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Improved muscle fatty acid composition and oxidative stability in lambs grazing on sainfoin pasture

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ABSTRACT

As a mode of animal management, grazing on pasture has the potential to improve animal performance and provide healthy meat. However, there is little information about the effects of lamb meat quality grazed on sainfoin pasture. Therefore, the present study was conducted to compare the fatty acid composition and oxidative stability of growing lambs. The twenty-four lambs were randomly divided into grazing on sainfoin pasture (GS) or feeding indoors pelleted total mixed ration (FI). The results show that GS had the higher polyunsaturated fatty acids (PUFA), especially n-3 PUFA ($P = 0.002$), and beneficial for nutritional index of fatty acid. Corresponding that GS had lower the Thiobarbituric acid reactive substance (TBARS) in raw ($P = 0.005$) and cooked meat ($P = 0.008$). The GS had higher total phenols ($P = 0.021$), ferric reducing antioxidant power (FRAP) ($P = 0.048$) and α-Tocopherol of meat ($P = 0.004$). In conclusion, grazing on sainfoin pasture in lambs can improve muscle fatty acid composition and oxidative stability than feeding indoors.

Introduction

An important theme of healthy meat production is to explore the factors that determine the nutritional value of meat and its impact on human health and diseases (De Smet & Vossen, 2016; Font-i-Furnols & Guerrero, 2022). As well as, it was deemed that animal management mode was a relative rationally feasible measure to provide healthy meat. Intensive sheep production is a common production mode in traditional agricultural areas of most developing countries (Herrero et al., 2013). However, a number of studies indicated that grazing can more efficiently utilize pasture resources and improve meat quality, especially in fatty acid composition, which is favored by consumers (Schulze et al., 2021; Spigarelli et al., 2020; Vahmani et al., 2020).

Legumes forage can have a positive effect on the performance of animals used in meat production (Copani et al., 2016). The sainfoin (*Onobrychis viciifolia*) as high-quality forage is rich in tannins and non-tannin polyphenols (Regos et al., 2009). Lobón reported that lactating ewes grazing on sainfoin improved the meat color stability and fatty acid content of suckling lamb, which may be due to the improvement of lipid oxidation by the forage components (Lobón et al., 2017). The oxidative stability of meat depends on the balance between oxidant and antioxidant components (Luciano et al., 2019). Oxidant components represented by readily oxidizable substrates, such as polyunsaturated fatty acids (PUFA). Antioxidant components comprise of both endogenous defense systems and antioxidants directly or indirectly from the diet, such as vitamins, tannins, phenols (Benzie & Choi, 2014; Gruffat et al., 2020). Therefore, there is no doubt that sheep can obtained these antioxidant components by grazing on sainfoin pasture. However, to the best of our knowledge, there are no report on meat quality of growing lambs grazing on sainfoin pasture. We hypothesized that grazing on sainfoin pasture may be beneficial to lamb meat. The present study was carried out to compare the differences of fatty acid composition and oxidative stability of lamb grazing on sainfoin pasture or feeding indoors pelleted total mixed ration.

Materials and methods

This experiment site located in the Grassland Agriculture Experimental Demonstration Zone, Anding District, Dingxi City, Lanzhou University in China (35°31′14″N, 104°19′32″E, elevation 2138 m, annual

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mean temperature 5.9 °C, annual rainfall 328.3 mm). The sainfoin pasture was established in 2018, and the lamb raising trials (including grazing and feeding indoor) were conducted from July to September 2020. All experimental protocols were approved by the Animal Care Committee of Lanzhou University in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals (CY-20,200,301).

Sward and animal management

Twenty-four 6-month-old male non-castrate Small Tail Han sheep (23.26 ± 3.02 kg of initial live weight) were randomly divided into grazing on sainfoin pasture group (GS) or feeding indoors pelleted total mixed ration group (FI). The adaptation period was 15 days and the experimental period was 60 days.

Grazing on sainfoin pasture sowed by strip cropping (row spacing: 30 cm, depth: 3 to 4 cm, sowing rate: 0.67 kg/hm^2). Grazing was started when the height of the second stubble was about 10 cm. The 0.1133 hm^2 test area of sainfoin pasture were selected and divided into 3 plots, each grazed of 4 lambs as one group. Lambs were allowed to graze unlimitedly. To protect sheep from hot weather and direct sunlight, grazing communities have water tanks and activity pergola, and the lambs can freely drink, lick nutrition blocks, without supplementary feed.

The FI lambs were kept in the pen individually, and allowing visual contact. The lambs received pelleted total mixed ration two times a day (at 7:00 am and 6:00 pm) during the trial period. The feeding amounts were adjusted as the fact that there was no experimental diets left in the trough within 2 h after ingesting. The chemical and fatty acid composition of experimental feeds were shown in Table 1.

Slaughter process and sampling

The sainfoin from grazing pasture was sampled 5 consecutive days, and the inedible part of the collected samples were discarded. The pelleted total mixed ration samples were also collected once a week. Each collection of sainfoin and pelleted subsamples were stored at 4 °C. At the end of the experiment, each subsample were mixed and dried in a forced-draft oven at 65 °C for 72 h to analyzed chemical composition.

At the end of the experiment, all lambs were slaughtered according to the halal slaughter method. Samples of longissimus thoracis (LTL) from the right half-carcass were collected. Subsequently, one part was collected vacuum-packaged and stored at −80 °C until analyses of fatty acids (FAs), and the other part was thawed overnight at 4 °C under vacuum pending oxidative stability measurements.

Table 1
Chemical and fatty acid composition of the experimental feeds.

	Sainfoin	Pelleted total mixed ration[1]
Chemical composition		
Dry matter (DM), g/100 g fresh weight	32.58	86.20
Organic matter, g/100 g DM	91.18	86.00
Crude protein, g/100 g DM	14.23	16.30
Neutral detergent fiber, g/100 g DM	57.43	20.40
Acid detergent fiber, g/100 g DM	38.67	12.31
Metabolizable Energy, MJ/kg DM	5.58	10.50
Fatty acids (% total FAs[2])		
16:0	17.89	14.94
18:0	3.30	1.95
18:1 n-9	12.60	15.68
18:2 n-6	14.38	14.38
18:3 n-3	46.44	0.28

Except for metabolizable energy, the others are actual measurements.
[1] Pelleted total mixed ration consisted of 45 % corn, 25 % grass meal, 20 % cottonseed meal and 10 % soybean meal.
[2] FAs: Fatty Acids.

Samples analysis

Lipid analysis and evaluation

Intramuscular fat (IMF) was measured on Soxhlet extractor method by Automatic fat meter (XT15i, ANKOM, United States of America). The FAs in the meat samples were determined and analyzed with methyl xanthate as internal standard. The analysis was performed as described by O'Fallon et al. (2007). In brief, the 0.3 g of LTL freeze-dried muscle powder was mixed with 0.7 mL of KOH (10 mol/L) and 6.3 mL of methanol to a centrifuge tube, then placed and bathed the centrifuge tube at 55 °C for 1.5 h, and shaken for 5 s every 20 min to make the sample penetration, dissolution and hydrolysis normal. After cooling in bath at room temperature, 0.58 mL H_2SO_4 (5 mol/L) was added and the tube turned upside down, then placed and bathed at 55 °C for 1.5 h. After fatty acid methyl ester (FAME) was formed, 2 mL n-hexane was added. The FAs profile was analyzed using a gas chromatograph (Thermo Fisher TRACE1300; Thermelfeld, Milan, Italy) with the Scion-fame column (100 m × 0.25 m × 0.20 μm, SCION, New Zealand). The injection volume, the shitter ratio, the temperature of the injector and the detector were 1 μL, 50:1, 240 °C and 250 °C, respectively. The flow rates of air, hydrogen and compensating gas (nitrogen) were 400, 40 and 20 mL/min, 50 °C for 4 min, and increased to 175 °C at 13 °C/min for 27 min, then at 3 °C/min up to 215 °C for 27 min. Identification and quantification were based on 37FAME samples (Supelco, United States of America) and odd chain branched chain fatty acids standard (Larodan Fine Chemicals, Malmo, Sweden).

The nutritional value of FAs were calculated referring to the methods by Gruffat et al. (2020). The parameters included saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), n-6 and n-3 PUFA, PUFA/SFA, n-6 PUFA/n-3 PUFA, 18:2 n-6/18:3 n-3 ratios, atherosclerosis index (AI), thrombosis index (TI), Δ5 plus Δ6 desaturase activity and peroxide index (PI).

Antioxidant status of meat

Preparation of muscle extract for antioxidant status measurements. The antioxidant status measurements were conducted according to the procedures as reported by Luciano et al. (2019). After overnight at 4 °C in vacuum, and the meat samples were divided into six subsamples (2 cm thick slices) covered with polyvinyl chloride film. Three slices of raw meat samples were stored in the dark at 4 °C, and lipid oxidation and colour stability were measured at 0 (after 2 h of blooming), 3 and 6 days after storage. The remaining three slices were bathed at 75 °C for 30 min (cooked meat), then immersed in ice bath and stored in the dark at 4 °C. The lipid oxidation of cooked meat samples were measured at 0 day (after 2 h of blooming), 2 and 4 days.

Colour stability was measured in raw meat using a WR-10 spectrophotometer (d/8° geometry, Shenzhen Weifu Photoelectric Technology Co., LTD, Shenzhen, China) setting to illuminant D65 and 10° standard observer. The descriptors lightness (L^*), redness (a^*) and yellowness (b^*) were recorded. The C^* (saturation) and H^* (hue angle) were calculated by formula:

$$C* = \left(a*^{\wedge 2} + b*^{\wedge 2}\right)^{\wedge 1/2}$$

$$H* = \arctan\left(b* / a*\right)$$

Thiobarbituric acid reactive substance. Thiobarbituric acid reactive substance (TBARS) is expressed as malondialdehyde content to evaluate the degree of lipid oxidation. Meat samples (2.5 g) and distilled water (12.5 mL) were added to a homogenizer with a running speed of 9500 r/min for homogenization, and then added 10 % (w/v) trichloroacetic acid, vortexed and filtered through filter paper. The supernatant (4 mL) was mixed with 1 mL of 0.06 mmoL thiobarbituric acid (TBA) solution. The sample was bathed at 80 °C for 90 min, and the absorbance at 532 nm was recorded. Quantification was based on external calibration curves of

TEP (1, 1, 3, 3-Tetraethoxypropane) in 5 % aqueous trichloroacetic acid (ranging from 0 to 65 nmol/4 mL).

Total phenolic compounds concentration. The phenolic compounds concentration in meat were determined as described the methods by Luciano et al. (2011b). In brief, the meat samples stored at –80 °C were defrosted and homogenized. Then, the tissue homogenate was filtered through filter paper. The concentration of total phenolic compounds was measured using Folin-Jocalto reagent. Transfer the meat extract to a centrifuge tube and add pre-diluted Folin-Jocalto reagent to the tube. After 1 min, sodium carbonate was added to remove precipitation, and the absorbance at 590 nm was recorded. Quantification was based on external calibration curves of TEP tannic acid (ranging from 0.50 μg/mL). The results were expressed as mg of tannic acid equivalents (TAE)/g of meat.

Ferric reducing antioxidant power (FRAP assay). FRAP analysis of muscle extracts were performed as described by Benzie and Strain (1996). FRAP reagent was prepared by mixing 10 vol of acetate buffer (300 mM, pH 3.6) with 1 vol of 10 mmol TPTZ solution (2,4,6-tris-(2-pyridyl) triazine in 40 mM HCl) and 1 vol of 20 mM ferric chloride in water and freshly prepared. Firstly, the meat samples were defrosted and homogenized. Then, 300 μL of distilled water, 100 μL of tissue homogenate and 3.0 ml of warm FRAP reagent (37 °C) were added to a glass test tube, then bathed at 37 °C for 4 min, and the absorbance at 593 nm was recorded. Quantification was based on external calibration curves of $FeSO_4{\cdot}7H_2O$ solution (ranging from 0 to 1000 μM). The results are expressed as 1 mole Fe^{2+} equivalent per gram of meat.

The content of α-Tocopherol. The meat samples were defrosted and washed with pre-cooled phosphate buffer solution (PBS, 0.01 M, pH = 7.4) to remove the surface blood stains, then homogenized with PBS (the volume of the solution depends on the weight of the meat sample, 9 mL PBS is suitable for 1 g meat sample). The tissue homogenate was moved to a 1.5 mL centrifuge tube and centrifuged at 4°C 5000 g for 10 min. After centrifugation, the supernatant was removed and placed in a new centrifuge tube and stored in −20°C. The samples were determined by sheep α-Tocopherol Elisa kit (αT-Elisa; Jiangsu Boshen Biotechnology Co., LTD, Shenzheng, China).

Statistical analysis

After preliminarily arranging the data through Excel 2013, the analyses were performed using SPASS 25.0. Data on meat fatty acids content and antioxidant indexes were analyzed by one-way ANOVA text. Data on colour stability in raw meat (*L*, a*, b*, C** and *H**) and lipid oxidation (TBARS values) in both raw and cooked meat were analyzed by a general linear model procedure. The fixed factors were sheep management mode, the time of storage (days 0, 3 and 6 for raw meat; and days 0, 2 and 4 for cooked meat) and their interactions, and the individual animal was included as a random factor. The $P < 0.05$ was used as the standard to declare the significant difference.

Results

Nutritive value and intramuscular fatty acids

The organic matter (OM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of sainfoin are higher than pelleted total mixed ration. The major FAs present in the pelleted total mixed ration are 16:0, 18:1 n-9 and 18:2 n-6, whereas 18:3 n-3 accounted for the largest proportion of sainfoin (Table 1).

As Tables 2 and 3 shown that GS had the higher contents of 19:0 ($P = 0.011$), 20:5 n3 ($P < 0.001$) and 22:6 n3 ($P = 0.003$). There was no significant differences on SFA ($P = 0.430$), MUFA ($P = 0.425$) and PUFA ($P = 0.744$) between two groups, but the n-3 PUFA ($P = 0.002$) and n-6 PUFA/n-3 PUFA ($P = 0.004$) were differences. The treatment had no effect on the concentration of the 18:2 n-6/18:3 n-3($P = 0.918$), AI ($P = 0.180$), TI ($P = 0.172$), Δ6 plus Δ5 desaturases ($P = 0.585$) and PI ($P = 0.314$). The content of PUFA/SFA ($P = 0.446$), Δ6 plus Δ5 desaturases and PI was higher in GS than in FI, and the AI and TI show opposite trend.

Table 2
Interactive effect of the management mode on fatty acids in meat.

	GS[1]	FI[1]	SEM[2]	*P*-value
IMF[3], g/kg of meat	39.1	45.9	0.16	0.080
FA[3], mg/kg of meat				
4:0	1.27	1.51	0.12	0.380
6:0	0.18	0.44	0.09	0.227
8:0	1.47	1.49	0.14	0.952
10:0	6.29	10.9	2.18	0.328
11:0	0.26	0.41	0.10	0.529
12:0	6.63	15.6	4.20	0.322
13:0	2.07	2.59	0.33	0.467
14:0	121	207	57.7	0.543
c9 14:1	10.4	12.7	3.08	0.730
15:0	20.7	15.4	2.90	0.405
iso 15:0	3.64	17.1	6.35	0.361
anteiso15:0	5.10	7.26	1.54	0.526
16:0	1160	1649	256	0.382
16:1	69.1	87.9	13.8	0.537
t-9 16:1	14.2	18.0	3.21	0.603
17:0	91.6	91.1	13.1	0.986
iso 17:0	91.7	113.9	17.4	0.565
18:0	1086	1251	203	0.717
anteiso 18:0	140	200	36.1	0.466
18:1 n-9c	1911	2534	368	0.440
c-11 18:1	58.4	72.6	8.36	0.450
t-11 18:1	5.27	7.27	1.33	0.509
18:2 n-6c	403	481	34.1	0.283
18:2 n-6t	6.11	20.67	6.33	0.330
18:3 n-3	86.2	36.8	13.3	0.053
c-6, c-9, c-12 18:3	8.13	8.38	0.42	0.816
19:0	2.32	0.34	0.41	0.011
20:0	8.77	40.8	16.3	0.399
20:1	5.12	7.94	1.02	0.191
20:2	2.41	4.34	0.57	0.108
20:3 n-3	1.19	1.18	0.08	0.964
20:3 n-6	28.5	19.2	3.98	0.307
20:4 n-6	181.6	176.8	15.9	0.892
20:5 n-3	62.3	15.0	9.50	<0.001
21:0	993	993	0.20	0.121
22:0	1.74	17.0	6.44	0.183
22:1 n-9	3.05	2.83	0.19	0.608
22:2	0.80	0.85	0.10	0.839
22:6 n-3	26.9	12.9	2.98	0.003
23:0	5.00	5.03	0.27	0.962
24:1	4.59	6.30	0.97	0.418
Summary				
SFA[4]	3652	4640	572	0.430
MUFA[4]	2060	2749	395	0.425
PUFA[4]	800	775	34.2	0.744
n-3 PUFA[4]	176	66	23.3	0.002
n-6 PUFA[4]	620	704	33.4	0.236

[1] GS: Grazing on sainfoin pasture; FI: Feeding indoors pelleted total mixed ration.
[2] SEM: Standard Error of Mean.
[3] IMF: Intramuscular Fat; FA: Fatty Acid.
[4] SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; SFA = 4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 13:0 + 14:0 + anteiso15:0 + 15:0 + iso15:0 + 16:0 + 17:0 + iso17:0 + 18:0 + anteiso18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:0; MUFA = *c*-9 14:1 + 16:1 + *t*-9 16:1 + 18:1 n-9c + *c*-11 18:1 + *t*-11 18:1 + 20:1 + 22:1 n-9 + 24:1; PUFA = 18:2 n-6c + 18:2 n-6t + 18:3 n-3 + *c*-6, c-9, c-12 18:3 + 20:2 + 20:3 n-3 + 20:3 n-6 + 20:4 n-6 + 20:5 n-3 + 22:2 + 22:6 n-3; n-3 PUFA = 18:3 n-3 + 20:3 n-3 + 22:6 n-3 + 20:5 n-3.

Table 3
Nutritional value of fatty acids in meat from lambs feeding indoors or grazing on sainfoin pasture.

	GS[1]	FI[1]	SEM[2]	*P*-value
Nutritional value				
PUFA[3]/SFA[3]	0.24	0.19	0.03	0.446
n-6 PUFA[3]/n-3 PUFA[3]	3.66	11.82	1.75	0.004
18:2 n-6/18:3 n-3	49.61	50.14	2.01	0.918
Nutritional index				
Atherogenic index[4]	0.47	0.65	0.06	0.180
Thrombogenic index[4]	1.86	3.27	0.50	0.172
Δ6 plus Δ5 desaturases[4]	0.47	0.41	0.05	0.585
Peroxidability index[4]	12.38	8.85	1.63	0.314

[1] GS: Grazing on sainfoin pasture; FI: Feeding indoors pelleted total mixed ration.
[2] SEM: Standard Error of Mean.
[3] PUFA: Polyunsaturated Fatty Acids; SFA: Saturated Fatty Acids; PUFA = 18:2 n-6c + 18:2 n-6t + 18:3 n-3 + *c*-6, c-9, c-12 18:3 + 20:2 + 20:3 n-3 + 20:3 n-6 + 20:4 n-6 + 20:5 n-3 + 22:2 + 22:6 n-3; SFA = 4:0 + 6:0 + 8:0 + 10:0 + 11:0 + 12:0 + 13:0 + 14:0 + anteiso15:0 + 15:0 + iso15:0 + 16:0 + 17:0 + iso17:0 + 18:0 + anteiso18:0 + 19:0 + 20:0 + 21:0 + 22:0 + 23:0; n-6 PUFA = 18:2 n-6t + 18:2 n-6c + *c*-6, c-9, c-12 18:3 + 20:3 n-6 + 20:4 n-6; n-3 PUFA = 18:3 n-3 + 20:3 n-3 + 22:6 n-3 + 20:5 n-3.
[4] Atherosclerosis Index (AI): AI = (12:0 + 14:0 + 16:0) / (MUFA + *n*-6 PUFA + *n*-3 PUFA); Thrombosis Index (TI): TI = (12:0 + 14:0 + 16:0) / (0.5 × MUFA + 0.5 × *n*-6 PUFA + 3 × *n*-3 PUFA + (n-6 PUFA/ *n*- 3 PUFA)); Δ5 plus Δ6 desaturase activity = (20:2n-6 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3) / (18:2n-6 + 18:3n -3 + 20:2n-6 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3) × 100; Peroxidation index (PI) is: PI = (∑dienoic acid to total fatty acid ratio × 1+∑trienoic acid to total fatty acid ratio × 2 +∑tetraenoic acid to total fatty acid ratio × 3+∑pentaenoic acid to total fatty acid Fatty acid ratio × 4+∑ hexaenoic acid to total fatty acid ratio × 5) × 100.

Meat colour stability and lipid oxidation

In the light of these findings, the effects of time of storage and management mode on the oxidative stability were presented in Table 4. The *a** ($P = 0.002$), *b** ($P = 0.020$) and *C** ($P = 0.002$) were affected by the time of storage. The management mode have a significant effect on the *L** of the meat colour ($P < 0.001$). Similarly, the time affected the development of TBARS, which was measured in both raw and cooked meat ($P < 0.001$). The management mode affected TBARS in both raw meat ($P = 0.005$) and cooked meat ($P = 0.008$). The TBARS of raw meat were found to be affected by T × M ($P < 0.001$).

The *L** value of GS is 36.67, which is significantly lower than FI. Furthermore, the *C** values of GS were higher than the FI. It can be found that, the TBARS in meat was increased between 0 and 6 days of storage, regardless of whether GS or FI. However, from day 0 to 3 and from day 3 to 6, the TBARS values of lambs in the FI increased, while the TBARS value in GS tended to be stable. Fig. 1 shows that TBARS always increased regardless of the management mode. In addition, at days 3 and 6 of refrigeration, TBARS of GS were lower than that of FI ($P < 0.05$).

Antioxidant indexes

As Table 5 shown, the management mode had significant effects on the content of total phenolic compounds ($P = 0.021$) and FRAP ($P = 0.048$) in the meat of lambs. The content of total phenolic compounds

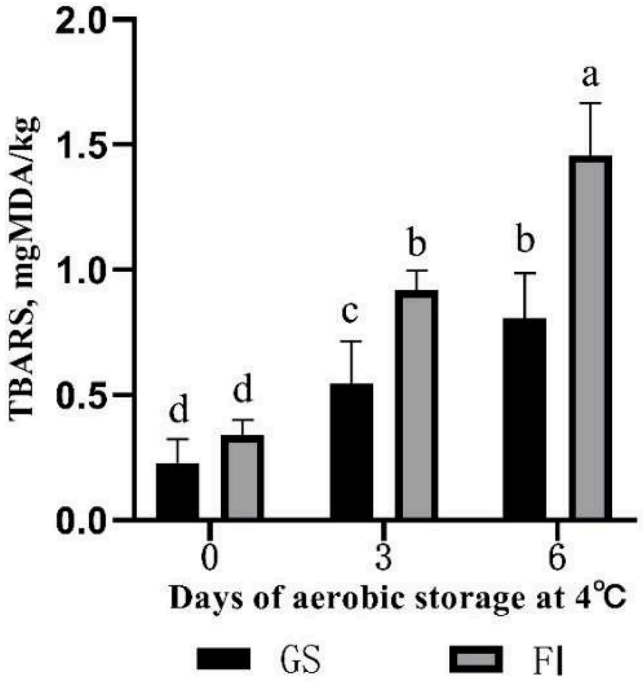

Fig. 1. Interactive effect of the time of storage (days 0, 3 and 6) and management mode on lipid oxidation (TBARS values) measured in raw meat slices stored aerobically at 4 °C. Mean values and standard error bars are presented. [a, b,c,d] Different superscript letters indicate differences ($P \leq 0.05$) between values tested using the Tukey's adjustment for multiple comparisons. GS: Grazing on sainfoin pasture; FI: Feeding indoors pelleted total mixed ration. TBARS: Thiobarbituric acid reactive substance.

Table 4
Effect of the time of storage and management mode on the colour stability of raw meat and on lipid oxidation of cooked meat stored aerobically at 4 °C.

	Time of storage[1]			Management mode		SEM[3]	*P*-value		
	0	3 (2)	6 (4)	GS[2]	FI[2]		Time of storage	Management mode	T × M[4]
Raw meat									
*L**[5]	39.71	40.87	40.69	36.67^{B}	44.18^{A}	0.50	0.324	< 0.001	0.330
*a**[5]	8.53	8.55	8.14	9.71	7.10	1.49	0.002	0.928	0.196
*b**[5]	9.15	9.02	8.24	9.84	7.76	0.63	0.020	0.051	0.813
*C**[5]	12.92	12.84	11.77	14.04^{A}	10.98^{B}	1.37	0.002	0.321	0.091
*H**[5]	0.77	0.85	0.88	0.90	0.76	0.05	0.241	0.414	0.242
TBARS[5], mg/kg	0.29^{c}	0.73^{b}	1.13^{a}	0.53^{B}	0.91^{A}	0.06	< 0.001	0.005	< 0.001
Cooked meat									
TBARS[5], mg/kg	0.62^{c}	1.17^{b}	1.97^{a}	1.12^{B}	1.39^{A}	0.05	< 0.001	0.008	0.800

[a,b,c]Within row, different superscript letters indicate differences ($P \leq 0.05$) between days of storage tested using the Tukey's adjustment for multiple comparisons.
[A,B]Within row, different superscript letters indicate differences ($P \leq 0.05$) between different management modes using the Tukey's adjustment for multiple comparisons.
[1] Time of storage: days 0, 3, 6 (raw meat); days 0, 2, 4 (cooked meat).
[2] GS: Grazing on sainfoin pasture; FI: Feeding indoors pelleted total mixed ration.
[3] SEM: Standard Error of Mean.
[4] T × M: Time of storage × Management mode.
[5] *L**: lightness; *a**: redness; *b**: yellowness; *C**: saturation; *H**: hue angle; TBARS: Thiobarbituric acid reactive substance.

Table 5
Effects of management mode on antioxidant indexes in meat.

	GS[1]	FI[1]	SEM[2]	*P*-value
Total phenols, mg/g	0.92	0.74	0.04	0.021
FRAP[3], μmol/g	1.82	1.44	0.10	0.048
α-Tocopherol, μg/g	3.01	0.37	0.53	0.004

[1] GS: Grazing on sainfoin pasture; FI: Feeding indoors pelleted total mixed ration.
[2] SEM: Standard Error of Mean.
[3] FRAP: Expressed as the amount of Fe^{2+}substances contained in each gram in meat.

and FRAP in lambs under GS were 0.92 mg/g and 1.82 μmol/g, respectively, which were higher than 0.74 mg/g and 1.44 μmol/g in the FI. The content of α-Tocopherol in GS was 3.01 μg/g, which was significantly higher than that in FI ($P = 0.004$).

Discussion

Meat fatty acid composition and nutritional value

Under grazing conditions, changes in diet composition and forage availability can affect animal growth rates and patterns, altering energy metabolism and body composition, which can directly or indirectly affect animal meat quality (Ådnøy et al., 2005; Cooper et al., 2004). Generally, ruminants that consume pasture diets have been shown to produce a more desirable fatty acid composition than those fed grain and offered potential to be further enhanced by using specific plant species (Campidonico et al., 2016; Daley et al., 2010; Yang et al., 2022). Higher IMF levels may be caused by animal breed or other factors, which can increase the proportion of SFA in total fatty acids and affect meat tenderness and taste (Luo et al., 2019). Recently studies have elucidated the molecular mechanism of lipid transformation, providing insights into lipid transformation and quality of sheep (Jia et al., 2021). The lambs on red clover pasture increased PUFA concentrations in meat compared to ryegrass, but found no differences in oxidative stability of muscle between treatments (Girard et al., 2016). There were significant differences in total PUFA content in this study, and the grazing on sainfoin pasture was significantly higher than the feeding indoors. It can be found that grazing had the higher PUFA content of meat compared with feeding indoors, especially n-3 PUFA, 18:3n3, 20:5n3 (EPA) and 22:6n3 (DHA). This may be the bioactive components of legumes forage (such as polyphenol oxidase and tannins), reduce the saturation of PUFA in the rumen, thereby increase the efflux of 18:3n-3 from the rumen and promotes the deposition of fatty acids in the meat (Bessa et al., 2015; Lourenco et al., 2007; Rivaroli et al., 2019).

In comparison to concentrate-based diet, forage-based diet is also associated to a higher proportion of PUFA, specifically n-3 PUFA in ruminant meat (Wood et al., 2008). Although PUFA, SFA and MUFA were not significantly different between two groups, but the higher levels of PUFA and lower levels of SFA and MUFA were observed in grazing than feeding indoors. The higher PUFA contents can inhibit the activity of the 9-desaturase complex, which is a key enzyme to converts SFA into MUFA, thus reducing the synthesis of MUFA (Ben Amor et al., 2018). Legume forage can stay in the rumen for a longer period of time after being consumed by ruminants because of their fibrous qualities, which lead to a higher level of n-6 PUFA biohydrogenation (Baldi et al., 2019). Therefore, the n-6 PUFA content of meat in the grazing was lower than that of feeding indoors, possibly for similar reasons.

Several studies have shown that EPA can prevent the occurrence of cardiovascular disease in humans, while DHA can promote retinal growth and brain development, and enhance brain power (Zhang et al., 2019). The results of this study found that the content of EPA in the meat of the grazing was 416 % higher than feeding indoors, which indicated that the muscle fatty acid composition value of the sheep in the moderate grazing treatment was much higher than that in the feeding indoors. The n-6 PUFA/n-3 PUFA is considered as an important nutritional index of fatty acids, and its ratio below 4 is considered to be better, and it can reduce the incidence of cardiovascular disease in humans (Vahmani et al., 2015). In this study, the ratio was less than 4 for grazing lambs with higher nutritional value, but 11.82 for feeding indoors which was higher than the recommended value.

Two representative indexes of AI and TI risk associated with lamb nutritional health were calculated based on fatty acid composition and content. Lower levels of AI in food are beneficial for human diseases such as hypertension, hyperlipidemia and diabetes. The AI index of the grazing lambs was 0.47 in this study, which was lower than the 0.63 of feeding indoors and lower than previous studies (Aurousseau et al., 2007). The higher TI in meat products, the stenosis of the human arterial lumen and the formation of thrombosis may occur after eating. The results of this study showed that the TI of grazing lambs was 1.86, which was 47 % lower than feeding indoors, and also greatly reduced the risk of TI for humans. The results suggest that meat from the grazing has very low thrombotic and atherosclerotic effects and lower cardiovascular and coronary heart disease risk.

Meat oxidative stability

Meat colour is the preferred apparent indicator for consumer judgments of meat quality, and dietary treatments also influence the colorimetric characteristics of meat (Atti et al., 2013). In general, the colour of fresh meat should be bright red or pink. The L^* of meat colour increases with aging of the meat sample, which is related to the depth of mitochondrial respiration and oxygen penetration into the exposed muscle surface that remains in the muscle after slaughter. The L^* increases as the meat becomes darker in colour. The a^* can reflect the positive correlation between the amount of hemoglobin in muscle. Colour b^* reflects the freshness of meat, which is negatively correlated with the freshness of meat, and the freshness of meat decreases with the increase of b^* (Calnan et al., 2016). It has been recently discovered that meat colour may also be caused by factors other than myoglobin, the light scattering in meat is a complex result of the interaction of various structures and phenomena, such as myofilament lattice spacing and sarcomere length within myofibrils, protein composition and distribution in the sarcoplasm (Purslow et al., 2020).

Dietary strategies that promote vitamin E deposition in meat and can improve the oxidative stability of meat. Vitamin E in meat at a certain concentration can effectively delay the oxidative deterioration of mutton, but it is also affected by other factors, such as temperature and storage conditions (Luciano et al., 2011a). Forage-based diets have a positive impact on both the fatty acid profile and the oxidative stability of meat, and legumes forage was found to increase lipid oxidative stability during high oxidative challenges (Descalzo & Sancho, 2008; Luciano et al., 2019). Ponnampalam showed that vitamin E concentration drops to a certain critical threshold, the pro-oxidative factors in the muscle will have negative effect on meat oxidative stability, such as oxidative polyunsaturated fatty acids and heme iron (Ponnampalam et al., 2016). Zhang showed that free iron rather than heme iron mainly induces oxidation of lipids and proteins in meat cooking (Zhang et al., 2022). In this study, the content of α-Tocopherol in the muscles of lambs in the grazing was significantly higher than that in the feeding indoors. The degree of meat lipid oxidation in grazing group was lower than that of feeding indoors on the 0, 3 or 6th day.

The antioxidant factors of meat (such as oxidizable substrates and catalysts) are related to the homeostasis between various antioxidant substances (endogenous system and dietary-derived exogenous antioxidants) (Zhang et al., 2013). In this study, α-Tocopherol and total phenolic content were mainly determined to understand the results of the oxidative stability of meat. Compared with γ-Tocopherol, α-Tocopherol is the main form of vitamin E in forage feed and animal muscle, and it is easy to measure (Valenti et al., 2019), therefore the

content of α-Tocopherol represents vitamin E. In this study, the content of α-Tocopherol was higher in the grazing group than feeding indoors. This may be due to the difference in α-Tocopherol content in sainfoin compared to pelleted total mixed ration (Elgersma et al., 2015). Conservation techniques can cause drastic changes in the antioxidant content of forage, for example, vitamin E and carotenoids in the following order: fresh forage, silage, and hay (Prache et al., 2003).

Conclusions

This study demonstrated that grazing on sainfoin pasture improved the fatty acid composition and nutritional value of lamb meat, and promoted the deposition of healthier fatty acids. In addition, lamb grazed on sainfoin pasture can produce meat colour preferred by consumers, and higher meat oxidative stability compared to feeding indoors management mode. Therefore, lambs grazed on sainfoin pasture can produce healthier meat by fatty acid composition and oxidative stability.

Ethics statement

All experimental protocols used in this study were approved by the Animal Care Committee of Lanzhou University, China (CY-20,200,301), in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals.

CRediT authorship contribution statement

Zijian Li: Writing – original draft, Validation, Software, Investigation, Formal analysis. **Chao Peng:** Validation, Investigation, Formal analysis. **Hucheng Wang:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Xianbai Liu:** Validation, Investigation, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

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