Dietary Linseed Supplementation Improves Meat Quality of Sheep by Altering Muscle Fiber Characteristics and Antioxidative Capacity

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Research

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Abstract

Background: In ruminants, due to the hydrogenation of the rumen, muscles contain a large amount of saturated fatty acids (SFA), which have a negative effect on meat quality. Linseed as a common oil crop which is rich in polyunsaturated fatty acid (PUFA), it affected the deposition of PUFA. Unfortunately, PUFA can exert a negative influence on the oxidative stability of meat. Fortunately, linseed is not only rich in PUFA, but also rich in phenols, which are a good source of antioxidants. Therefore, linseed may be can serve as an effective feed additive to improve meat quality of sheep. The aim of this investigation was to establish the effects of dietary linseed supplementation on carcass traits, meat quality, muscle fiber characteristics and antioxidative capacity of sheep.

Results: Results of this study indicated that linseed supplementation significantly increased the loin-eye area, crude protein and ash. Reduced pH24h, L* and shear force. Moreover, linseed supplementation affected the relative content and enriched the kinds of volatile flavor substances. Increased mRNA expression of MyHC I and MyHC IIx, and a decrease in cross-sectional area (CSA) and muscle fiber diameter was also observed. Additional changes included enhanced activity of succinic dehydrogenase (SDH), decreased activity of lactate dehydrogenase (LDH), increased total antioxidative capacity (T-AOC) activity. The mRNA expression of glutathione peroxidase (GSH-PX) and catalase (CAT) were increased while malondialdehyde (MDA) decreased.

Conclusions: The results suggest that linseed is an effective feed additive in improving meat quality. The underlying mechanism(s) for its effectiveness may be partly due to a change in muscle fiber characteristics and antioxidative capacity.

Background

As consumers' requirements for meat quality increase, it becomes a major problem for livestock producers to provide safer, healthier, and more delicious meat. Compared with pasture feeding, sheep maintained in feed lots do exhibit improved carcass weight but unfortunately also exhibit a decrease in meat quality [1, 2]. Therefore, improving the meat quality of feed lots sheep has attracted worldwide attention. Previous research has reported that diet nutrition had an effect on meat quality of sheep [3–6]. For example, dietary rumen-protected betaine supplementation was shown to increase the average daily gain and antioxidant status of muscle in male Hu sheep [7]. Also, according to PE Simitzis, MA Charismiadou, M Goliomytis, A Charalambous, I Ntetska, E Giamouri and SG Deligeorgis [5], incorporation of flavonoids in sheep diets improved plasma and meat antioxidant capacity. Growth performance and carcass trait were also affected by dietary inulin supplementation [8]. These results indicate that improvement of meat quality with dietary supplementation is possible.

As a common oil crop, linseed is rich in polyunsaturated fatty acid (PUFA), such as α-linolenic acid and linoleic acid. In ruminants, due to the hydrogenation of the rumen, muscles contain a large amount of saturated fatty acids (SFA), which is not conducive to the improvement of meat quality. According to HV

Le, DV Nguyen, QV Nguyen, BS Malau-Aduli, PD Nichols and AEO Malau-Aduli [9], n-3 LC-PUFA contents were increased in lambs by dietary flaxseed supplementation. Furthermore, when linseed was added to the diets of bulls [10], pigs [11], and chickens [12], it affected the deposition of PUFA. Unfortunately, PUFA can exert a negative influence on the oxidative stability of meat. Fortunately, linseed is not only rich in PUFA, but also rich in phenols, which are a good source of antioxidants [13]. Improving antioxidation can prevent the occurrence of peroxidation reactions thereby reducing the generation of offensive flavors in meat.

Previous research has indicated potential associations between muscle fiber types and meat quality. It is now well established from various studies that muscle fiber characteristics are related with meat color, tenderness, postmortem pH, etc. [14–16]. In this respect no investigations on the effect of linseed supplementation on meat quality of sheep have been performed.

Therefore, the aim of this investigation was to assess the effect of linseed supplementation into the diet of sheep with respect to on carcass traits, meat quality, muscle fiber characteristics and antioxidative capacity.

Materials And Methods

Animals and diets

Animal experiments were approved by the Inner Mongolia Agricultural University Animal Experiment Committee and conducted in accordance with the Guide to Animal Experiment System of College of Animal Science, Inner Mongolia Agricultural University.

A total of twenty-four Sunit sheep with an average body weight of 16.5 ± 1.6 kg from Inner Mongolia, China were weaned for 90 d and then randomly allocated into control (C) and linseed supplemented (L) groups. Three replications of four sheep per replicate were used for each group. The C group was fed a basic diet, which consisted of whole plant silage and sunflower cake supplemented with commercial fattening feed. The L group diet consisted of a similar diet but supplemented with 8% linseed. The nutrient contents of the diets are shown in Table 1. Water was provided *ad libitum* throughout the experiment. The feeding trials lasted for 90 d; a feed intake and weight gain were recorded throughout.

Table 1
Nutrient contents of basal diet (dry matter basis)

Nutrient levels	Content (%)
Crude protein	18.00
Crude fat	4.20
Crude fiber	3.80
Neutral detergent fiber	35.85
Acid detergent fiber	15.63
Ash	9.00
Calcium	1.25
Phosphorus	0.50
NaCl	0.75

Sample collection at slaughter and carcass traits

All sheep were sacrificed in a local abattoir (Bayannur Agriculture and Animal Husbandry, Inner Mongolia, China) after the feeding experiment was completed. After slaughtering and bleeding, the heads, hoofs, internal organs, hides, and lymph fluid were removed. Carcass weight was determined to calculate percent dressing. Back fat depth, and loin-eye area were determined at the 12th and 13th ribs of the left carcass half. The *M. longissimus lumborum (LL)* muscles between the 10th and 12th ribs of the left carcass half were collected for quality measurements. Within 30 min postmortem, the LL muscle located at the 9th and 10th ribs of the left carcass half were assessed for muscle fiber characteristics RNA extraction and enzyme activity were also assessed. Samples for RNA extraction and enzyme activity were immediately frozen in liquid nitrogen and stored at -80 °C. Samples for muscle fiber analysis were cut into 0.5 cm × 0.5 cm × 1.0 cm blocks, dehydrated in pre-cooled isopentane for 30 s, immediately frozen in liquid nitrogen and stored at − 80 °C.

Meat quality

LL muscle from the 10th and 12th ribs were divided into four samples. The first sample was stored at − 20 °C and used to determine flavor and nutritional quality. The second sample was used to measured pH. Third sample and forth samples were used to measure meat color, cooking loss and shear force respectively.

Volatile flavors were determined using solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS) as described by V Vasta, G Luciano, C Dimauro, F Rohrle, A Priolo, FJ Monahan and AP Moloney [17] with some modifications. Samples (5 g) were grounded with a grinder and placed into a 15 mL vials capped with PTFE septa.

The SPME fibre (DVB/CAR/PDMS 50/30 mm; 57328-U; Supelco, Bellefonte, USA) was exposed to each sample and placed in vial for 40 min at 60 °C. After adsorption, the fibre was inserted into the injection port at 250 °C for 3 min. For the GC (TRACE 1300, Thermo Fisher Scientific, USA) analysis: the injector operated in splitless mode at 250 °C. The initial temperature was 40 °C, held for 5 min, heated up to 200 °C at 5 °C / min, held for 5 min, then heated up to 250 °C at 20 °C / min, held for 5 min. Helium was used as the carrier gas and the flow rate is 1.0 ml/min. The mass spectrum was acquired at 70 eV, and the scan mass range was m/z 30 ~ 400 m/z. Volatile compounds were identified by comparison with the mass spectra from the library database (NIST MS Search 2.0). The results of volatiles compound were expressed as the percentage (%) of each compound in total identified compounds.

The protein content was analyzed by Kjeldahl (GB 5009.5–2016) and intramuscular fat content was analyzed by Soxhlet (GB 5009.6–2016). The moisture was determined by drying to a constant weight in an oven (105 °C), and the ash on the sample residue was measured after drying at 550 °C for 12 hours in a muffle furnace.

The pH was determined at 45 min and 24 h post-mortem using a pH meter (pH-Star; Ingenieurbüro R. Matthäus, Ebenried). Meat color was evaluated at 45 min post-mortem by measuring the lightness (L), red (a *), and yellow (b *) values using a chromatic meter (CR-410, Konica Minolta, Japan). Samples were subsequently chilled at 4 °C for 24 h. Each sample was weighed (W1, g), then heated at 85 °C for 40 min in sealed plastic bag using a water bath. After cooling, absorb surface moisture with filter paper and weigh sample again (W2, g), and cooking loss (%) was calculated as (W1 − W2)/W1 × 100. To determine the shear force, samples were chilled at 4 °C for 24 h, heated in a sealed plastic bag using a water bath at 75 °C for 45 min. After cooling, any surface moisture was absorbed with filter paper. Rectangular cores (3 cm × 1 cm × 1 cm), parallel to the longitudinal orientation of the muscle fibers, were taken and analyzed. Measurements were performed at least 8 times using a tenderness meter (C-LM3B, Northeast Agricultural University, Harbin, China).

Muscle fiber characteristics

Transverse serial muscle fiber sections (10 μm), obtained using a cryostat microtome (MEV, SLEE, Germany) at − 25 °C, were incubated using myofibrillar adenosine triphosphatase (mATPase) staining methods (pH 4.60) to classify the muscle fiber types [19]. Approximately 1500 fibers per sample, which randomly selected from no tissue disruption and freeze damage, were detected and defined as fiber type I, fiber type IIA, fiber type IIB. All samples were analyzed by image analysis program (Laica QWin V3 Processing-Analysis Software, Leica, Germany). The cross-sectional area (CSA) of the muscle fiber was determined as the ratio of the total measured area to the total number of fibers count. The muscle fiber density was expressed as the mean number of fibers/mm^2. The total number of muscle fibers measured as the loin-eye area multiplied by the muscle fiber density.

RNA extraction, cDNA synthesis and real-time PCR

Total RNA was prepared from LM using Trizol Reagent (TaKaRa, Dalian, China) according to the instructions of the manufacturer. Total RNA was extracted using Trizol Reagent (TaKaRa, Dalian, China)

according to the instructions of the manufacturer. The purity and concentration of total RNA was detected by a microspectrophotometer (ND-1000, gene, China). Ratios of 260 nm and 280 nm absorption (A260/A280) between 1.8 and 2.0 was considered good quality of extracted RNA. The integrity of extracted total RNA was detected using 1% agarose gel electrophoresis. Total RNA was reversed transcribed into cDNA using PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China) according to manufacturer's instructions. The mRNA expression levels of all genes were examined by real-time quantitative polymerase chain reaction (RT-PCR) using TB Green™ Premix Ex Taq^{TM} (Tli RNaseH Plus) (TaKaRa, Dalian, China) and RT-PCR detection system (Roche, China). All primers were synthesized commercially by Sangon Biotech (Shanghai) and shown in Table 2.

Table 2
Primers used for real-time quantitative PCR

Gene name	Primer sequence (5'–3')	Product length (bp)	Accession no.
GAPDH	F: CTCAAGGGCATTCTAGGCTACACT	180	NM_001190390.1
	R: GACCATGAGGTCCACCACCCTGT		
MyHC	F: AAGAACCTGCTGCGGCTG	250	AB058898
	R: CCAAGATGTGGCACGGCT		
MyHCa	F: GAGGAACAATCCAATACAAATCTATCT	173	AB058896
	R: CCCATAGCATCAGGACACGA		
MyHCb	F: GACAACTCCTCTCGCTTTGG	247	XM_027974883.1
	R: GGACTGTGATCTCCCCTTGA		
MyHCx	F: GGAGGAACAATCCAATGTCAAC	178	AB058897
	R: GTCACTTTTTAGCATTTGGATGAGTTA		
SOD	F:5'GGCAGAGGTGGAAATGAAGAAAGT	94	NM_001145185.2
	R:5' CAGGGAATGTTTACGGGGCAAT		
CAT	F:5' CGTGACCCTCGTGGCTTT	190	XM_012096208.3
	R:5' GACGCAGGCTCCAGAAGT		
GPH-PX	F:5' GCAGGAGCCAGGGAGTAATG	207	XM_027970173.1
	R:5' ACACAGCCGTTCTTATCAATCAGG		

Enzyme activities and malondialdehyde (MDA) content

Malondialdehyde (MDA), succinic dehydrogenase (SDH), lactate dehydrogenase (LDH), total superoxide dismutase (T-SOD), catalase (CAT), glutathione peroxidase (GSH-PX) and total antioxidative capacity (T-

AOC) were evaluated using kits obtained from Nanjing Jiancheng Bioengineering Institute of China (Nanjing, Jiangsu, China).

Statistical analysis

All analyses were carried out using IBM SPSS Statistics 19.0. The Shapiro Wilk test and Levene test were used to test the normality of data distribution and homogeneity of variance. All data were analyzed using one-way ANOVA; each result was reported as the average ± standard error. $P < 0.05$ or $P < 0.01$ signified significance between means.

Results And Discussion

Carcass characteristics and meat quality

Linseed supplementation had no effect ($P > 0.05$) on carcass weight, average daily food intake, average daily gain, dressing loss, backfat thickness, however, it did increase ($P < 0.05$) loin-eye area which is mainly used to evaluate carcass meat production (Table 3). It was also observed that linseed supplementation significantly reduced pH_{24h} ($P < 0.01$), L* ($P < 0.05$) and shear force ($P < 0.01$), but had no effect ($P > 0.05$) on pH_{45min}, a*, b* and cook loss (Table 4). Additionally, crude protein and ash significantly increased ($P < 0.01$) in the linseed group (Table 5).

Table 3
Effect of dietary linseed supplementation on carcass traits of sheep

Item	Control group	Linseed group	P-value
Initial weight, kg	16.69 ± 1.80	16.03 ± 0.91	0.272
Final weight, kg	31.96 ± 4.82	33.51 ± 2.41	0.331
Carcass weight, kg	14.07 ± 2.27	14.08 ± 1.38	0.983
Dressing percentage, %	43.94 ± 1.66	42.04 ± 2.85	0.883
backfat thickness, mm	3.97 ± 0.83	4.43 ± 1.29	0.331
Loin-eye area, cm^2	12.24 ± 2.42	14.78 ± 2.10	0.028
Average daily food intake, kg/d	1.90 ± 0.13	1.89 ± 0.15	0.258
Average daily gain, kg/d	0.17 ± 0.04	0.19 ± 0.03	0.225

Table 4
Effect of linseed supplementation on sheep muscle quality

Item	Control group	Linseed group	P-value
pH_{45min}	6.40 ± 0.20	6.32 ± 0.39	0.555
pH_{24h}	5.73 ± 0.16	5.45 ± 0.18	0.001
L*	35.13 ± 0.85	33.78 ± 1.41	0.026
a*	17.14 ± 1.13	18.09 ± 1.43	0.093
b*	2.92 ± 0.53	2.77 ± 0.55	0.526
Shear force, N	86.23 ± 15.76	69.78 ± 7.41	0.005
Cook loss, %	39.58 ± 3.42	39.82 ± 7.44	0.922

Table 5
Effect of linseed supplementation on the chemical composition of sheep muscle

Item (%)	Control group	Linseed group	P-value
Intramuscular Fat	2.53 ± 0.97	2.03 ± 0.85	0.211
Crude Protein	21.69 ± 1.11	22.92 ± 0.97	0.01
Moisture	76.63 ± 2.46	75.76 ± 1.26	0.296
Ash	1.64 ± 0.27	2.11 ± 0.33	0.001

Several reports have shown that dietary supplementation with linseed had no significant effect on growth performance, carcass traits, meat quality and chemical composition[20–24]. In the present study, however, linseed supplementation did exert a significant effect on carcass traits, meat quality and chemical composition. Conflicting results might be related to the level of linseed supplementation and the duration of the feeding trial [25]. For example, A Guerrero, C Sañudo, MM Campo, JL Olleta, E Muela, RMG Macedo and FAF Macedo [26] reported that a diet supplemented with 10% linseed and fattening for either 30 or 50 d could improve the fatty acid composition, productive, carcass characteristics and meat quality of cull ewes. According to R Marino, M Caroprese, G Annicchiarico, F Ciampi, MG Ciliberti, Ad Malva, A Santillo, A Sevi and M Albenzio [4], dietary linseed supplementation improved the meat tenderness and agrees with our current observations, which showed that dietary supplementation with linseed exhibited a lower shear force. The pH of muscle after slaughter is an important indicator of glycolytic activity; and was the basis for identifying potential PSE and DFD. In agreement with CE Devine, AE Graafhui, PD Muir and BB Chrystall [27], the pH_{45min} and pH_{24h} fell within the normal range. The pH_{24h} in the linseed group was lower than that of the control group. This difference in pH may account for the difference in muscle fiber composition between the two groups since muscle fiber type is related to the ultimate pH [28, 29]. In

the case of meat color, supplementation with linseed only reduced L* values, which is contrary to previous [4, 30].

Major volatile flavors in LL muscle including alcohols, aldehydes, ketones and hydrocarbons are given in Table 6. Aldehydes were the most, prominent followed by alcohols. A total of 47 volatile compounds were detected in current study, of which 33 volatiles were detected in the control group. In contrast, the linseed group contained 38 volatiles. The relative content of 1-pentanol, hexanal, nonanal, 2,3-octanedione and allyl 2-ethyl butyrate in the control group were significantly higher than that in the linseed group ($P < 0.05$). Dietary linseed supplementation significantly increased the relative content of Z-10-Pentadecen-1-ol, pentanal, 2-Octenal, (E)-, decanal, butane and 2-heptanone ($P < 0.05$).

Table 6
Effect of linseed supplementation on volatile flavor substances in sheep muscle

Item	Compound	Control group (%)	Linseed group (%)	P-value
Alcohols		39.48 ± 6.12	44.04 ± 8.14	NS
	2-Penten-1-ol, (E)-	0.58 ± 0.35	ND	ND
;	1-Pentanol	7.30 ± 1.49	4.50 ± 1.72	0.003
	3-Methyl-2-butanol	3.06 ± 1.88	ND	ND
	Cyclopentanol, 3-methyl-	ND	1.83 ± 0.77	ND
	1-Hexanol	4.10 ± 1.62	4.08 ± 1.33	NS
	1-Heptanol	4.47 ± 1.03	4.63 ± 1.57	NS
	3-Octyn-2-ol	ND	0.22 ± 0.52	ND
	1-Octen-3-ol	10.41 ± 4.81	9.07 ± 5.08	NS
	2-Octen-1-ol, (Z)-	1.90 ± 0.64	2.04 ± 0.79	NS
	Cyclohexanol, 2,4-dimethyl-	ND	0.74 ± 0.37	ND
	1-Hexanol, 2-ethyl-	1.66 ± 0.86	2.06 ± 0.80	NS
	1-Octanol	7.18 ± 2.09	8.11 ± 1.98	NS
	2,4-Decadien-1-ol	ND	1.23 ± 0.56	ND
	4-Methyl-5-decanol	ND	2.03 ± 1.53	ND
	Z,Z-2,5-Pentadecadien-1-ol	0.71 ± 0.44	0.50 ± 0.24	NS
	Z-10-Pentadecen-1-ol	0.66 ± 0.22	1.50 ± 0.20	0.005
	2-Hexadecanol	1.52 ± 0.80	1.82 ± 0.99	NS
Aldehydes		39.22 ± 11.29	37.44 ± 9.71	NS
	Butanal, 3-hydroxy-	0.89 ± 0.36	2.37 ± 0.91	NS
	Glutaraldehyde	ND	1.67 ± 0.80	ND
	Pentanal	1.02 ± 0.26	4.37 ± 2.09	0.003
	Hexanal	12.38 ± 5.61	0.88 ± 0.46	0.000
	Benzaldehyde	1.71 ± 0.97	ND	ND
	2-Heptenal, (Z)-	ND	3.38 ± 0.79	ND

Note: ND, not detected; NS, nonsignificant

Item	Compound	Control group (%)	Linseed group (%)	P-value
	Heptanal	4.72 ± 1.31	4.81 ± 1.31	NS
	2-Octenal, (E)-	0.78 ± 0.28	1.41 ± 0.47	0.005
	Octanal	4.27 ± 1.79	4.08 ± 1.39	NS
	2,4-Nonadienal	ND	0.62 ± 0.31	ND
	2-Nonenal, (E)-	0.44 ± 0.12	0.48 ± 0.09	NS
	Nonanal	11.12 ± 2.60	7.73 ± 3.24	0.012
	2,4-Decadienal, (E,E)-	ND	0.62 ± 0.19	ND
	2-Decenal, (E)-	0.96 ± 0.32	1.08 ± 0.36	NS
	Decanal	0.97 ± 0.35	1.42 ± 0.45	0.023
	Undecanal	0.73 ± 0.15	ND	ND
	2,4-Dodecadienal, (E,E)-	ND	1.31 ± 0.83	ND
	Dodecanal	ND	0.87 ± 0.28	ND
	Tetradecanal	ND	0.93 ± 0.25	ND
Hydrocarbons		5.41 ± 1.26	10.31 ± 3.72	0.004
	Butane	3.18 ± 0.84	9.57 ± 1.26	0.001
	Pentane, 3-methyl-	1.85 ± 0.74	ND	ND
	Cyclopentane, methyl-	1.99 ± 0.63	ND	ND
Ketones		5.01 ± 2.65	4.35 ± 1.89	NS
	2-Heptanone	1.10 ± 0.16	1.42 ± 0.40	0.047
	2,3-Octanedione	3.47 ± 1.56	1.45 ± 0.66	0.002
	3-Undecanone	ND	0.65 ± 0.18	ND
Others compounds		4.77 ± 1.49	1.13 ± 0.37	0.000
	Phenol	ND	1.03 ± 0.65	ND
	Hexanoic acid, ethyl ester	ND	0.54 ± 0.38	ND
	Allyl 2-ethyl butyrate	3.07 ± 1.50	1.13 ± 0.38	0.000
	Formamide, N, N-dibutyl-	0.68 ± 0.24	ND	ND

Note: ND, not detected; NS, nonsignificant

Item	Compound	Control group (%)	Linseed group (%)	P-value
	Cyclohexaneacetic acid, 2-phenyl-	0.52 ± 0.15	ND	ND
Note: ND, not detected; NS, nonsignificant				

Aldehydes, which had a low threshold and were mostly derived from fat oxidation and degradation, moreover, it played an important role in the formation of mutton flavor [31]. Linseed significantly increased the relative content of pentanal and enhanced the fruity odors of mutton [32]. However, the relative content of hexanal was significantly reduced in linseed group, indicating that the control group had stronger grass like odors [32]. Hexanal was derived from the oxidation of linoleic acid and arachidonic acid. The reason why the relative content of hexanal in the control group was higher than that in the linseed group may be related to the antioxidant capacity of meat. The increase of antioxidant capacity can reduce the degree of oxidation of unsaturated fatty acids, thereby reducing the content of hexanal, the product of linoleic acid oxidation [3, 33]. Linseed significantly increased the relative content of 2-octenal, (E)-,which provided a stronger meat and fatty and green odors [32]. Relative content of nonanal in the control group was significantly higher than that in the linseed group. Nonanal is associated with various odors in mutton including fat, floral and citrus [34]. 2,4-Decadienal, (E,E)- was a product of linoleic acid, which had a meaty and grilled odors, and was a unique flavor substance in the linseed group [33]. The relative content of decanal in the linseed group was significantly higher in the control group ($P < 0.05$), contributing to stronger odors of soap, orange peel and tallow in mutton [32]. Undecanal were the special flavor substances in the control group, which exhibited fat, wax and soapy odors [34]. Dodecanal was endemic to the linseed group and has been reported to have an onion and yeast odor [34].

The formation of meat flavors is generally related to low thresholds of volatile compounds. For example, aldehyde compounds, produced by the oxidation of fat were reported by [35] to have low thresholds with respect to smell. Compared with glycolytic muscle fiber, oxidized muscle fibers had higher a content of phospholipid [36] which were one of the main substances affecting meat. flavor Free amino acids are also vital flavor precursors in meat [37]. D Mashima, Y Oka, T Gotoh, S Tomonaga, S Sawano, M Nakamura, R Tatsumi and W Mizunoya [38] reported that there was a positive correlation between the MyHC and total free amino acid concentrations. These results revealed that muscle fiber type had a strong effect on the flavor of meat. In addition, PUFA in meat were prone to oxidation and were important precursors of volatile flavor substances. But excessive oxidation could cause bad smell. According to ND Cameron and MB Enser [39], PUFA in meat was easily oxidized which was inversely related to flavor. Therefore, the difference in flavor may be related to the antioxidant capacity. The antioxidant system and the oxidizing system restrict each other to prevent the occurrence of lipid peroxidation.

Muscle fiber characteristics and MyHC isoforms mRNA expression level

Representative photomicrographs of control and linseed fed groups are shown in Fig. 1. Muscle fibers were classified into types: I (black), IIA (white) and IIB (brown) using myosin ATPase staining. Muscle fiber characteristics are shown in the Table 7. Linseed supplementation decreased CSA ($P < 0.01$), increased total fiber number and density ($P < 0.01$). The fiber diameters of types I, IIA and IIB were observed to decrease ($P < 0.01$) with linseed supplementation, but had no effect on muscle fiber numbers and area composition ($P > 0.1$). According to the polymorphism of the myosin heavy chain (MyHC), muscle fibers can be divided into four types: MyHC I (slow-twitch oxidative), MyHC IIa (fast-twitch oxidative), MyHC IIx (fast-twitch oxidative-glycolytic) and MyHC IIb (fast-twitch glycolytic) [40]and serve as a fast, reliable and accurate method to identify muscle fiber types. As shown in Fig. 2, linseed supplementation increased the mRNA expression of MyHC□and MyHC □x ($P < 0.01$), but had no effect on the mRNA expression level of MyHC□a and MyHC □b. These results suggest that the number of oxidized muscle fibers were higher in the linseed group. Muscle from the linseed group contained increased ($P < 0.01$) activity of SDH which is a key enzyme involved in aerobic metabolism. In addition, a decrease ($P < 0.01$) in LDH activity, major enzymes involved in anaerobic glycolysis, were observed (Fig. 3). These results suggest that a diet supplemented with linseed increased the oxidative metabolism of muscle. Several reports have shown that the of number muscle fibers contributed to no significant changes in postnatal mammalian muscles [41, 42]. Therefore, muscle fiber hypertrophy in postnatal lies with the total number of muscle fibers within a muscle [42]. This finding was consistent with that of AJ Fahey, JM Brameld, T Parr and PJ Buttery [43] who found that maintenance, nutrition, and mobility of lambs during the postnatal period had no effect on the muscle fibers numbers and fiber types However, nutrition did have a significant effect on muscle fiber diameter. Fewer number of muscle fibers may lead to an increase in the muscle fiber diameter [44]. This finding was also reported by F Gondret, L Lefaucheur, H Juin, I Louveau and B Lebret [45]. In current study, linseed supplementation resulted in higher total number of muscle fiber and lower muscle fiber diameters. In the control group lower numbers of muscle fiber and higher muscle fiber diameters were consistent with previous research results. A relationship between the number and size of muscle fibers and muscle mass has been reported [15, 46]. Several reports have shown that mean CSA fibers [47] and muscle fibers diameter [48] were negatively related to tenderness. Moreover, a previous study has shown that shear force was negatively related to tenderness [49]. All in all, as the muscle fiber diameter and CSA increased, the shear force increased, and the tenderness decreased. In our study, dietary linseed supplementation decreased CSA and the diameter of muscle fibers, meanwhile the lower shear force value was observed in linseed group. These results fit well with the previous several studies. According to G-D Kim, J-Y Jeong, E-Y Jung, H-S Yang, H-T Lim and S-T Joo [50], the higher proportion of fast-twitch glycolytic (IIB) fibers may lead to L* values and the rate and extent of pH decline were increased. A study in Simmental hybrids found that MyHC I and MyHC IIa were negatively correlated with and intramuscular fat (IMF) content and meat shearing force (MSF), MyHC IIx was inverse associated with MSF [51]. Our present study indicated that dietary supplementation with linseed increased the mRNA expression level of MyHC I and MyHC IIx, along with decreased CSA and muscle fiber diameter, meanwhile enhanced the activity of SDH and decreased the activity of LDH, which may partly explain why linseed can improve the meat quality of sheep as demonstrated in the current study.

Table 7
Effect of linseed supplementation on muscle fiber characteristics

Trait	Control Group	Linseed Group	P-value
Mean CSA fibers (μm^2)	1435.09 ± 172.79	1104.31 ± 215.03	0.001
Total muscle fiber number ($\times 10^3$)	853.46 ± 238.02	1200.89 ± 154.23	0.001
Density of muscle fibers (mm^2)	688.01 ± 111.13	901.41 ± 167.08	0.003
Muscle fiber number composition (%)			
Type Ⅰ	8.89 ± 2.45	9.80 ± 3.34	0.485
Type ⅡA	32.51 ± 5.87	35.73 ± 7.18	0.274
Type ⅡB	57.68 ± 5.64	55.39 ± 6.78	0.274
Muscle fiber area composition (%)			
Type Ⅰ	8.11 ± 2.18	6.91 ± 1.72	0.139
Type ⅡA	38.19 ± 9.41	40.67 ± 9.50	0.554
Type ⅡB	53.71 ± 8.52	52.42 ± 9.40	0.942
Mean diameter of muscle fibers			
Type Ⅰ	41.85 ± 5.90	34.04 ± 5.24	0.005
Type ⅡA	44.95 ± 3.18	40.21 ± 4.24	0.009
Type ⅡB	38.28 ± 3.43	32.67 ± 3.69	0.002

Muscle antioxidative capacity

Dietary supplementation with linseed had no effect ($P > 0.10$) on muscle CAT and GSH-PX enzyme activity but did result in greater ($P < 0.01$) T-AOC activity (Table 8) and the mRNA level of GSH-PX ($P < 0.01$) and CAT ($P < 0.05$) (Fig. 4), and decreased T-SOD enzyme activity and mRNA level (Table 9, Fig. 4). Moreover, MDA content was decreased ($P < 0.01$) by dietary supplementation with linseed. Lipid peroxidation had a negative effect on meat quality [52]. Therefore, it is common knowledge that inhibit oxidation and reduce lipid peroxidation by dietary natural antioxidants were effective for improving meat quality. Previous studies had shown that flaxseeds are rich in phenolic substances which can directly participate in anti-free radicals active [13]. I Moñino, C Martínez, JA Sotomayor, A Lafuente and MJ Jordán [53] reported that sheep which are rich in polyphenols in the diet, free radical scavenging ability will also be enhanced. Because of MDA was a secondary product of lipid oxidatirron, MDA content could reflect the degree of lipid peroxidation in the tissue [54]. Therefore, MDA may indicate an antioxidant capacity. In current study, T-AOC activity and the mRNA level of GSH-PX and CAT were increased, MDA was decreased in linseed group, which results indicated that dietary linseed supplementation can

enhance antioxidant capacity of sheep. This finding was consistent with that of LB Pouzo, AM Descalzo, NE Zaritzky, L Rossetti and E Pavan [55] who found that by supplementing with low levels of flaxseed, the antioxidant capacity of beef can be improved. SOD was the first enzyme to fight oxidative stress, the content of flaxseed oil and fat were as high as 30–45%, and the oil and fat may act as a pro-oxidant to inhibit the activity of SOD, which may be the reason for the decreased SOD activity of linseed group [56, 57]. Moreover, at the same time point, aldehyde compounds which was considered to be a characteristic product of the oxidation process [35] had changed was observed in linseed group, which may be related to increased antioxidant capacity. In general, we speculate that the improvement of meat quality and the change of flavor substances may be attributed to the enhancement of antioxidant capacity.

Table 8
Effect of linseed supplementation on antioxidant activity

Item	Control group	Linseed group	P-value
T-SOD, U/mg prot	41.86 ± 2.35	37.53 ± 3.79	0.008
CAT, U/μg prot	4.86 ± 0.61	4.41 ± 0.46	0.564
GPH-PX, U/mg prot	36.99 ± 3.23	38.83 ± 2.92	0.678
T-AOC, U/mg prot	0.35 ± 0.04	0.53 ± 0.03	0.001
MDA, nmol/mg	2.96 ± 0.13	1.23 ± 0.11	0.001

Conclusions

The aims of the present study were to determine the effects of dietary linseed supplementation on carcass traits, meat quality, muscle fiber characteristics and antioxidative capacity of sheep. The results indicated that linseed supplementation served as an effective feed additive and improved the carcass traits and meat quality of sheep. In this respect, dietary linseed supplementation promoted the conversion of muscle fiber type from glycolytic to oxidized, decreased CSA and muscle fiber diameter, increased activity of SDH and decreased the activity of LDH concomitant with enhanced antioxidative capacity. The latter attribute may aid in understanding the relationship between meat quality and linseed supplementation.

Abbreviations

SFA: Saturated fatty acids

PUFA: Polyunsaturated fatty acid

CSA: Cross-sectional area

SDH: Succinic dehydrogenase

LDH: Lactate dehydrogenase

T-AOC: Total antioxidative capacity

GSH-PX: Glutathione peroxidase

CAT: Catalase

LL: M. longissimus lumborum

GC-MS: Gas chromatography mass spectrometry

SPME: Solid phase microextraction

mATPase: Myofibrillar adenosine triphosphatase

RT-PCR: Real-time quantitative polymerase chain reaction

MDA: Malondialdehyde

T-SOD: Total superoxide dismutase

MyHC: Myosin heavy chain

IMF: Intramuscular fat

Declarations

Ethics approval and consent to participate

Animal experiments were approved by the Animal Experiment Committee and conducted in accordance with the "Guide to Animal Experiment System of College of Animal Science, Inner Mongolia Agricultural University". The experiments were carried out in accordance with recommendations made by the European Commission (1997) with the aim of minimizing animal suffering.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Author contributions are as follows: Conceptualization (Yanru Hou, Chang Liu, Lin Su and Ye Jin); Investigation (Yanru Hou, Chang Liu, Lin Su, Lihua Zhao, Lu Dou, Yanping Bai and Duo Yao); Funding acquisition (Ye Jin); Writing – original draft (Yanru Hou); Writing – review & editing (Yanru Hou, Chang Liu, Lin Su , Lihua Zhao and Ye Jin). All authors read and approved the final version of this manuscript.

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Figures

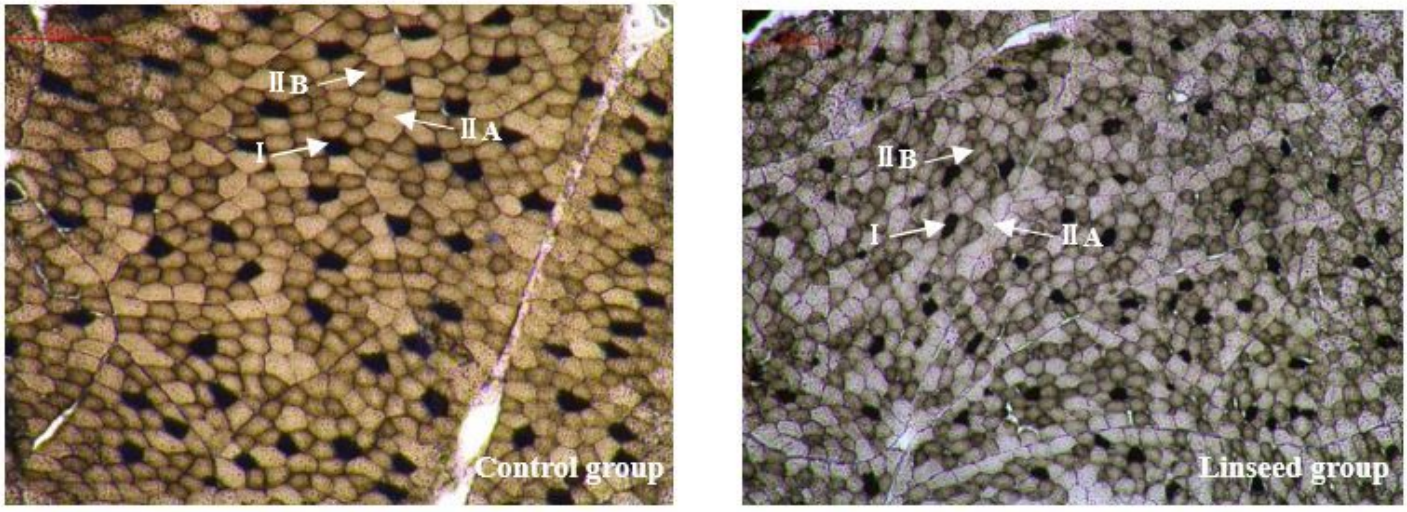

Figure 1

Cross-sections of LL muscle stained for myosin ATPase after pre-incubation in pH 4.60-4.65. Magnification of 200× was used (bar=200 μm). I: fiber type I, black; IIA: fiber type IIA, white; IIB: fiber type IIB, brown.

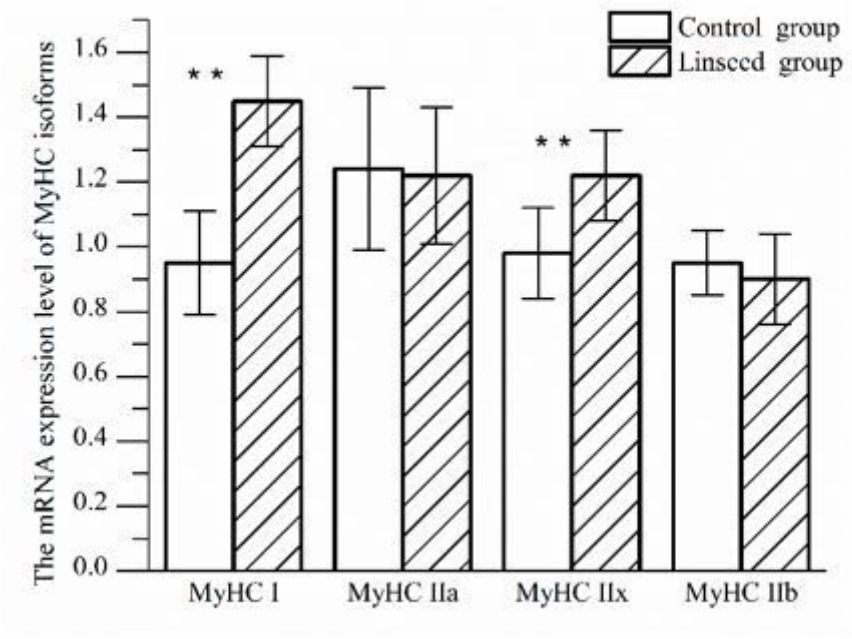

Figure 2

Effect of linseed supplementation on MyHC isoforms of LL muscle ** Significantly different (P<0.01) from control.

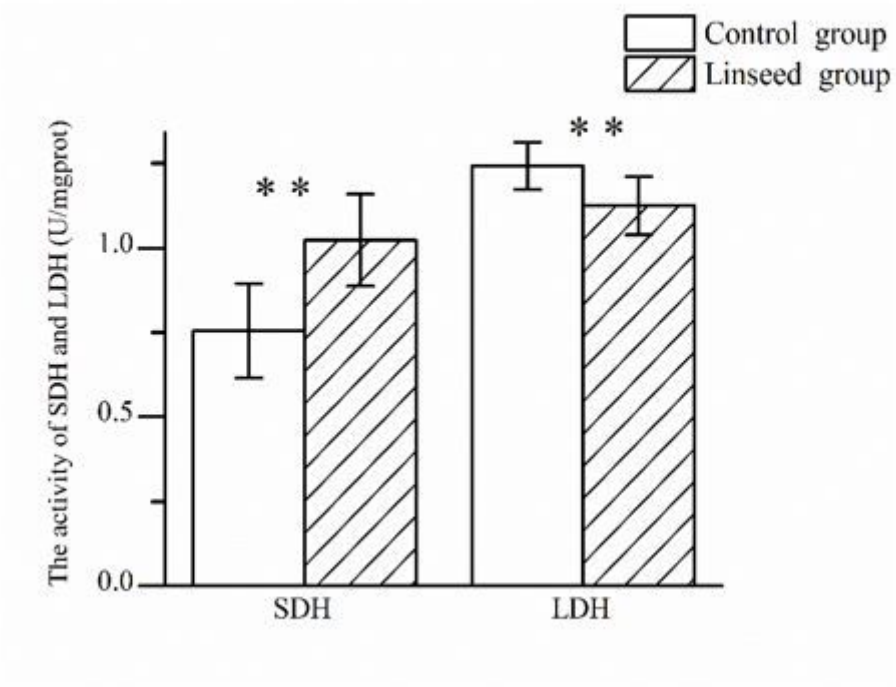

Figure 3

Effect of linseed supplementation on SDH and LDH activities of LL muscle ** Significantly different (P<0.01) from the control.

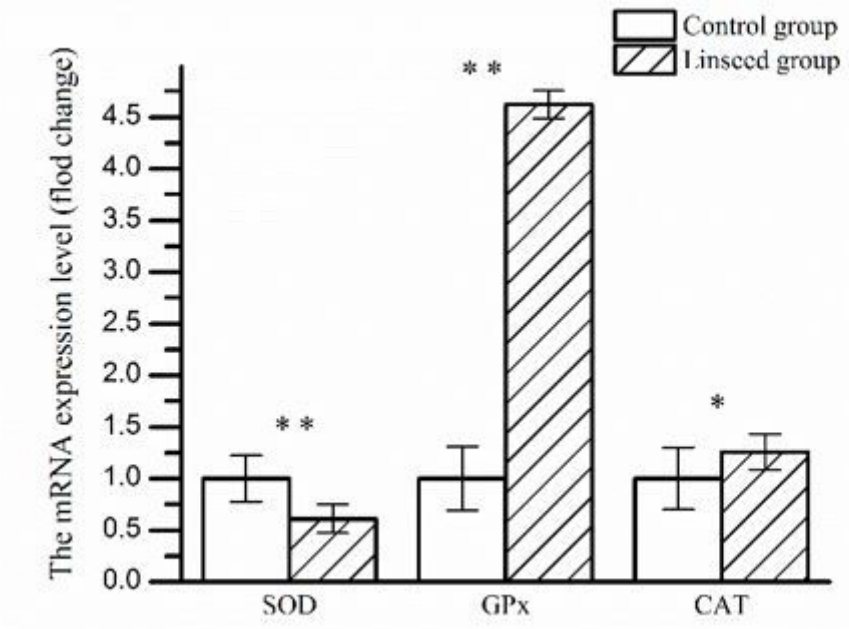

Figure 4

Effect of linseed supplementation on the mRNA expression level of antioxidant genes of LL muscle * Significantly different (P<0.05), ** significantly different (P<0.01) from the control.