15.8	14.5
ΣMUFA ⁴	21.1	28.9	30.1	31.6	32.2
ΣPUFA ⁵	39.4	50.8	51.8	52.4	53.8

See Table I. ²Calculated according to NRC (1977). C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1c, vaccenic acid; C18:1n-9, oleic acid; C18:2n6, linoleic acid; C18:3n-3, linolenic acid; C20:0, arachidic acid; C22:0, behenic acid; C22:1, Erucic acid; C20:5n3, eicosapentaenoic acid; C22:6n3, docosahexaenoic acid. ³Saturated fatty acid. ⁴Mono unsaturated fatty acid. ⁵Polyunsaturated fatty acid.

Fatty acids analysis

For FA analysis of diets and meat (*longissimus dorsi* muscles), lipids from freeze dried, grounded samples were extracted with chloroform-methanol (2:1 v/v; Folch *et al.* (1957) with slight modification as described by Khan *et al.* (2011). After extraction, FAs in the residual fat were esterified, using acid and base catalyzed methods as described by Khan *et al.* (2015b). Fatty acid methyl esters (FAMES) analysis was performed by gas chromatography–mass spectrometry (GC–MS; Shimadzu-QP 2010 plus, Japan) equipped with electron impact (EI) detector. Separations of FAs were carried out on capillary column TRB-FFAP (30m × 0.32 mm × 0.25 μm) using Helium as carrier gas. Column temperature was held at 50°C for 1 min, and then the temperature was raised up to 150°C at the rate of 15°C per min. Then the temperature was

increased to 175°C at the rate of 2.50°C and hold for 5 min and finally increased to 220°C at the rate of 2.50°C per min and kept for 5 min. The peaks were identified by 37 components of the FAME standard mix (S37, Supelco, Bellefonte, PA, USA) accompanied by MS library.

Statistical analysis

The effect of diets on the performance, carcass yield, dressing percentage, meat physicochemical quality and FA profile of rabbit meat was determined using PROC MIXED Procedure of the Statistical Analysis System (SAS, SAS Institute Inc., Cary, NC)

$$Y_{ij} = \mu + D_i + \epsilon_{ij}$$

Where, Y_{ij} is the response of the dietary treatments, μ is the population mean, F_i is the fixed effect of diets (i = control, LCF, HCF, LFO and HFO diets) and ϵ_{ij} is the random error.

When significant effects were observed post-hoc analysis were carried out using Tukey-Kramer test to computed pair-wise differences in the treatment means.

RESULTS

Data on the chemical composition and FA contents of experimental diets are summarized in Table II. The ether extract content was higher in diets supplemented with flaxseed as compared to the control group. The flaxseed supplementation to the basal diet reduced the contents of myristic acid (C14:0), palmitic (C16:0) and total SFA, and increased the contents of total UFA, C18:3n-3 and total polyunsaturated FA (PUFA).

Table III.- Effect of concentration and technological form of flaxseed supplementation on the growth performance and carcass yield of indigenous rabbits.

	Control	Diets ¹				SEM ²	Sig [*]
		LCF	HCF	LFO	HFO		
Final weight(g)	1205	1207	1214	1205	1210	1.57	ns
AWG (g/d)	18.2	18.4	18.6	18.3	18.5	0.08	ns
Feed intake (g/d)	99.9	100.2	101.3	99.2	100.9	0.964	ns
FCR	5.47	5.45	5.43	5.42	5.44	0.057	ns
C. wt (g)	659	663	669	661	666	3.57	ns
Dressing (%)	54.5	54.6	54.9	54.5	54.7	0.34	ns
Liver (g)	55.2	54.5	56.3	57.1	56.4	2.37	ns
Kidney (g)	8.98	9.23	9.28	9.49	9.20	0.31	ns
Heart (g)	4.20	4.34	4.21	4.30	4.24	0.06	ns
Lungs (g)	9.12	9.20	8.99	9.10	9.34	0.04	ns

See Table I. ²Standard error of the mean. *P > 0.05. FCR, feed conversion ratio; AWG, average weight gain; C. wt, carcass weight; Sig, significance.

Data on the effect of type and concentration of flaxseed

supplementation on the growth performance, carcass yield and organ weight are summarized in [Table III](#). Live body weight, feed consumption and feed conversion efficiency did not differ ($P > 0.05$) due to different concentrations of flaxseed supplementation. Similarly, carcass weight, dressing percentage and organ weight did not differ due to dietary treatments. [Table IV](#) summarizes data on physicochemical characteristics of rabbits as affected by the type and concentration of flaxseed supplementation. The meat physical quality such as pH, percentage of water release and cooking losses did not differ ($P > 0.05$) due to flaxseed supplementation. The contents of moisture, crude protein and ash also did not differ; however, the contents of fat were higher ($P < 0.05$) in the supplemented groups.

Table IV.- Effect of concentrations and technological form of flaxseed supplementation on the physico-chemical quality of indigenous rabbits.

	Control	Diets ¹				SEM ²	Sig [*]
		LCF	HCF	LFO	HFO		
Chemical quality (Fore legs, %)							
Moisture	70.6	71.5	71.7	70.9	71.6	0.747	ns
Protein	21.4	21.7	20.9	21.1	20.7	0.366	ns
Fat	7.32 ^b	8.32 ^a	8.26 ^a	8.25 ^a	8.25 ^a	0.184	**
Ash	1.33	1.32	1.31	1.30	1.29	0.018	ns
Physical quality (hind legs)							
pH	5.65	5.68	5.67	5.63	5.61	0.213	ns
Release water (%)	15.9	16.1	15.6	15.4	15.8	0.457	ns
Cooking loss (%)	34.8	35.6	36.1	35.4	35.1	0.923	ns

^{1,2} see [Table III](#).

Data on the effect of the concentration and type of flaxseed supplementation on meat FA profiles of indigenous rabbits, as affected by the technological form and concentration of supplementation, are presented in [Table V](#). Inclusion of flaxseed in the diet reduced ($P < 0.05$) the contents of C14:0, C16:0, stearic acid (C18:0) and total SFA as compared to the control group. The contents of monounsaturated FAs such as myristoleic acid (C14:1c), palmitoleic acid C16:1c and oleic acid (C18:1c) were higher ($P < 0.05$) in the supplemented groups. Moreover, the contents of C18:2n-6, C18:3n-3 and total PUFA increased ($P < 0.05$) with the supplementation of flaxseed in the diet. The ratio of *n-6:n-3* FAs decreased in the supplemented groups as compared to control. Among the supplemented group the lowest ($P < 0.05$) concentration

of C14:0, C16:0, C18:0 and total SFA was recorded with the diets containing higher levels of flaxseed or flaxseed oil. Notably, rabbits supplemented with higher levels of flaxseed oil had higher ($P < 0.05$) contents of C18:2n-6, C18:3n-3 and total PUFA and lower ($P < 0.05$) *n-6:n-3* ratio as compared to other supplemented groups.

Table V.- Effect of concentrations and forms of flaxseed supplementation on the meat fatty acid profile (% of total methyl esters) of rabbit meat.

Fatty acids	Control	Diets ¹				SEM ²	Sig ³
		LCF	HCF	LFO	HFO		
C14:0	3.42 ^a	2.99 ^b	2.62 ^c	2.79 ^b	2.60 ^c	0.081	**
C16:0	22.0 ^a	17.7 ^b	14.9 ^c	14.7 ^c	13.8 ^c	0.464	**
C18:0	12.2 ^a	7.36 ^b	5.29 ^c	6.00 ^b	5.93 ^c	0.297	**
C20:0	1.52 ^a	2.44 ^a	5.36 ^b	7.05 ^b	2.10 ^a	0.881	*
C22:0	0.24 ^a	0.15 ^b	0.15 ^b	0.14 ^b	0.16 ^b	0.008	*
C14:1c	1.38 ^c	2.34 ^b	2.46 ^{ab}	2.90 ^a	2.95 ^a	0.087	**
C16:1c	2.66 ^b	2.38 ^c	2.90 ^{ab}	2.35 ^c	3.36 ^a	0.038	*
C18:1c	15.2 ^c	19.5 ^b	21.9 ^a	19.9 ^b	21.0 ^{ab}	0.329	***
C18:1n9T	1.23 ^b	1.75 ^a	1.56 ^{ab}	1.44 ^{ab}	1.41 ^{ab}	0.078	≠
C22:1	0.10 ^b	0.52 ^a	0.58 ^a	0.45 ^{ab}	0.32 ^{ab}	0.058	*
C18:2 n6	22.5 ^a	19.2 ^b	21.4 ^b	20.9 ^b	21.8 ^a	0.276	**
C20:5n3	0.94 ^c	1.09 ^b	1.13 ^b	1.11 ^b	1.33 ^a	0.020	***
C18:3n3	2.95 ^d	7.91 ^c	13.5 ^b	9.45 ^c	15.4 ^a	0.296	***
C20:4n6	3.05 ^a	2.91 ^{ab}	2.99 ^a	2.61 ^b	2.99 ^a	0.089	*
C20:3n6	0.23 ^c	0.26 ^b	0.34 ^a	0.21 ^c	0.33 ^a	0.005	***
C22:6n3	0.07 ^c	1.15 ^b	1.40 ^a	1.23 ^b	1.58 ^a	0.095	*
SFA ⁴	39.4 ^a	30.6 ^b	28.3 ^c	30.7 ^b	24.6 ^c	0.499	**
MUFA ⁵	20.6 ^b	26.5 ^a	29.4 ^a	27.0 ^a	29.1 ^a	1.044	*
PUFA ⁶	29.7 ^c	32.5 ^d	40.8 ^b	35.5 ^c	43.4 ^a	0.126	***
<i>n-6: n-3</i>	6.51 ^a	2.20 ^b	1.54 ^c	2.01 ^b	1.37 ^c	0.056	**

See [Table II](#), $P < 0.1$; *, $P < 0.05$, ** $P < 0.001$; *** $P < 0.0001$. ⁴Saturated fatty acid = C14:0 + C16:0 + C18:0 + C20:0 + C22:0. ⁵Mono unsaturated fatty acid = C14:1c + C16:1c + C18:1c + C18:1n9T + C22:1. ⁶Polyunsaturated fatty acid = C18:2 n6 + C20:5n3 + C18:3n3 + C20:4n6 + C20:3n6 + C22:6n3. *n-6: n-3* = (C18:2 n6 + C20:4n6 + C20:3n6) / (C20:5n3 + C18:3n3 + C22:6n3).

DISCUSSION

Dietary supplementation of unsaturated fat can increase the content of UFAs with a concomitant decrease in SFA in animal products, and render it more beneficial to human health. More interest has been given by meat researchers to enhance the contents of PUFA, particularly the *n-3* FAs in meat. The present study reports the first comprehensive data set on the FAs profile of indigenous rabbits in Northern Pakistan, and the transfer efficiency of dietary PUFA into rabbit meat. The database can be used to devise management and feeding strategies for the rabbits.

The results showed that there was an excellent relationship between the content and composition of dietary PUFA with that of the *longissimus dorsi* muscle in rabbits.

In agreement with earlier findings (Trebusak *et al.*, 2014), dietary supplementation of flaxseed did not significantly alter the diet intake, growth, carcass weight and composition of rabbits. Except fat, the contents of all other nutrients in the carcass were not altered by the supplementation of flaxseed. In agreement with our findings, Bianchi *et al.* (2006) found higher total fat deposition in the muscles with flaxseed supplementation. The cost incurred per kg of diet in PKR was highest (36.2) for HCF, followed by HFO (34.5), LCF (32.0), LFO (31.2), and control group (26.6), respectively. However, consumers pay premium prices for omega-3 enriched eggs, and it is expected that high consumer prices may be possible for omega-3 enriched meat and milk in the future.

Research has established that changes in the lipid composition of animal diet can alter the content and composition of meat fat (Bourre, 2004). In the present study, the PUFA concentrations in the *longissimus dorsi* muscles were increased by dietary inclusion of crushed flaxseed or flaxseed oil. On the other hand, the content of SFA decreased by feeding diets enriched with crushed flaxseed or flaxseed oil. Our results are consistent with earlier findings of Trebusak *et al.* (2011), who observed a higher PUFA and lower SFA contents with a more favorable *n-6*: *n-3* FAs ratio in rabbit meat, with supplementation of flaxseed or sunflower in the basal diet. These findings highlight that inclusion of flaxseed in rabbit diets can improve the nutrition value of rabbit meat. Similarly, Gelibolu *et al.* (2018) reported positive effects of mannanoligosaccharide fed fish on the meat fatty acid profile. Research has shown that the concentration of C14:0 and C16:0 in human diet affects the level of low-density lipoprotein concentration in the plasma (Wood *et al.*, 2004). On the other hand, C18:1cis-9 is desired for hypocholesterolemic action (Molkentin, 2000). Our results indicate that supplementation of flaxseed in rabbit diets can favorably modulate the FA profile of rabbit meat by lowering the concentration of C14:0 and C16:0, and by increasing the concentration of C18:1cis-9 as compared to control group. Linoleic and C18:3n-3 are the essential FAs due to their important functions in the structure of cellular membranes and metabolic processes. Their consumption is therefore desirable (Martin *et al.*, 2006). Flaxseed is a rich source of the aforementioned essential FAs, which was reflected in the composition of the muscle in the rabbits. The *n-6*: *n-3* FAs ratio is highly influenced by the FA composition of the diet fed to the animals (Raes *et al.*, 2004). Lowering the ratio of *n-6* to *n-3* FA in food products have been recommended to prevent or modulate lifestyle

diseases in humans (Russo, 2009). The ratio of *n-6* to *n-3* FA in food should range between 1 and 4 (Simopoulos, 2001). In accordance with various studies, degenerative diseases, such as diabetes, arthritis and cancer, are related, in part, to the disproportion of *n-6* and *n-3* FA concentrations in the human diet, especially when there is a high concentration of *n-6* FA and a lack of *n-3* FAs (Oliveira *et al.*, 2011). In this study, more favorable *n-6*: *n-3* FAs ratio was observed in rabbits receiving flaxseed supplementation.

CONCLUSIONS

This study provides the first dataset on carcass yield and quality, physicochemical characteristics, and FA profile of indigenous rabbits. The results show that indigenous rabbits contain a high proportion of PUFA (29.7% of total fatty acids), and that supplementation of flaxseed favorably modulates the FAs composition of rabbit meat, with no negative effect on their growth performance and meat physicochemical quality. Moreover, lowest ($P < 0.05$) concentration of medium chain and total saturated fatty acids, and higher concentration of PUFA was recorded with the diets containing higher levels of flaxseed or flaxseed oil. Compared to crushed flaxseed, the supplementation of oil was more effective in reducing saturated FAs and increasing PUFA level.

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Statement of conflict of interest

We certify that there is no conflict of interest with any funding organization.

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