The effect of periodic energy restriction on growth performance, serum biochemical indices, and meat quality in sheep

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ABSTRACT: Energy is a key factor regulated by the neuroendocrine system. The objective of this study was to compare growth performance, serum biochemical indices, carcass traits, meat quality, and nutrient composition of lambs fed restricted metabolizable energy (ME) intakes to mimic the seasonal changes of the natural grasslands of northwest China. Nineteen male Dorper × Small Tailed Han lambs were assigned to treatments, control (CON) fed at $1.0 \text{ MJ/W}^{0.75} \times \text{d}^{-1}$ throughout the 180 d study, and restricted ME (RES) fed by sequentially restricting ME every 30 d (0.56, 0.84, 1.0, 0.84, 0.56, and $0.28 \text{ MJ/W}^{0.75} \times \text{d}^{-1}$). Lambs were harvested at the end of the study. Lambs fed the CON diet demonstrated higher ($P < 0.05$) average daily gain (ADG) compared with RES-fed lambs (158.79 and 57.01 g/d, for CON and RES, respectively), although ADG were lower in each period with the last period being a negative ADG for RES-fed lambs resulting in a loss of body weight. Both treatments demonstrated lower ADG in the last 3 study periods compared with the first 2 periods, which may be explained by cold stress ($< -3^\circ \text{C}$) increasing the lamb's energy requirement. Feeding lambs a restricted ME diet resulted in lower ($P < 0.05$) blood serum glucose,

triglycerides, total protein, calcium, phosphorus, immunoglobulin G, immunoglobulin M, and immunoglobulin A concentrations compared with CON-fed lambs, but both treatments were similar ($P > 0.05$) for cholesterol and high-density lipoprotein-cholesterol. Feeding lambs a restricted ME diet increased ($P < 0.05$) meat pH, while reducing the cooked meat rate compared with CON-fed lambs. Thus, the carcass yield, rib eye muscle area, and shear force were lower ($P < 0.05$) for RES-fed lambs compared with CON-fed lambs. The meat water concentration was higher ($P < 0.05$), whereas the concentrations of dry matter, crude fat, crude protein, and carbohydrate were lower ($P < 0.05$) for lambs fed a RES ME diet compared to CON-fed lambs. These results demonstrate that sequentially restricting ME intake, as natural grasslands might experience during seasonal forage quality and quantity changes, resulted in a reduction of body weight because lambs were mobilizing body reserves as a source of ME. The reduction in concentrations of key serum nutrients, as well as carcass traits and meat composition, demonstrate the body is responding to the meet the lambs ME requirements, which has implications for both meat quality and the animal's physiological functions.

Key words: energy restriction, growth performance, lambs, metabolism, serum biochemical indices

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INTRODUCTION

Sheep graze on the natural grasslands in the semiarid and arid pastoral area of Northwest China. Sheep production is highly dependent on the grassland quantity and quality (Selvaggi et al., 2014; Toro-Mujica et al., 2015). The metabolizable energy (ME) concentration of grass changes with the grazing season and is influenced by temperature and rainfall (Chen et al., 2017). In the warm season (June to October), the ME concentration of grass is greater than the sheep's maintenance requirement, while ME is deficit during the cold season from November to May (Yang et al., 2012). Thus, the grazing sheep must deposit reserves during the warm season and then mobilize those reserves [through body weight (BW) and body condition loss] during the cold season for survival (Obeidat et al., 2002; Craine et al., 2009; Wiecek et al., 2010; Skiba et al., 2012). Consequently, grass quantity and quality influences ME balance and glucose homeostasis (Daza et al., 2007). Furthermore, ME intake dictates growth performance (Cameron and Speakman, 2011; Kim et al., 2017) and meat composition and quality (Arsenos et al., 2002; de Araújo et al., 2017). Lamb growth performance is a major production management indicator while meat quality and flavor are related to consumer satisfaction. Although ME intake effects on BW gain/loss are well known, little scientific information exists linking changes in growth performance, serum biochemical indexes, and meat quality when the ME density of grass changes with the grazing season. The experimental objective was to simulate changes in grass pasture ME density during the 4 seasons via ME restriction on lamb growth performance, blood serum biochemical indexes, carcass traits, and meat quality and composition. These results could be useful to demonstrate ME balance and nutrient metabolism of sheep, which depends upon the quantity and quality of Northwest China's natural grasslands in providing guidance to herders on feeding management.

MATERIALS AND METHODS

Animals and Treatments Description

This experiment was conducted at the Lintao Huajia Animal Husbandry Co., Ltd., Gansu, China (35°42'N, 103°88'E, 2,055 m average above sea level). The regional climate is temperate, with a mean annual temperature of 7.80 °C and a mean annual rainfall of 565 mm, which has been

previously published (Song et al., 2017). The animals were managed, fed, and harvested according to the Chinese Standards for the Use and Care of Research Animals (He et al., 2016), the laboratory animals guideline of welfare and ethics (Standardization Administration of China, 2016) via the Gansu Agricultural University, Lanzhou, China, and harvested according to the Chinese National Standards of Human Food Animal Harvesting and Processing (Chinese Ministry of Agriculture, 2001).

Briefly, 18 male Dorper × Small Tailed Han lambs with BW (22.79 ± 1.42) kg and age (3.0 ± 0.5) mo were randomly assigned to 2 treatments [Control (CON) and Treatment (RES)], 18 lambs were arranged in order from 1,2,...18 by weight, then continuous 18 random numbers were taken from any one starting point and direction in the list of random numbers and copied under the animal numbers in turn. The random numbers were ranked from small to large and assigned in sequence to the animal sequence number, the first 9 lambs with random numbers are CON and the last 9 lambs are RES. All lambs in each group were fed as individuals. The CON ration (Table 1) was fed at $1.0 \text{ MJ/W}^{0.75} \times \text{d}^{-1}$ throughout the trial and the RES were fed with the ME intake being restricted sequentially every 30 d for 6 periods (0.56, 0.84, 1.00, 0.84, 0.56, and $0.28 \text{ MJ/W}^{0.75} \times \text{d}^{-1}$), which is similar to that of animals grazing a typical grass pasture from the grass turning green (April to May) to the grass wilting (December to March of following year) on northwest China's rangeland. The daily nutrient requirements were met for all lambs, except minerals and energy as designed, throughout the experiment (NRC, 2007). At the end of the 180 d experimental period, all lambs were harvested with the CON and RES lamb's weights being 51.60 ± 2.15 and 32.30 ± 1.85 kg, respectively. In this experiment, the RES-fed ME was maintained at 0.28 to $1.00 \text{ MJ/W}^{0.75} \times \text{d}^{-1}$, which is similar to that of animals grazing a typical grass pasture during the 4 seasons of northwest China's rangeland (Liang et al., 2015).

Animal Performance and Serum Biochemical Indices

During the experiment, lambs were weighed and a blood sample (glass tube without anticoagulant, 10 mL; Kangjian Medical Technology Co., LTD., Jiansu, China) was collected from the saphenous vein at 8 a.m. every 10 d before feeding. Daily BW gain was calculated based on the 10 d BW gain and the 3 measurements were average for individual

Table 1. Ingredient composition (% of DM) of diet and nutrient concentrations

Ingredient	Percentage, % of DM ¹	Nutrient levels	
Corn	19.84	Crude protein, g•kg ⁻¹	183.00
Wheat bran	3.20	Crude fat ² , g•kg ⁻¹	68.75
Soybean meal	7.88	Total calcium, g•kg ⁻¹	6.96
Rapeseed meal	7.88	Total phosphorus, g•kg ⁻¹	5.82
Premix ³ , 1%	0.40	ME ⁴ , MJ•kg ⁻¹	11.20
Limestone	0.40		
Salt	0.40		
Corn straw silage	60.00		

¹DM = dry matter.

²Fatty acid profile of the basal diet was 13.60, 8.20, 25.30, 26.80, 14.50, and 11.60% for C16:0, C18:0, C18:1, C18:2, C18:3, and other fatty acids, respectively.

³The premix composition was Vitamin A 15,000 IU, Vitamin D 5,000 IU, Vitamin E 50 mg, Fe 9 mg, Cu 12.5 mg, Mn 130 mg, Zn 100 mg, Se 0.3 mg, I 1.5 mg, Co 0.5 mg per kg.

⁴ME = metabolizable energy.

average daily gain (ADG) in each 30 d feeding stage. Furthermore, all lambs were weighed 3 consecutive times at the beginning and end of the experiment to ensure accurate beginning and ending mean BW.

Blood samples without anticoagulant were centrifuged at 2,810 ×g at 20 °C for 10 min and the supernatant was removed with a pipette (500 to 5,000 µL; Eppendorf Ltd., Hamburg, Germany) and stored in EP tubes, which are microcentrifuge tubes made of polypropylene (1.5 mL; Kangjian Medical Technology Co., LTD., Jiansu, China), at -20 °C for conducting further analyses at a later date (Venkatesan et al., 2006). Blood serum samples were thawed using a 37 °C water bath before measuring the biochemical indexes of total protein (TP), glucose (GLU), calcium (Ca), magnesium (Mg), phosphorus (P), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) using an automatic Biochemical Analyzer (BX-3010; Sysmex Co., Ltd., Chuo-ku, Kobe, Hyogo, Japan) according to the procedures of each kit as described by Li and Wu (2007). All kits were purchased from Huaxin Technology Co., Ltd. (Fanyu, Guanddong, China). Standards were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Serum TP concentrations were measured using the biuret method (Kit 170314); GLU concentrations were measured using a glucose oxidase method (Kit 161212); Ca and Mg concentrations were measured using the 2 toluidine blue method; P concentrations were measured using ammonium molybdate procedure; TG concentrations were measured using the glucose oxidase-peroxidase (GOD-POD) enzyme colorimetric method (Kit 1635503); HDL-C concentrations were measured using the method of direct enzyme (Kit

377AFL); and IgG, IgA, and IgM concentrations were measured using immune colorimetric method (Kits 377AFL, 377AFL, and 377AFL), separately.

Harvest Date, Carcass Traits, and Meat Quality

All the lambs were weighed, following withholding feed for 24 h and water for 2 h, and then harvested at the Lintao Huajia Animal Husbandry Co., Ltd. after the 180 d feeding experiment using the Islam exsanguination method (D'Agata et al., 2010). After exsanguination, a small cut was made through the hide with a scalpel and air was pumped between the hide and the subcutaneous fat (SF) to separate the wool and hide from the SF. Then, the head, hooves, and tail fat were separated, weighed, and sampled. Then, the abdominal wall was cut along the middle line of the abdomen with the viscera being removed with the kidney and kidney fat retained. After 30 min, the carcasses were weighed and then spilt longitudinally. The dressing percentage was calculated as: dressing percentage = (carcass weight/live weight) × 100%.

The left half-carcass was divided into 2 halves between the 12th and 13th rib of the spine with the left posterior half-carcass used for carcass quality analysis. Carcass quality parameters were analyzed according to the procedures described by Zhao (2000). A cross-sectional area of the 12th and 13th rib of the spine was drawn on sulfur paper and repeated twice and the area was determined using a planimeter (KP-21C; Koizumi Co. Ltd., Tsuchiura, Japan). The 2 measurements were averaged and reported as rib eye muscle area. Followed by measuring the total tissue thickness between the 12th and 13th rib and 11 cm from the back midline of the carcass was measured with a Vernier caliper (0

to 100 mm, Links HMCT Group, Harbin, China) and reported as GR (GR = the total tissue thickness between the 12th and 13th rib and 11 cm from the back midline of the carcass), which is the index of carcass fat content.

Meat quality sampling location was determined according to the methods described by Zhao (2000), and meat quality parameters were analyzed according to the methods described by Honikel (1998) and Zhao (2000). The left longissimus dorsi was used for the measurement of pH and dehydration rate; the right longissimus dorsi was used for the measurement of nutrient concentrations. The musculus semitendinosus was used for the measurement of the cooked meat rate and shear force. The left longissimus dorsi between the 5th and 10th rib was removed and taken to the laboratory where the pH and shear force were measured within 2 h postmortem. An incision was made into the muscle, and pH was measured with a pH meter (PB-10; Sartorius Co. LTD., Gottingen, German); an average of 3 random readings were reported for pH. Then, a 2.532 cm diameter and 1.5 cm thick sample of the left longissimus dorsi at the 13th ribs was collected using a Round sampler ($d = 2.532$ cm, Tengba instrument technology Co. Ltd., Shanghai, China), weighed, and placed in the middle of 36 layers of neutral filter paper followed by adding a piece of plastic pad on the top and bottom. Thirty-five kilograms of pressure were applied and held for 5 min. The pressure was released, the sample removed, and weighed. The loss in weight was used to calculate the dehydration rate as [(weight original sample – weight after pressing)/weight original sample] $\times 100$]. A fresh approximately 15 mm thick and weighing approximately 80 g musculus semitendinosus sample was collected from the left hind leg and placed in a plastic bag and stored at 15 to 16 °C for approximately 24 h, followed by storage at 4 °C for approximately 24 h, followed by warming to room temperature for approximately 1 h, and then cooked in an 80 °C water bath to an internal temperature of 70 °C for approximately 45 min. Cooked samples were allowed to cool for 30 min at room temperature and then weighed. The cooked meat rate was calculated as the cooked meat rate = (cooked meat weight/uncooked meat weight) $\times 100\%$. After weighing, the cooked meat samples were cut to approximately 2.5 cm thickness and 5 cm in length along the vertical direction of the muscle fiber, followed by cutting a round 1.27 cm diameter ($d = 1.27$ cm, Tengba Instrument Technology Co. Ltd.), following the muscle fiber direction and subjected to measurement of shear

force using an astral construct instrument (CT 3; Brookfield Co. Ltd., Middleboro, MA, USA) equipped with a 5 kg load cell and a crosshead speed of 6 mm/s. An average of 6 random readings were collected and reported as shear force. The right longissimus dorsi of the 11th and 13th ribs was vacuum packaged and transported to the laboratory and stored at –20 °C for later measurement of nutrient concentrations.

The right longissimus dorsi samples of the 11th and 13th ribs were stored at –20 °C, were thawed at room temperature, ground using a 4 mm screen using a meat grinder (AMG199AM3-200; Breko Co. Ltd., Wuhan, China), and analyzed in triplicate for moisture by drying a weighed sample at 105 °C (DHG-9030; Yiheng Co. Ltd., Shanghai, China) for 2 h, took it out and put in a dryer and cooled to room temperature, and weighed. Following drying at 105 °C for 1 h, it was taken out and put in a dryer and cooled to room temperature, weighed, and repeated the operation until reaching a constant weight (the difference between the 2 consecutive weights was less than 1 mg; Pregenolatto and Pregenolatto, 1985). Ash concentrations were determined placing a weighed sample in a muffle furnace (BSX2-6-12TP; Yiheng Co. Ltd.) at 600 °C for 4 h. Crude protein concentrations were determined using an automatic Kjeldahl nitrogen detector (Kjeltec 8400; Foss Co. LTD, Hilleroed, Denmark; AOAC, 1984). Total lipid concentrations were determined using the method of Bligh and Dyer (1959).

Statistical Analyses

All individual animal data (experimental unit) were subjected to least squares analysis of variance for a completely random design (CRD) using the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) as a CRD. The linear statistical model used was:

$$Y_{ij} = \mu + T_i + D_j + T_i \times D_j + e_{ij}$$

where Y_j is dependent variable, μ overall mean, T_j treatment, D_j day of experiment, $T \times D_{ij}$ the interaction of treatment by day of experiment, and e_{ij} is residual random error. Fixed effects were treatment, days of the experiment, and the interaction of treatment with days. The experimental day was analyzed as a repeated measure having an autoregressive covariance structure. Treatment means are presented as least squares means and significant differences were declared when $P < 0.05$ and trends declared at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Body Weight and Daily Weight Gain

Limiting energy intake resulted in lambs fed the CON diet having ($P < 0.05$) greater ADG compared with lambs fed the RES diet (158.79 vs. 57.01 g/d, for CON vs. RES, respectively). Energy restriction resulting in the decreased ADG is similar to previous research (Lasa et al., 2007; Tripathi et al., 2007). Per the study design, ADG were ($P < 0.05$) greater for lambs fed CON diet compared with lambs fed RES diet at the end of each phase. During the first 3 trial phases, lambs fed both treatments had gradually increasing ADG (Table 2 and Fig. 1) from 151.01 to 228.39 g/d, and 82.53 to 151.97 g/d, for CON vs. RES, respectively. However, in the last 3 trial phases, lambs fed RES diet demonstrated gradually decreasing to becoming a negative ADG (-38.33 g/d) in the last trial phase. Although the CON-fed lambs also demonstrated decreasing ADG in the last 3 phases, the ADG was always positive (122.41 to 130.30 g/d). This ADG reduction

for lambs fed CON was unexpected. The average temperature abruptly decreased to -5°C at stage 4 and then began to warm slowly by the end of the study, but the CON-fed lambs' minimum ADG (122.41 g/d) appeared in stage 5, lower than stage 4 and stage 6 (133.40 vs. 130.30 g/d, respectively). This may be explained by cold stress and the mobilization of body reserves for heat generation during the low temperature followed by compensatory replenishment of body reserves. The ADG reduction for lambs fed RES gradually decreased in the last 3 stages, because energy intake decreased gradually and the compensatory replenishment of body reserves following the low ambient temperatures.

The results of altering the feeding program resulted in the lambs fed CON gaining BW throughout the trial reaching a final BW of 51.60 kg in contrast to the RES-fed lambs that plateaued followed by losing BW due to the negative ADG during the last phase resulting in a final BW of 32.30 kg. The study design achieved the goal of mobilizing BW as an ME source when sheep were grazing a typical grass pasture in the cold season of northwest

Table 2. The growth performance of lambs fed at Control (CON) or restricted intake (RES)

Item	Period, days	CON ¹	RES ²	SEM ³	P-values
Average daily gain (g)	30	151.01	82.53	17.434	0.000
	60	187.23	105.44		<0.0001
	90	228.39	151.97		<0.0001
	120	133.40	38.02		<0.0001
	150	122.41	2.40		<0.0001
	180	130.30	-38.33		<0.0001
	Average	158.79	57.01	12.916	<0.0001

¹CON = Lambs fed Control ME intake of $1.0 \text{ MJ/W}^{0.75} \cdot \text{d}^{-1}$ throughout the 180 d.

²RES = Lambs fed Treatment ME intakes of 0.56, 0.84, 1.0, 0.84, 0.56, and 0.28 $\text{MJ/W}^{0.75} \cdot \text{d}^{-1}$, sequentially changed every 30 d.

³SEM = standard error of mean.

Figure 1. The average daily gain and body weight for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

China's rangeland, which altered the meat fatty acid composition resulting in an improved n-3 fatty acid profile, and reduced unsaturated fatty acid profile (Song et al., 2017). Therefore, research to determine the appropriate grazing lamb harvest time not only result in an improved n-3 fatty acid profile, but also retain its unsaturated fatty acid profile. Moreover, to harvest grazing lambs is a good option that aligns with people habits of eating mutton during the cold seasons.

Grass energy is the most important factor to regulate animal growth performance, while the animal's body fat mobilization for oxidation as an ME source is an important regulator of fat deposition (Shatha and Peter, 2017). Grass DM quantity generally varies dynamically with the seasons, throughout the late spring through early fall, while the nutritional

composition increases until the plant reaches maturity and then decreases during the later growing season, which is one of the main nutritional factors affecting the grazing sheep's growth (Luccia et al., 2003; Wiecek et al., 2010; Skiba, 2012) and production performance. The trial ME intake mimics the extreme fluctuations in pasture quantity and quality that can lead to fluctuations in lamb growth based on either positive or negative energy balance; thus, ME intake within certain ranges either increases the daily gain and growth performance of sheep or conversely, mobilizes the body fat as a ME source.

Serum Biochemical Indices

Limiting ME intake results in lower ($P < 0.05$) serum Ca (2.28 vs. 2.15 mmol/L) except stage 1, and P (2.57 vs. 2.22 mmol/L) concentrations except first 2 stages for lambs fed RES compared to lambs fed the CON during the course of the trial (Table 3 and Fig. 2). Certainly, restricting feed intake in this study reduce the nutrient intake of Ca and P. Therefore, future studies using energy restriction in sheep should have different mineral mixes balanced for each phase to ensure that the lamb's Ca and P requirements are still being met (NRC, 2007).

The lambs fed CON had greater ($P < 0.05$) GLU concentrations during the course of the trial compared to the lambs fed RES except stage 3 (Table 3 and Fig. 3), which resulted in the trial mean GLU concentrations being greater ($P < 0.05$) for lambs fed CON compared to lambs fed RES (4.35 vs. 3.84 mmol/L). The degree of energy restriction within each phase was indicative of the serum GLU concentrations. Serum GLU increased

Table 3. Slaughter performance of the lambs fed at Control (CON) or restricted intake (RES)

Slaughterperformance	CON ¹	RES ²	SEM ³	P-values
Dressing percentage ⁴ , %	44.28	33.76	0.623	< 0.001
Loin eye area, cm ²	36.31	23.40	2.113	< 0.001
GR ⁵ value, cm	1.54	0.92	0.112	< 0.001
pH, value	6.81	7.08	0.104	0.018
Shear force, kg	5.83	7.47	0.372	0.001
Dehydration rate, %	3.06	3.16	0.143	0.509
Cooked meat rate, %	61.56	60.34	0.431	0.012

¹CON = Lambs fed Control ME intake of 1.0 MJ/W^{0.75}•d⁻¹ throughout the 180 d.

²RES = Lambs fed Treatment ME intakes of 0.56, 0.84, 1.0, 0.84, 0.56, and 0.28 MJ/W^{0.75}•d⁻¹, sequentially changed every 30 d.

³SEM = standard error of mean.

⁴Dressing percentage = Carcass weight/BW × 100%.

⁵GR = Total tissue thickness between the 12th and 13th rib, 11 cm from the midline.

Figure 2. Serum Ca and P concentrations for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

for lambs fed both CON and RES as ME intake was increased per phase 1 through 3 of the study, but lambs fed RES demonstrated decreasing serum GLU concentrations with decreasing ME intake. As would be expected, the more severe the degree of ME restriction and mobilization of BW and fat would result in lower serum GLU concentrations. In contrast, no differences ($P > 0.10$) were detected in mean TG concentrations (0.41 vs. 0.43 mmol/L) for lambs fed both treatments. However, an interaction of treatment by day was detected ($P < 0.05$) for lambs fed RES having increasing TG concentrations during the first 3 phases of the study, but then decreasing as the ME restriction became more severe compared to the lambs fed CON. This observation can be explained by the animal's

energy intake and status (positive and negative) that is critical to serum TG concentrations because as energy intake increases the liver will synthesize TG for export to the body for energy storage, but during energy mobilization, the fat depots will be exporting energy in the blood in the form of free fatty acids and not as TG. Analyzing blood samples from this trial for nonesterified fatty acids (NEFA) or β -hydroxy butyrate may have added valuable information and understanding of body tissue mobilization.

Serum CHO (1.81 and 1.74 mmol/L) and HDL-C (0.03 vs. 0.05 mmol/L) were similar ($P > 0.05$) for lambs fed both treatments and was not influenced by trial day (Table 3 and Fig. 4). Serum TP concentrations were lower ($P < 0.05$) for lambs

Figure 3. Serum glucose (GLU) and triglyceride (TG) concentrations for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

Figure 4. Serum cholesterol (CHOL) and high-density lipoprotein (HDL) concentrations for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

fed RES compared with CON except stage 1 and 3, and IgM were lower ($P < 0.05$) throughout the trial (Table 3 and Fig. 5). Limiting energy intake for lambs fed RES resulted in lower ($P < 0.05$) serum IgG (18.06 vs. 15.71 g/L) and IgA (5.45 vs. 5.04 mmol/L) concentrations, but concentrations did rise with energy intake in the first phase, but then remained constant for the remaining trial days (Table 2 and Fig. 6).

In the present study, these data taken together indicate that restricting the ME intake for RES-fed lambs reduced Ca, P, GLU, TG, TP, Ca, P, IgG, IgM, and IgA concentrations compared to lambs

fed the CON diet, while CHOL and HDL-C were not significantly ($P > 0.10$) different between treatments, which is similar to the results observed when feeding high- and low-energy diets (Nicklas et al., 1997). Raeinisarjaz et al. (2001) reported that GLU concentrations were significantly different while CHOL concentrations were not significantly different between the high- and low-energy diets. Noakes and Clifton (2000) reported that diets resulting in smaller reductions in HDL-C to the point that these differences were no longer apparent appear to be dependent on both the dietary fat content and the duration of energy restriction. Changes in

Figure 5. Serum total protein (TP) and immunoglobulin M (IgM) concentrations for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

Figure 6. Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) concentrations for lambs fed at Control (C) or restricted (RES) metabolizable energy intakes.

dietary ME intake, whether via restricted feeding or grass quantity and quality, are a metabolic signal influencing cellular metabolism (Luccia et al. 2003; Skiba, 2012) that involve a number of processes including signaling, stabilization, hormone synthesis, and formation of cellular membranes (Zhang et al., 2015), besides energy balance and GLU homeostasis due to lipogenic enzyme activity changes (Daza et al., 2007). Serum Ca and P regulate the biological activity of macromolecules and tyrosine kinase-mitogen protein kinase signaling pathway (MAPK), which is closely related to the cell differentiation and proliferation regulation (Steers, 2016). The reduction in serum Ca, P, IgG, and IgA for RES-fed lambs may indicate a slower metabolism via metabolic regulation and reduced immunity compared to CON-fed lambs.

Serum TG concentrations are a reflection of body lipid metabolism. High serum concentrations of TG and total CHOL combined with low HDL-C concentrations are closely associated with fat accumulation (Targher et al., 2008). Several studies have shown that low-fat diets not only increase TG concentrations, but also induced a shift toward smaller, denser LDL-particles compared to high-fat diets (Campos et al., 1995; Dreon et al., 1995; Krauss and Dreon, 1995). Physiologically, serum TG roles are to maintain the dynamic lipoprotein balance for tissue repair, maintain blood pH, and as necessary provide energy via oxidation (Steers, 2016). Moreover, Lasa et al. (2007) reported that serum CHO, TG, and GLU were significantly reduced during energy restriction, in which GLU was negatively and TG was positively correlated with Leptin, which plays a key role in the regulation of metabolism to regulate energy homeostasis, neuroendocrine, and metabolic functions (Singhal et al., 2002; Vijay et al., 2009; Dardeno et al., 2010). On the contrary, some researchers have reported that serum LDL, HDL, and TG were not significantly affected by feeding a high- or low-fat diet (Egret et al., 2011), which may be related to the

severity and duration of the ME restriction and BW (Critser et al., 1995). Changes in dietary ME intake, whether via restricted feeding or grass quantity and quality, is a metabolic signal influencing fat metabolism (Luccia et al. 2003; Skiba, 2012). Therefore, sheep adipose tissue metabolism is closely associated with an evolutionary adaptive mechanism to survive the grassland ME changes due to environmental conditions (stress). The grass ME concentration changes with the grazing season, which results in periodic changes in sheep's metabolic energy supply, i.e., fat deposition during the grass growing seasons vs. fat mobilization and oxidation for energy to survive environmental (drought, mature, etc.) and dormant conditions.

Carcass Traits and Meat Nutritional Composition

Limiting energy intake for lambs fed RES diet decreased ($P < 0.05$) carcass yield, rib eye muscle area, GR value, shear force, and cooked meat rate and increased ($P < 0.05$) meat pH compared to lambs fed CON diet (Table 4). While an energy-restricted feeding program may alter meat fatty acid composition (Song et al., 2017), a lower carcass yield and meat quality may not be economically feasible without a fatty acid premium paid to the livestock producer. Previous studies reported that energy restriction decreased slaughter weight, chilled carcass weight, carcass yield, and dressing percentage (Serrano et al., 2009; Metzger et al., 2011), which agrees with these data. The increase in carcass pH for lambs fed RES compared to lambs fed the CON, may be due to the lower muscle glycogen reserves (Pettigrew and Esnaola, 2001) that ultimately increased muscle pH (Metzger et al., 2009). Song et al. (2017) in the companion study reported that feeding lambs a restricted ME program reduced fat deposition and intramuscular fat content, which would contribute to the reduction in dressing percentage and an increase in shear

Table 4. Nutrient composition of the meat from lambs fed at Control (CON) or restricted intake (RES)

Nutrition composition	CON ¹	RES ²	SEM ³	P-values
Water, %	70.46	72.81	0.779	0.009
Dry matter, %	29.54	27.19	0.752	0.009
Crude ash, %	1.03	1.04	0.019	0.587
Crude fat, %	9.24	8.03	0.375	0.007
Crude protein, %	18.77	17.65	0.409	0.007
Carbohydrate, %	0.48	0.47	0.145	0.003

¹CON = Lambs fed Control ME intake of 1.0 MJ/W^{0.75}•d⁻¹ throughout the 180 d.

²RES = Lambs fed Treatment ME intakes of 0.56, 0.84, 1.0, 0.84, 0.56, and 0.28 MJ/W^{0.75}•d⁻¹, sequentially changed every 30 d.

³SEM = standard error of mean.

force. However, others (Kannan et al., 2006) have reported that shear force was not affected by dietary energy level, which could be influenced by the length and severity of the ME restriction.

Limiting energy intake for lambs fed RES diet resulted in higher ($P < 0.01$) water percentage, but lower ($P < 0.01$) concentrations of dry matter (DM), fat, protein, and carbohydrate in the harvested meat compared to lambs fed the CON diet, while crude ash concentrations were similar for lambs fed both treatments (Table 5). While the study objective was to use a restricted ME feeding program to alter the fatty acid composition of the meat, these data would indicate that other nutrients are being altered as well. Future studies should be conducted to evaluate

a more subtle restrictive energy feeding to achieve the desired goals. Previous research (Aharoni et al., 1995; Luccia et al., 2003; Rodríguez-González et al., 2016) has demonstrated that feeding higher energy rations increased DM, protein, and lipids compared to feeding a lower energy ration. The concentration of intramuscular fat has been shown to be important for meat palatability and flavor (Fisher et al., 2000; Hopkins et al., 2006). In addition, Serrano et al. (2009) reported that Iberian $\times$ Duroc pigs restricted to 82% of ad libitum feed intake at 152 to 201 d and restricted to 72% of ad libitum feed intake from 202 to 263 d, that the meat's nutrient composition was similar to pigs fed ad libitum feed intakes. In agreement, Costa et al. (2013) proposed

Table 5. The serum biochemical indices of lambs fed at Control (CON) or restricted intake (RES)

Item	Period, days	CON ¹	RES ²	SEM ³	P-values	Item	Period, days	CON ¹	RES ²	SEM ³	P-values
Ca concentration (mmol/L)	30	2.11	2.14	1.517	0.225	P concentration (u/L)	30	2.49	2.32	0.180	0.345
	60	2.22	2.08		0.013		60	2.54	2.37		0.336
	90	2.26	2.18		0.061		90	2.57	2.22		0.056
	120	2.30	2.20		0.036		120	2.64	2.22		0.023
	150	2.34	2.16		0.041		150	2.54	2.07		0.010
	180	2.42	2.12		0.000		180	2.66	2.11		0.004
	Average	2.28	2.15	0.057	0.042		Average	2.57	2.22	0.144	0.026
Glucose concentration (mmol/L)	30	3.77	3.27	0.167	0.004	Triglyceride concentration (mmol/L)	30	0.46	0.44	0.042	0.616
	60	4.60	3.97		0.000		60	0.49	0.44		0.429
	90	4.35	4.44		0.562		90	0.43	0.67		<0.0001
	120	4.58	4.19		0.022		120	0.38	0.36		0.616
	150	4.39	3.70		<0.0001		150	0.36	0.28		0.050
	180	4.43	3.47		<0.0001		180	0.44	0.27		<0.0001
	Average	4.35	3.84	0.109	0.003		Average	0.43	0.41	0.023	<0.0001
Cholesterol concentration (mmol/L)	30	1.60	1.60	0.117	1.000	High-density lipoprotein-cholesterol (HDL-C) concentration (mmol/L)	30	0.99	1.12	0.075	0.310
	60	1.73	1.68		0.637		60	0.96	1.07		0.152
	90	1.89	1.85		0.770		90	1.02	1.11		0.249
	120	1.74	1.84		0.381		120	1.04	1.10		0.432
	150	1.78	2.01		0.057		150	1.06	1.13		0.366
	180	1.73	1.89		0.170		180	1.04	0.99		0.449
	Average	1.74	1.81	0.099	0.509		Average	1.02	1.09	0.055	0.251
Total protein concentration (g/L)	30	66.82	64.97	1.517	0.225	Immunoglobulin M concentration (mg/dL)	30	20.93	18.71	0.508	<0.0001
	60	65.63	61.78		0.013		60	21.16	19.10		0.000
	90	67.86	64.97		0.060		90	22.49	19.83		<0.0001
	120	68.54	65.30		0.036		120	22.71	20.47		<0.0001
	150	69.09	65.93		0.041		150	22.68	20.49		<0.0001
	180	69.58	63.99		0.000		180	22.49	19.28		<0.0001
	Average	67.92	64.49	1.217	0.012		Average	22.08	19.65	0.233	<0.0001
Immunoglobulin G concentration (mg/dL)	30	15.33	12.78	0.272	<0.0001	Immunoglobulin A concentration (mg/dL)	30	4.28	3.93	0.084	0.000
	60	18.67	16.00		<0.0001		60	5.42	5.22		0.020
	90	18.67	16.11		<0.0001		90	5.67	5.31		<0.0001
	120	18.11	16.44		<0.0001		120	5.69	5.30		<0.0001
	150	19.00	16.89		<0.0001		150	5.83	5.36		<0.0001
	180	18.56	16.04		<0.0001		180	5.82	5.09		<0.0001
	Average	18.06	15.71	0.110	<0.0001		Average	5.45	5.04	0.038	<0.0001

¹CON = Lambs fed Control ME intake of 1.0 MJ/W^{0.75}•d⁻¹ throughout the 180 d.

²RES = Lambs fed Treatment ME intakes of 0.56, 0.84, 1.0, 0.84, 0.56, and 0.28 MJ/W^{0.75}•d⁻¹, sequentially changed every 30 d.

³SEM = standard error of mean.

that the genetic background is the major determinant of carcass composition and meat quality, such that dietary nutrient differences have limited effects on carcass composition. Whether energy restriction affects meat quality and nutrient composition could be related to the length and severity of the ME restriction (Critser et al., 1995).

CONCLUSIONS

The ME density of natural grasslands in Northwest China changes with the seasons such that grazing sheep can be subjected to extreme seasonal fluctuations in pasture ME quantity and quality, which becomes a metabolic signal that influences sheep production. These data indicate that dietary ME restriction sequence used in this study mimics the seasonal variation in forage quantity and nutrient density that resulted in a reduction in BW due to the negative ADG and cold stress (temperature sharply dropped $<-3^{\circ}\text{C}$). Lambs fed an energy-restricted feeding sequence program to mimic changes in grassland ME content demonstrated reduced serum Ca, P, GLU, TG, TP, Ca, P, IgG, IgM, and IgA concentrations. Feeding lambs a restricted ME intake reduced carcass yield and meat quality. These data demonstrate that sheep grazing on natural grass experiencing seasonal grass availability and nutrient variations influence ADG and BW loss leading to a decline in meat quality, as well as serum indices of metabolic processes, but improve the fatty acid profile for human consumption. The ME restriction approach to influence meat quality and fatty acid profiles may need to be more subtle than used in the current study. These data provide information for providing recommendations on energy and mineral supplementation for year-round lamb management on pasture to maintain meat quality and animal physiological functions.

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