Influence of pasture or total mixed ration on fatty acid composition and expression of lipogenic genes of longissimus thoracis and subcutaneous adipose tissues in Albas White Cashmere Goats

Xue Wang, Tiemei Wu, Sumei Yan, Binlin Shi, Ying Zhang and Xiaoyu Guo

College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia 010018, China

ABSTRACT

The aim of this study was to evaluate pasture or total mixed ration (TMR) feeding of adult and kid Albas White Cashmere Goats (AWCG), considering the FA profiles and lipogenic gene expressions in the longissimus thoracis (LT) and subcutaneous adipose tissue (SAT). In the first trial, 60 adult female goats from Inner Mongolia White Cashmere Goat Breeding Farm were used. In the second trial, 60 AWCG castrated male kid goats from Inner Mongolia White Cashmere Goat Breeding Farm were used. FA was determined with gas chromatography, and gene expressions were analysed using qRT-PCR. In the first trial, the levels of C16:0, C18:0, C18:2 \(\alpha-6/\alpha-3\) PUFA, ACC, FAS, SCD, SREBP-1c and C/EBP \(\alpha\) were significantly higher, but the levels of C18:3 \(\alpha-3\), C20:5 \(\alpha-3\), C22:6 \(\alpha-3\), \(\alpha-3\) PUFA, PUFA and P/S as well as the genes expression of LPL, HSL and PPAR \(\alpha\), were significantly lower in LT muscle and SAT from TMR-fed adult goats compared to pasture-fed adult goats. In the second trial, the TMR-fed kid goats had higher levels of C17:0, C18:0, C18:2 \(\alpha-6/\alpha-3\), MUFA, \(\alpha-6/\alpha-3\) PUFA, ACC, FAS, SCD and C/EBP \(\alpha\) in LT muscle and SAT, but had lower levels of C18:3 \(\alpha-3\), C20:5 \(\alpha-3\), C22:6 \(\alpha-3\), \(\alpha-3\) PUFA, LPL, HSL and PPAR \(\alpha\), than the pasture-fed kids. In addition, TMR feeding increased final body weight and average daily gain of adult female goats and kid goats.

1. Introduction

Fat and fatty acids (FA) in muscle and adipose tissue are the major factors significantly affecting meat quality, particularly its nutritional value and palatability (Wood et al. 2008). In fact, subcutaneous and intramuscular adipose tissues are the most important fat deposits concerning meat quality traits. Currently, consumers are eager to obtain high quality and healthy meat. Attention has been given to lipids that contain different types of FA associated with health problems. Total saturated fatty acids (SFA) in the human diet are associated with several diseases, including coronary heart diseases (Hooper et al. 2015), whereas poly-unsaturated fatty acids (PUFA) might have health benefits for consumers (Dilzer and Park 2012). In particular, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) have anti-atherogenic, antithrombotic and anti-inflammatory effects (Deckelbaum and Torrejon 2012). Goetsch et al. (2011) in reviewing the literature noted that concentrate feeding in confinement increased the level of SFA and monounsaturated fatty acids (MUFA) in confined goats compared to grazing goats.

Albas white cashmere goats (AWCG) are raised for cashmere and meat in the plateau region of Ordos, Inner Mongolia, China. AWCG adapt well to the arid desert, semi-desert pasture conditions, and they are well known for their excellent pure white, thin, long and soft cashmere. Moreover, with the growing demand for mutton, AWCG meat has created consumer affection because of its unique flavour, and this breed has become one of the main sources of Inner Mongolia mutton production. Population of white cashemere goats in Inner Mongolia was estimated to be 70.80 million with the AWCG constituting about 62% of the population (Inner Mongolia statistical year book 2012). The overall mean of reproduction rate of AWCG is 1.23 kid/doe/year (Liu et al. 2010). The average body weight of adult female cashmere goats, weaned male kid goats and weaned female kid goats.
goats in Inner Mongolia region is 27.4, 21.98 and 19.00 kg, respectively (Jin 2009). Most of the weaned kid AWCG and thirty percent of adult AWCG are used for meat production, due to the restrictions of the pasture grazing capacity and breeding stocks in cashmere goats' husbandry. However, overgrazing contributes to a decrease of grassland productivity and damage to the ecological balance (Zhang et al. 2014). Therefore, indoor feeding instead of traditional grazing on pasture is becoming important in goat husbandry in Inner Mongolia, China. Perea et al. (2011) reported that the feeding system affected both the chemical composition and the FA profile of lamb meat. However, few data are available regarding effects of pasture and total mixed ration (TMR) feeding on FA profiles affecting mutton quality of AWCG under Inner Mongolian conditions, and the biochemical processes of the complex traits of fat content and FA composition from AWCG mutton are not yet fully understood.

The expression level of adipogenic and lipogenic genes in adipose tissues is regulated by a number of transcription factors, which are known to play a key role in lipid metabolism of goat adipocytes. In small ruminants, the transcription factors sterol regulatory element-binding proteins (SREBP1-c), peroxisome proliferator-activated receptors-γ (PPARγ) and CCAAT/enhancer-binding proteins alpha (C/EBPα) regulate the expression of adipogenic and lipogenic genes (Toral et al. 2013; Wei et al. 2014; Gallardo et al. 2015). The current study was conducted to evaluate the effects of feeding regimen on FA profiles and lipid metabolism genes of the intramuscular fat and adipose tissues from adult and kid AWCG to provide a theoretical basis for improving mutton quality by dietary modification under TMR feeding conditions.

2. Materials and methods
2.1. Experimental design, animals and diets
All procedures involving animals were evaluated and approved by the guidelines of the Animal care and Use Committee of the Inner Mongolia Agriculture University (Hohhot, Inner Mongolia, China). This study was conducted in Inner Mongolia White Cashmere Goat Breeding Farm, Wulan Town, Etuoke Banner, Ordos City, Inner Mongolia Autonomous Region, China (39°12 N, 107°97 E). The site was located in the north of the Maowusu desert, which belongs to the interim zone of the loess plateau and ordo platform and has a typical temperate continental monsoon climate; the steppe type is classified as arid and semi-arid grassland. The area receives an annual average rainfall of 250 mm, mostly occurring from July to September, and mean annual temperature of 7.0°C. Frost-free period lasting 120–150 d every year.

The study included two parts. The first trial was designed as a single factorial treatment. Sixty five-year-old adult female AWCG with body weight (BW) averaging 40.38 ± 0.84 kg were selected from the Inner Mongolia White Cashmere Goat Breeding Farm and randomly assigned to two groups (pasture-fed group and TMR-fed group) of 30 animals each. A total of 40-hectares of grassland in Inner Mongolia White Cashmere Goat Breeding Farm were used for goats grazing. The grazing method was continuous stocking and rest period was excluded from April to June. No fertiliser had been used on this site. The vegetation coverage and height were low and the grass was sparse. The botanical composition of the pasture was herbage (mainly Stipa capillata and Cleistogenes Keng), compositae (mainly Artemisia aurata) and shrub (Caragana, Aompipitanthus mongolicus, Ceratoides lanata, Salsola collina) (Bai 2014). In our experimental period, the pasture grass was in the heading stage and maturity stage. The pasture-fed group was allowed to graze from 7 am to 6 pm each day. The TMR-fed group composed of two units of 15 adult female goats was kept indoors and received TMR based on maize straw, sunflower plate powder and concentrate including distillers grains, corn, soybean meal, cottonseed meal and dried distillers grains with solubles. TMR was offered in two equal meals at 08:30 and 14:30 hours. All goats had free access to water. During the feeding trial, body weight (BW) was measured every 15 d interval before the morning feed was offered. The length of this experiment was 60 d from August to September, divided into early period (1–30 d) and late period (31–60 d).

The second trial was conducted at the Inner Mongolia White Cashmere Goat breeding Farm. Sixty AWCG castrated male kids (20.36 ± 0.32 kg of BW, four months old) were assigned to two groups (pasture-fed group and TMR-fed group) of thirty animals each. The pasture-fed group was allowed to graze natural pasture, which covered an area of 30-hectares in Inner Mongolia White Cashmere Goat breeding Farm and was different from adult goats grazed pasture but had a similar botanical composition and management strategy. The TMR-fed group composed of two units of fifteen lambs was kept indoors and received TMR based on alfalfa hay, maize straw, sunflower plate powder and concentrate. TMR was offered in two equal meals at 08:30 and 14:30 hours. All goats had free access to water. During the feeding trial, BW was measured
every 30 d interval before the morning feed was offered. The length of this experiment was 90 d from August to October, divided into early period (1–30 d), middle period (31–60 d) and late period (61–90 d). The ingredients and chemical composition of TMR and grass in two experiments are given in Tables 1 and 2, respectively.

### 2.2. Sampling and slaughtering procedures

#### 2.2.1. Faeces sampling

Six kid goats and six adult goats from the grazing flocks were identified for faecal collection. The same animals were used in all the three periods to avoid individual variations. They were harnessed with faecal bags at the first five consecutive days and used for collection of diet and faeces samples, which was following 7 d allotting to acclimatisation of animals for harness in each period. Faecal bags were emptied in the morning (07:00 hours), afternoon (13:00 hours) and evening (18:00 hours) to estimate daily faecal output. Representative samples (10%) of faeces were pooled separately for 5 days collection period and stored at −20 °C to use for future chemical analyses.

#### 2.2.2. Diets sampling

Samples of TMR were collected into separate vale bags at the beginning of each period and stored at −20 °C until chemical analysis. The vegetation picked up by the goats harnessed with faecal bags during grazing was snatched by the operator before it was contaminated by saliva and masticated (Sankhyan et al. 1999) at the first five consecutive days and the last five consecutive days in each period. Samples of vegetation were mixed and pooled for 10 days

---

### Table 1. Ingredients and chemical composition of the diets offered to TMR-fed adult and kid goats.

| Feed ingredients, % | Adult goats | Kid goats |
|---------------------|-------------|-----------|
|                     | 1–30 d      | 31–60 d   | 1–30 d | 31–60 d | 61–90 d |
| Alfalfa hay         | 0.0         | 0.0       | 10.8   | 0.0      | 0.0 |
| Maize straw         | 4.6         | 4.0       | 4.0    | 4.2      | 7.7 |
| Sunflower plate powder | 36.8     | 28.0      | 29.4   | 32.6     | 25.0 |
| Distillers grains   | 25.3        | 19.8      | 16.2   | 13.5     | 16.5 |
| Corn                | 22.7        | 42.6      | 18.7   | 30.1     | 40.8 |
| Soybean meal        | 0.0         | 0.0       | 4.7    | 1.5      | 0.0 |
| Cottonseed meal     | 3.5         | 2.7       | 5.6    | 4.7      | 2.4 |
| Distillers dried grains with solubles | 5.1 | 1.2 | 7.9 | 10.8 | 5.6 |
| CaHPO4              | 0.1         | 0.0       | 0.2    | 0.2      | 0.2 |
| Sodium chloride     | 0.5         | 0.4       | 0.5    | 0.4      | 0.5 |
| Premix*             | 0.5         | 0.3       | 1.0    | 1.0      | 0.5 |
| Sodium bicarbonate  | 0.5         | 0.6       | 0.6    | 0.6      | 0.6 |
| Total               | 100.0       | 100.0     | 100.0  | 100.0    | 100.0 |

**Chemical composition**

|                       | Adult goats | Kid goats |
|-----------------------|-------------|-----------|
| DE, MJ/kgDM           | 12.1        | 12.4      | 11.5   | 11.9     | 12.1 |
| CP, g/kgDM            | 157.3       | 130.7     | 174.6  | 155.5    | 131.1 |
| Ca, g/kgDM            | 6.0         | 4.5       | 7.1    | 4.7      | 4.3 |
| P, g/kgDM             | 2.4         | 1.8       | 2.9    | 2.3      | 1.8 |
| NDF, g/kgDM           | 326.7       | 280.1     | 333.7  | 298.5    | 298.1 |
| ADF, g/kgDM           | 210.0       | 168.6     | 207.1  | 172.7    | 169.4 |
| ADL, g/kgDM           | 102.3       | 78.5      | 87.5   | 86.3     | 78.6 |
| Ash, g/kgDM           | 112.7       | 88.4      | 100.6  | 94.9     | 83.2 |
| EE, g/kgDM            | 35.4        | 33.1      | 33.7   | 38.9     | 35.6 |
| DMI, kg               | 1.9         | 2.0       | 0.9    | 1.0      | 1.0 |

**DE**: digestible energy; **CP**: crude protein; **P**: phosphorous; **Ca**: calcium; **NDF**: neutral detergent fibre; **ADF**: acid detergent fibre; **ADL**: acid detergent lignin; **EE**: ether extract; **DMI**: dry matter intake.

*Provided per kg of premix, vitamin K and water-soluble vitamins only for kid goats), Iron (Fe) 4 g, Copper (Cu) 0.8 g, Zinc (Zn) 5 g, Manganese (Mn) 3 g, Iodine (I) 30 mg, Selenium (Se) 30 mg, Cobalt (Co) 25 mg, vitamin A (VA) 600,000 U, vitamin D (VD3) 250,000 U, vitamin E (VE) 2500 U, vitamin K (VK3) 180 mg, vitamin B1 (VB1) 35 mg, vitamin B2 (VB2) 850 mg, vitamin B6 (VB6) 90 mg, Nicotinic acid 2200 mg, p-panthenolic acid 1700 mg, vitamin B12 (VB12) 3 mg, Biotin 14 mg, Folic acid 15 mg.

### Table 2. Nutrient content of grasses offered to pasture-fed adult and kid goats.

| Chemical composition | Adult goats | Kid goats |
|----------------------|-------------|-----------|
|                     | 1–30 d      | 31–60 d   | 1–30 d | 31–60 d | 61–90 d |
| DE, MJ/kgDM         | 11.5        | 10.8      | 11.9   | 11.9     | 11.7 |
| CP, g/kgDM          | 99.7        | 89.5      | 100.7  | 89.5     | 68.8 |
| Ca, g/kgDM          | 16.1        | 14.7      | 14.4   | 11.5     | 8.6 |
| P, g/kgDM           | 1.7         | 1.4       | 1.1    | 1.2      | 1.0 |
| NDF, g/kgDM         | 619.8       | 650.1     | 505.7  | 528.0    | 547.5 |
| ADF, g/kgDM         | 396.7       | 404.3     | 274.0  | 314.9    | 317.8 |
| ADL, g/kgDM         | 76.6        | 79.4      | 75.3   | 79.6     | 81.1 |
| Ash, g/kgDM         | 75.4        | 85.4      | 76.2   | 86.2     | 84.7 |
| EE, g/kgDM          | 24.4        | 22.3      | 24.1   | 22.9     | 20.2 |
| DMI, kg             | 1.8         | 2.0       | 1.0    | 1.1      | 1.1 |

**DE**: digestible energy; **CP**: crude protein; **P**: phosphorous; **Ca**: calcium; **NDF**: neutral detergent fibre; **ADF**: acid detergent fibre; **ADL**: acid detergent lignin; **EE**: ether extract; **DMI**: dry matter intake.
collection period and stored at −20°C to use for future chemical analyses. Percentage of contribution for each forage species is presented in Table 3.

### 2.2.3. Slaughtering procedures and tissues sampling

At the end of the each experiment, a total of 16 goats including 8 goats from the pastured-fed group and 4 goats from each unit of the TMR-fed group were randomly selected and slaughtered by exsanguination. Before being slaughtered, the goats were prevented from consuming food for 24 h and from drinking for 2 h. Immediately after death, longissimus thoracis (LT) muscle and subcutaneous adipose tissue (SAT) samples for genes expression analysis were collected from the left side of carcase at the 5th lumbar vertebra levels under sterile conditions, snap frozen in liquid N2 and stored at −80°C until RNA extraction. Second samples of LT and SAT samples 50 g were vacuum-packed and stored at −20°C until lipid extraction and determination of FA composition.

### 2.3. Chemical analysis

#### 2.3.1. Proximate analysis of diets and faeces

The grass, TMR feed and faeces samples were analysed for the proximate chemical composition by drying them in oven at 60°C till constant weight according to the method of the Association of Official Analytical Chemists (AOAC 2004). Nitrogen was determined by Kjeltc Auto Analyzer and then converted to crude protein (CP = N × 6.25, AOAC, 1995, method no. 984.13). Ether extract (EE, AOAC, 1995, method no. 920.39) was determined by extracting the sample with diethyl ether (60−75°C) using a Soxhlet extractor. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991) with an Ankom 220 Fiber Analyser (Ankom Co., U.S.A.) and were expressed inclusive of residual ash. Heat-stable amylase was not used in the NDF determination. Samples were ashed in a muffle furnace at 550°C for 4 h to determine the ash content. Gross energy (GE) was measured using an adiabatic calorimeter bomb (IKA C7000, Staufen). Meanwhile, representative samples of faeces, grass and TMR feed were dried in oven at 105°C for 48 h to measure dry matter (DM). The grass ingredients and faecal samples were also analysed for Acid Insoluble Ash (AIA) using the 4N HCl procedure described by Vogtmann et al. (1975).

#### 2.3.2. Calculation of dry matter intake

Feed intake of stall-fed animals were recorded daily based on the amount of feed offered and refusals, and then dry matter intake (DMI) was calculated according to the percentage of DM in the feed (presented in Table 1). Dietary intake of pasture-fed animals were assessed by 4N-HCl AIA marker in combination with total faecal collection, according to the following equation:

\[ \text{DMI} = \text{faecal output} \times \% \text{AIA in faeces} / \% \text{AIA in grasses} \]

where faecal output is expressed as dry matter amount (presented in Table 2).

#### 2.3.3. Measurement of FA

Fatty acid methyl esters were produced from 0.5 g sample (feed, muscle or adipose tissue) according to the method of O’Fallon et al. (2007). For the determination of FA concentration, a GC-2014 gas chromatograph (Shimadzu International Trading Co., Ltd, Kyoto, Japan) with a HP-88 column (Agilent, 100 m × 0.25 mm ID × 0.2 μm film thickness) was used. The injector temperature was 250°C, and the column was programmed to run at 120°C for 10 min, warm to 230°C at 3.2°C/min, and hold for 35 min to achieve a satisfactory separation. Nitrogen was the carrier gas with a flow rate of 1.75 ml/min and split ratio of 1:50.

### 2.4. RNA extraction and real-time PCR

Total RNA was extracted from the 200 mg of frozen LT muscle and SAT samples using the Trizol reagent (TaKRa, No.D9108A) according to the manufacturer’s recommendations. Total RNA was purified using NucleoSpin mini kit columns combined with a DNA digestion on NucleoSpin membranes according to the manufacturer’s protocol (Machery-Nagel, Hoerdt, France). The extracted RNA was dissolved in DEPC-treated water, and the concentration, purity and integrity were assessed by using 2% agarose gel electrophoresis and microplate reader (SynergyH4, BioTek, U.S.A.) at 260/280 nm (OD260/OD280 = 1.8–2.0).

### Table 3. Percentage of contribution for each forage species.

| Species              | Adult goats | Kid goats |
|----------------------|-------------|-----------|
|                      | 1–30 d      | 31–60 d   |
|                      | 61–90 d     |
|                      | 1–30 d      | 31–60 d   |
|                      | 61–90 d     |
| Stipa capillata      | 3.4         | 0.4       | 2.7 | 1.6 | 25.2 |
| Cleistogenes Keng    | 0.5         | 32.2      | 1.6 | 33.9| 1.6  |
| Artemisia aurata     | 0.9         | 31.2      | 4.6 | 21.6| 16.8 |
| Caragana             | 3.8         | 0.4       | 7.5 | 0.8 | 16.8 |
| Ammopiptanthus mongolicus | 0.5     | 4.8       | 1.3 | 8.4 | 19.3 |
| Ceratoides lanata    | 80.5        | 20.4      | 69.9| 12.7| 10.0 |
| Saussola collina     | 10.1        | 10.2      | 12.0| 20.7| 10.0 |
First-strand cDNA was synthesised in a volume of 20 µL using 1 µg of total RNA and the PrimeScript® RT reagent (TaKaRa, No.DRR036A). β-actin was used as the internal control. The primers used are presented in Table 4. Real-time PCR reactions were carried out in 20 µL reactions containing 10 µL of 1 × SYBR Premix Ex Taq™ (TaKaRa, No. DRR081A), 2 µL cDNA, 0.4 µL each of 0.2 µM forward and reverse primers, and 7.2 µL RNase-free water. The reactions were performed in a BIO-RAD iCycler Thermal Cycler w/iQ5 Optical Module for Real-time PCR machine with an initial denaturing step of 95°C for 30 s followed by 40 cycles of 95°C for 30 s (denaturation), various annealing temperatures (designed in Table 3), 72°C for 20 s (extension) and then 51 cycles of 70°C for 0.06 s (drawing melting curve). The specificity of the PCR amplification was confirmed by melting curve analysis and 2% agarose gel electrophoresis of the PCR products. The efficiency of PCR amplification for each gene was calculated with the standard curve method (E = 10^{-1/slope}). The standard curves for each gene were generated by five-fold serial dilution of pooled cDNA. Each sample was run in triplicate, and averaged triplicates were used to assign cycle threshold (CT) values. The ΔCT values were generated by subtracting experimental CT values from the CT values for β-actin targets amplified with each sample. The group with the highest mean ΔCT value (lowest gene expression) per amplified gene target was set to zero, and the mean ΔCT values of the other groups were set relative to this calibrator (ΔΔCT). The ΔΔCT values were calculated as powers of 2^{-ΔΔCT}, to account for the exponential doubling of the PCR.

### 2.5. Statistical analysis

A t-test was performed to compare the effects of pasture vs. TMR feed on goat muscle and adipose tissue FA and lipogenic gene expression. The data means were considered significantly different at \( p < .05 \), and tendencies were considered at \( .05 < p \leq .10 \).\\

### 3. Results

#### 3.1. Fatty acid profile of experimental diets

FA profiles of the experimental diets are presented in Table 5. Pasture grass had higher levels of C14:0, C18:3 n3, PUFA and \( \alpha \)-6PUFA than TMR, but the C16:0, C18:0, C18:1 n9c, C18:2 n6c, MUFA and \( \alpha \)-6PUFA levels were lower than TMR. In addition, \( \alpha \)-6/\( \alpha \)-3 ratios of the pasture grass was lower, but P/S value was higher than that of TMR.

#### 3.2. Growth performance

The effects of pasture and TMR on BW and average daily gain (ADG) in adult goats are presented in Table 6. BWs during the whole experimental period were significantly higher in adult goats from the TMR-fed group than from the pastured-fed group (\( p < .05 \)), although adult goats fed TMR tended to (\( p = .059 \)) be higher than adult goats fed grasses on d15. Total weight gain and ADGs were greater (\( p = .001 \)) in TMR-fed adult goats than in pasture-fed adult goats, although the differences were not significantly during d 1 to d 15 and d 16 to d 30.
As shown in Table 7, BWs in TMR-fed kid goats were significantly higher than in the pasture-fed kid goats throughout the whole period \((p = .001)\). TMR-fed kid goats had a greater total weight gain than in pasture-fed kid goats \((p = .001)\). TMR feeding resulted in higher ADG at all trial periods except d 31 to d 60.

### 3.3. Fatty acid profiles of muscle and adipose tissue in adult goats

The effects of pasture and TMR on FA profiles of LT muscle in adult goats are presented in Table 8. TMR-fed goats had significantly increased proportions of C16:0, C17:0, C18:0, C14:1n9c, C18:1n9c, C18:2n6c, C20:4n6, \(\omega-6\)PUFA and \(\omega-6/\omega-3\) compared to pasture-fed goats \((p < .05)\), and C16:1n9c and MUFA showed a tendency \((p = .060, p = .080)\) to be higher in TMR-fed goats than in pasture-fed animals. In contrast, TMR-fed goats had lower levels of C14:0, C15:0, C18:1n9t, C18:2n6t, C20:3n6, C18:3n3, EPA, DHA, \(\omega-3\)PUFA, PUFA and P/S in LT muscle than pasture-fed goats \((p < .05)\).

The values for FA profiles in the SAT of adult goats are presented in Table 9. C10:0, C14:0, C16:0, C18:0, C14:1n9c, C16:1n9c, C18:3n6, C20:3n6, SFA, \(\omega-6/\omega-3\) and S/U in SAT from TMR-fed adult goats were significantly higher than from pasture-fed goats, whereas C18:1n9t, C18:2n6t, C18:3n3, C20:3n3, EPA, DHA, PUFA, UFA, \(\omega-3\)PUFA and P/S were significantly lower in SAT from TMR-fed goats compared to pasture-fed goats \((p < .05)\). C18:2n6c in TMR-fed adult goats showed an increased tendency \((p = .062)\) compared to pasture-fed goats.

### 3.4. Gene expression in muscle and adipose tissue of adult goats

The relative expression of genes in the LT muscle of TMR-fed adult goats compared to pasture-fed is presented in Table 10. The acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), the lipoprotein lipase (LPL), stearoyl-CoA desaturase (SCD), SREBP1-c and C/EBP \(\alpha\) genes showed a higher level of expression in the TMR-fed adult goats compared with the pasture-fed goats \((p < .05)\). Conversely, hormone-sensitive lipase (HSL) and PPAR\(\gamma\) genes expressions showed a significant reduction in the TMR-fed goats compared to pasture-fed \((p < .05)\).

The relative gene expression in the SAT of the TMR-fed adult goats compared to pasture-fed is shown in Table 11. The ACC, FAS, LPL, SCD, SREBP1-c and C/EBP\(\alpha\) genes showed higher expression levels in the TMR-fed adult goats compared to the pasture-fed \((p < .05)\). However, TMR promoted HSL and PPAR\(\gamma\) down-regulation in comparison to the pasture \((p < .05)\).

### 3.5. Fatty acid profiles of kid goats muscle and adipose tissue

The effects of pasture and TMR on FA profiles of LT muscle in kid goats are presented in Table 12.
Table 6. Effects of feeding system on BW (kg) and ADG (g) of AWCGs adult female goats.

| Item            | Pasture-fed | TMR-fed | SEM  | p value |
|-----------------|-------------|---------|------|---------|
| BW, kg          | n = 30      | n = 30  |      |         |
| 15 d            | 41.88       | 42.48   | 0.221| .059    |
| 30 d            | 44.43b      | 45.18a  | 0.195| .009    |
| 45 d            | 45.93b      | 49.38a  | 0.239| .001    |
| 60 d            | 46.68b      | 52.08b  | 0.212| .001    |
| Total gain      | 6.33b       | 11.70a  | 0.183| .001    |
| ADG, g          |             |         |      |         |
| 1–15 d          | 100.00      | 140.00  | 0.024| .216    |
| 16–30 d         | 170.00      | 180.00  | 0.016| .626    |
| 31–45 d         | 100.00f     | 280.00e | 0.020| .001    |
| 46–60 d         | 180.00      | 180.00  | 0.011| .001    |
| 1–60 d          | 105.00b     | 195.00f | 0.011| .001    |

*BW: body weight; ADG: average daily gain.

*bMeans within a row with different superscripts (a, b) are different between pasture- and TMR-fed goats (p < .05).

Table 7. Effects of feeding system on BW (kg) and ADG (g) of AWCGs kid goats.

| Item            | Pasture-fed | TMR-fed | SEM  | p value |
|-----------------|-------------|---------|------|---------|
| BW, kg          | n = 30      | n = 30  |      |         |
| 30 d            | 22.16b      | 23.94a  | 0.250| .001    |
| 60 d            | 25.09b      | 26.71a  | 0.256| .001    |
| 90 d            | 26.14b      | 30.22a  | 0.292| .001    |
| Total gain      | 5.78b       | 9.86a   | 0.237| .001    |
| ADG, g          |             |         |      |         |
| 1–30 d          | 60.14c      | 119.43c | 8.179| .001    |
| 31–60 d         | 97.82      | 94.25   | 9.384| .789    |
| 61–90 d         | 34.64a      | 115.05c | 7.058| .001    |
| 1–90 d          | 64.27c      | 109.58c | 3.980| .001    |

*BW: body weight; ADG: average daily gain.

*bMeans within a row with different superscripts (a, b) are different between pasture- and TMR-fed goats (p < .05).

C17:0, C18:0, C18:1n9c, C20:3n3, MUFA and ω-6/ω-3 of LT muscle were higher in TMR-fed kid goats than in the pasture-fed kid goats (p < .05). In LT muscle, C18:2n6c, SFA and S/U tended (p = .06, p = .07, p = .07) to be more abundant in TMR-fed kid goats than in pasture-fed kids. However, C14:0, C16:1n9c, C18:1n9t, C18:2n6t, C18:3n6, C20:3n6, C20:3n3, C18:3n3, EPA, DHA, PUFA, ω-3PUFA and P/S in the LT muscle of the TMR-fed kid goats had lower values than that of the pasture-fed kids (p < .05). UFA tended to be less abundant in TMR-fed than in pasture-fed kids (p = .08). The values for FA profiles in the SAT of kid goats are presented in Table 13. In SAT, TMR-fed kid goats relative to pasture-fed had higher contents of C10:0, C14:0, C17:0, C14:1n9c, C16:1n9c, C18:2n6c, C18:3n6, C20:3n6, MUFA, ω-6PUFA and ω-6/ω-3 (p < .05) but lower contents of C18:1n9t, C18:2n6t, C18:3n3, EPA, DHA and ω-3PUFA (p < .05). TMR-fed kid goats had a tendency toward increased C18:0 (p = .08) compared to pasture-fed kids.

3.6. Gene expression in muscle and adipose tissue of kid goats

The relative expression of the genes in the LT muscle of pasture-fed kid goats compared to TMR-fed is shown in Table 14. The expression levels of ACC, FAS, LPL, SCD, SREBP1-c and C/EBPα in LT muscle were significantly higher in the TMR-fed kid goats compared to the pasture-fed kids (p < .05). However, the HSL and PPARγ genes had significantly lower expression levels in the TMR-fed kid goats than the pasture-fed kids (p < .05).

The mRNA expression of the SAT genes of kid goats fed pasture and TMR is shown in Table 15. ACC, FAS, LPL, SCD and C/EBPα showed higher expression levels in TMR-fed kid goats compared to pasture-fed (p < .05). In reverse, HSL and PPARγ showed lower expression levels in TMR-fed kid goats compared to pasture-fed (p < .05). The expression levels of SREBP1-c had a tendency to be higher in TMR-fed kid goats (p = .062) in pasture-fed goats.
4. Discussion

4.1. Fatty acid profile

With the increasing demand for mutton, adult and kid cashmere goats have become one of the main sources of mutton production. However, the Inner Mongolia grassland of northern China is a seriously desertified region caused by a specific climate and grazing animals, which have decreased the biomass productivity.
Mongolian goat husbandry. The current study indicated that indoor feeding system improved production more efficiency than grazing. However, there is no information about the FA profiles of kid and adult AWCG meat, particularly in regard to whether their FA profiles are changed with TMR feeding compared with pasture-fed.

On the basis of this, we explored whether FA profiles was changed. In both feeding regimes, the predominant FAs in intramuscular fat and SAT were C16:0 and C18:0 as SFA, C18:1n9c as MUFA and C18:2n6c as PUFA. These results were similar to those reported by Lee et al. (2008). In a review, Salter (2013) noted that C14:0 and C16:0 have been shown to raise serum levels of low-density lipoproteins, a risk factor for cardiovascular disease in humans. In our present study, the levels of C16:0 in LT muscle and C14:0 in SAT were significantly higher in TMR-fed adult and kid goats, which also indicated TMR feed and pasture feed had significant effects on the FA profiles in tissues of goats, and the causes of these differences were associated with the FA profiles of the ingested diets. Ebrahimi et al. (2014) reported that the reduction of SFA in intramuscular tissue of Boer kid goats obtained by feeding flaxseed oil was mainly due to the inhibitory effect of α-linolenic acid (C18:3n3) and/or its biohydrogenation products on de novo FA synthesis. Firstly, in our study, pasture grass typically contained more C18:3n3 as primary FA than TMR, which could be one explanation for some of the decrease in individual and total SFA in tissues of the pasture-fed goats. Secondly, inclusion of pasture in the diet may lead to alterations in the ruminal environment other than increasing C18:3n3 supply. The type and the source of dietary carbohydrates could induce different ruminal conditions, leading to changes in microbial fermentation patterns (Noci et al. 2005). Our previous study found that compared with pasture feeding, TMR feeding significantly increased the production of acetic (p = .013) and propionic (p = .006) acid, but slightly decreased the ratio of acetico propionic (p = .360) in rumen of adult female goats (Sun et al. 2013). Acetic acid is the main substrate of de novo biogenesis of FA in the rumen. Propionate produced in the rumen could be utilised for gluconeogenesis, providing energy for de novo biogenesis of FA. Therefore, the content of SFA increased in tissues of our TMR-fed animals. Thirdly, dietary EPA and DHA, have been particularly successful in inhibiting the last step of biohydrogenation, the conversion of trans11-18:1 to C18:0 (Chilliard et al. 2007). This was an explanation for

Therefore, indoor feeding is becoming important in Mongolian goat husbandry. The current study indicated that indoor feeding system increased BW and ADG of goats due to the higher DE, CP and lower NDF and ADF contents in diets in concordance with Das et al. (2014), Shi et al. (2014) and Wang et al. (2014), which also indicated that indoor feeding system improved production more efficiency than grazing.

### Table 13. Effect of feeding system on SAT fatty acid profile of AWCGs kid goats (g/100 g total fatty acid).

| Fatty acid | Pasture-fed, n = 8 | TMR-fed, n = 8 | SEM | p value |
|-----------|-------------------|----------------|-----|--------|
| C1:0      | 0.083b            | 0.103*         | 0.003 | .001   |
| C12:0     | 0.121b(a)         | 0.129          | 0.003 | .120   |
| C14:0     | 0.139b            | 0.174*         | 0.004 | .001   |
| C14:1n9c  | 1.762b(a)         | 1.923*         | 0.034 | .010   |
| C15:0     | 0.389b            | 0.392a         | 0.007 | .780   |
| C16:0     | 10.401a           | 10.222*        | 0.233 | .620   |
| C16:1n9c  | 1.394b            | 1.761a         | 0.034 | .001   |
| C17:0     | 1.766b            | 1.917*         | 0.040 | .030   |
| C18:0     | 18.006a           | 18.184a        | 0.097 | .080   |
| C18:1n9t  | 6.667a            | 5.511b         | 0.123 | .001   |
| C18:1n9c  | 25.026a           | 25.865a        | 0.423 | .270   |
| C18:2n6   | 1.491a            | 1.267b         | 0.009 | .001   |
| C18:2n6c  | 12.837b           | 13.336*        | 0.087 | .044   |
| C18:3n6   | 0.162b(a)         | 0.195a         | 0.004 | .001   |
| C18:3n3   | 1.633a            | 1.348b         | 0.011 | .016   |
| C20:3n6   | 0.120a            | 0.103b         | 0.002 | .001   |
| C20:4n6   | 0.105b(a)         | 0.103a         | 0.001 | .120   |
| C20:5n3   | 0.016b(a)         | 0.010b         | 0.001 | .001   |
| C22:6n3   | 0.292a            | 0.232b         | 0.007 | .001   |
| SFAa      | 43.345b(a)        | 43.475*        | 0.233 | .730   |
| MUFAb     | 32.656b           | 34.270a        | 0.363 | .020   |
| PUFAa     | 16.880b           | 16.749*        | 0.094 | .360   |
| UFAa      | 56.308b           | 56.525a        | 0.304 | .640   |
| ω-6PUFAa  | 13.316b           | 13.793*        | 0.087 | .006   |
| ω-3PUFAa  | 2.072b            | 1.690b         | 0.009 | .001   |
| ω-6/ω-3   | 6.428b(a)         | 8.161*         | 0.044 | .001   |
| S/E       | 0.776a            | 0.769*         | 0.010 | .650   |
| P/S       | 0.386b(a)         | 0.385*         | 0.004 | .820   |

- SFA: total saturated fatty acids, sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0 and C18:0; MUF: total monounsaturated fatty acids, sum of ω-6PUFA and ω-3PUFA; ω-6PUFA: sum of C18:2n6, C18:2n6c, C18:3n6, C20:3n6 and C20:4n6; ω-3PUFA: sum of C18:3n3, C20:3n3, C20:5n3 and C22:6n3; UFA: total unsaturated fatty acids, sum of MUF and PUFA; ω-6/ω-3: ω-6PUFA/ω-3PUFA; S/U: SFA/UFA; P/S: PUFA/SFA.
- SEM: standard error of the mean.
- Means within a row with different superscripts (a, b) are different between pasture- and TMR-fed goats (p < .05).

### Table 14. Effect of feeding system on relative expression of lipogenic and lipolysis genes in the LT muscle of AWCGs kid goats.

| Gene symbol | Pasture-fed, n = 8 | TMR-fed, n = 8 | SEM | p value |
|-------------|-------------------|----------------|-----|--------|
| ACC         | 1.000b(a)         | 1.564*         | 0.013 | .001   |
| FAS         | 1.000b(a)         | 1.203*         | 0.026 | .001   |
| LPL         | 1.000b(a)         | 1.581*         | 0.040 | .001   |
| HSL         | 1.000b(a)         | 0.515b         | 0.008 | .001   |
| SCD         | 1.000b(a)         | 2.412b         | 0.020 | .001   |
| SREBP1-c    | 1.000b(a)         | 1.525b         | 0.028 | .001   |
| C/EBPα      | 1.000b(a)         | 1.230*         | 0.062 | .040   |
| PPARγt      | 1.000b(a)         | 0.863b         | 0.003 | .001   |

- PPARγ: Peroxisome proliferator-activated receptor gamma; SREBP1c: Sterol regulatory element binding transcription factor 1c; C/EBPα: CCAAT/enhancer-binding proteins alpha; FAS: Fatty acid synthetase; LPL: Lipoprotein lipase; ACC: Acetyl-CoA carboxylase; SCD: Stearoyl-CoA desaturase; HSL: Hormone-sensitive lipase.
- SEM: standard error of the mean.
- Means within a row with different superscripts (a, b) are different between pasture- and TMR-fed goats (p < .05).
C18:0 concentration in tissues from TMR-fed goats was higher than from pasture-fed goats. Finally, this dietary effect may be related to a change in the FA composition of adipocyte membranes, which is strongly influenced by the relative abundance of PUFA in the diet (Hulbert et al. 2005). Such changes could alter membrane fluidity, which is involved in the mechanisms of FA transport across membranes (Bojesen and Bojesen 1999), leading to the different contents of FAs in adipocyte. Therefore, in our study, the differences in FA composition in tissues between TMR- and pasture-fed goats were presented.

Consumption of ω-3PUFA has been shown to positively influence immune function, blood pressure, cholesterol and triglyceride levels, in addition to cardiovascular function in humans (Mozaffarian and Wu 2012). These benefits are almost certainly a consequence of the conversion of the C18:3ω-3PUFA to EPA and DHA. ACC and FAS are key enzymes in the de novo synthesis of SFA, and SC1D1, a key enzyme for FA desaturation (Dervishi et al. 2011). Previous studies have shown that C18:3n3 supplementation decreases gene expression of SREBP1-c, a transcription factor, which exerts a crucial function in the transcriptional control of genes related to FA synthesis (ACC and FAS), both in vivo and in vitro (Zhu et al. 2014). The regulation of the lipid metabolism in adipose tissues in response to dietary changes (different ω-3 and/or ω-6 PUFA supplementation) has been investigated in sheep and lambs (Dervishi et al. 2011; Gallardo et al. 2015), but there are only few studies in muscle and adipose tissues of goats. In the present study, higher ACC and FAS gene expression levels were detected in the LT muscle and SAT of AWCG adult and kid goats fed TMR.
compared with pasture-fed goats. This difference can be explained by up-regulated gene expression of SREBP1-c, a transcriptional activator of genes encoding enzymes involved in the de novo synthesis of FA such as ACC and FAS, which is consistent with C16:0 and total SFA in adult goats. The up regulation of SREBP1-c can be partly explained by the lower concentration of C18:3n3 in TMR compared with pasture grass (see Table 5). Grazing lambs showed lower levels of ACC, FAS and SCD1 gene expression in semitendinosus muscle compared with lambs grazing alfalfa with supplements/indoor feeding and consequently diminished levels of de novo synthesised FA (Conte et al. 2012). Ebrahimi et al. (2014) showed down-regulation of SCD gene in the semitendinosus muscle for a high C18:3n3 group compared with a low C18:3n3 group, which is in agreement with our present findings where down-regulation of SCD occurred in the pasture-fed goats with the highest C18:3n3 concentrations resulting in a decrease of C18:1c. LPL is a rate-limiting enzyme for the hydrolysis of the triacylglycerol (TG) core of circulating TG-rich lipoproteins, chylomicrons and very low-density lipoprotein. LPL-catalysed reaction products, FA and monoacylglycerol are taken up in part by adipose tissue and skeletal muscle and stored as neutral lipids (Wang and Eckel 2009). These results suggest that the LPL gene may be a genetic marker predictor of intramuscular fat deposition. TMR-fed adult and kid goats had a higher relative expression of LPL in the LT muscle and SAT than pasture-fed goats. HSL is an essential enzyme in lipolysis metabolism and energy homeostasis that can hydrolyse stored triacylglycerol (Lampidonis et al. 2011). Our study revealed that TMR-fed adult and kid goats had less HSL expression level in LT and SAT than pasture-fed goats, which indicated that the pasture grass energy level did not meet their growth requirements. Guo et al. (2015) noted that C/EBPβ is induced early to transactivate the expression of C/EBPα and PPARγ, two master transcription factors for terminal adipocyte differentiation during 3T3-L1 adipocyte differentiation. Only a few authors have studied the impact of dietary ω-3PUFA on transcription factor gene expression in goats. In Boer goats, a higher C18:3n3 in the diet upregulated PPARγ gene expression in the semitendinosus muscle compared with a lower C18:3n3 group (Ebrahimi et al. 2014). In the present study, according to dietary intake, ω-3PUFA intake of adult goats experienced pasture-fed was 16.40 g/day during d 1 to d 30, which was much higher than stall-fed adult goats 2.14 g/day. Similar trends was observed during d 31 to d 60; ω-3PUFA intake of kid goats experienced pasture-fed was 9.140 g/day, which was much higher than stall-fed kid goats 1.28 g/day. Similar trends was presented during d 31 to d 60 and d 61 to d 90. Our results clearly support a relationship between PPARγ gene expression and ω-3PUFA intake. PPARγ expression levels in muscle and adipose tissue from pasture-fed adult and kid goats were significantly higher than that in the TMR-fed goats. The expression levels of C/EBPα and LPL genes had significant correlations with fat deposition in tissues of sheep (Wei et al. 2014). The results herein presented for C/EBPα expression are higher in LT muscle and SAT from TMR-fed goats compared to pasture-fed goats.

5. Conclusions

The FA composition and the expression of lipid metabolism gene in LT muscle and SAT of AWCG were affected by the feeding regimens. Compared with TMR feeding, pasture feeding could improve the nutritional value of the chevon by significantly increasing the muscle ω-3 FA concentration, making it a healthier product.

Ethical approval

All procedures involving animals were evaluated and approved by the guidelines of the Animal care and Use Committee of the Inner Mongolia Agriculture University (Hohhot, Inner Mongolia, China).

Acknowledgements

The authors gratefully acknowledge the staff of the breeding farm of AWCG in Etuoke banner of Ordos in Inner Mongolia and all members of our research group in Inner Mongolia Agriculture University.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by National Key R&D Program of China [2017YFD0500504] and National Public Welfare Industry (agricultural) Special Funds for Scientific Research [201003061].

ORCID

Sumei Yan http://orcid.org/0000-0002-4107-2858

References

AOAC. 1995. Official Methods of Analysis. 16th ed. AOAC, Washington, DC, USA.
Sprecher H. 1981. Biochemistry of essential fatty acids. Prog Lipid Res. 20:13–22.
Sun G, P, Yan S, M, He H, Wu T, M. 2013. Effect of different feeding ways on rumen fermentation function in Albas White Cashmere goats. China Feed Ind. 13:51–54.
Toral PG, Bernard L, Delavaud C, Gruffat D, Leroux C, Chilliard Y. 2013. Effects of fish oil and additional starch on tissue fatty acid profile and lipogenic gene mRNA abundance in lactating goats fed a diet containing sunflower-seed oil. Animal. 7:948–949.
Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.
Vatansever J, Kurt E, Enser M, Nute GR, Scollan ND, Wood JD, Richardson RL. 2016. Shelf life and eating quality of beef from cattle of different breeds given diets differing in n-3 polyunsaturated fatty acid composition. Anim Sci. 71:471–482.
Vogtmann H, Pfirter HP, Prabucki AL. 1975. A new method of determining metabolisability of energy and digestibility of fatty acids in broiler diets. Br Poult Sci. 16:531.
Wang H, Eckel RH. 2009. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 297:E271–E288.
Wang D, Zhou L, Zhou H, Hou G, Li M, Shi L, Huang X, Guan S. 2014. Effects of nutrition level of concentrate-based diets on growth performance and carcass characteristics of Hainan black goats. Trop Anim Health Prod. 46:783–788.
Wei X, Xu X, C, Yang Y, X, Wang X, L, Chen Y, L, Niu W, Z, Kou Q, F. 2014. Differential expression of CCAAT/enhancer binding protein α(C/EBPα) and lipoprotein lipase (LPL) genes in tail adipose tissues of sheep (Ovisaries) with different types of tail. J Agric Biotechnol. 22:598–606.
Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RL, Hughes SJ, Whittington FM. 2008. Fat deposition, fatty acid composition and meat quality: a review. Meat Sci. 78:343–358.
Zhang XQ, Luo HL, Hou XY, Badgery WB, Zhang YJ, Jiang C. 2014. Effect of restricted time at pasture and indoor supplementation on ingestive behaviour, dry matter intake and weight gain of growing lambs. Livest Sci. 167:137–143.
Zhu JI, Luo J, Wang W, Yu K, Wang HB, Shi HB, Sun YT, Lin XZ, Li J. 2014. Inhibition of FASN reduces the synthesis of medium-chain fatty acids in goat mammary gland. Animal. 8:1469–1510.