



Original Research Article

Effects of dietary energy on growth performance, carcass characteristics, serum biochemical index, and meat quality of female Hu lambs



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ABSTRACT

This study evaluated the effects of dietary energy levels on growth performance, carcass traits, meat quality, and serum biochemical of female Hu lambs. Seventy female Hu lambs (aged 4 months) were randomly allotted to 5 dietary treatments. Lambs were fed diets with 5 levels of metabolizable energy (ME): 9.17 (E1), 9.59 (E2), 10.00 (E3), 10.41 (E4), and 10.82 MJ/kg (E5). The lambs were adapted to the experimental diets for 10 d and the experiment period lasted for 60 d. Dry matter intake and feed conversion ratio linearly ($P < 0.001$) increased and decreased ($P < 0.001$), respectively, with increasing dietary ME levels. Average daily gain (ADG) linearly ($P < 0.001$) increased with increasing dietary ME levels, with the highest final body weight ($P = 0.041$) observed in E4 group. Moreover, dietary energy level was associated with linear increases in serum total protein (TP) ($P < 0.001$), albumin (ALB) ($P = 0.017$), glucose (GLU) ($P = 0.004$), and low-density lipoprotein cholesterol (LDLC) ($P = 0.006$) concentrations, and it was associated with a quadratic decrease in serum triglyceride (TG) concentration ($P = 0.002$). Serum ammonia concentration, which was firstly decreased and then increased, was quadratically affected by dietary ME levels ($P = 0.013$). Compared with E1 group, lambs in E4 group had higher ($P < 0.05$) live weights, carcass weights, mesenteric fat ratio, non-carcass fat ratio, and larger loin muscle area, but lower ($P < 0.05$) meat colour a^* and b^* values, and lesser ($P < 0.05$) C17:0, C20:0, C18:1n-9t, C18:3n-3, and n-3 polyunsaturated fatty acids (PUFA), but greater ($P < 0.05$) C18:3n-6 and n-6:n-3 ratios in longissimus dorsi (LD) muscle tissue, and lesser ($P < 0.05$) C17:0, C18:3n-3, C22:6n-3, and n-3 PUFA in the biceps femoris (BF) muscle tissue. The results demonstrated that increasing dietary energy level improved the growth performance and affected carcass traits, serum biochemical indexes, and fatty acid profiles in different muscles of female Hu lambs. For 4-month-old female Hu lambs, the recommended fattening energy level is 10.41 MJ/kg.

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1. Introduction

With the improvement of living standards, the consumption of mutton in China has increased and gradually changed from cheap mutton to high-quality lamb. The Hu sheep is a breed found in China with special characteristics (Geng et al., 2003). It has attracted a lot of attention due to its high fertility, strong

adaptability, tender and delicious meat, and abundance of lysine, which is the first-limiting amino acid (AA) and important for humans. Since the nutrition requirement of sheep was first introduced by National Research Council (NRC) in 1953, feeding standards have been established in many countries. Nowadays, industrialised sheep rearing has become a trend. The formulation of feeding standards plays a critical role in promoting the development of the sheep mutton industry. Energy is the major dietary element responsible for nutrient utilization, productivity, and gain (Hosseini et al., 2008). Many studies have reported that high dietary energy levels increase the average daily gain (ADG) of lambs (Kabir et al., 2014; Yerradoddi et al., 2015), but a high-energy diet can lead to wastage of resources. In 1990, metabolizable energy (ME) requirements were calculated in Hu sheep at 4 stages of pregnancy (Chai, 1990). Yang et al. (1988) obtained the energy and protein requirements of Hu sheep at different physiological stages through experiments. This is a supplement and improvement to the research results of Chai (1990). In 2012, the energy requirements of Dupo sheep × Hu sheep (Duhu cross) F1 male sheep were established (Nie et al., 2012).

The reproductive traits of Hu sheep have been reported (Chong et al., 2018), but there are only a few reports on the standard of energy requirement of fattening Hu sheep. Other studies have shown that diet may influence carcass composition (Hornick et al., 1999), muscle pH, carcass cooling rates, and meat tenderness (Kannan et al., 2006) of ruminants. However, it is not clear whether dietary energy levels are at work.

Feeding standards are not immutable. With the development of experimental technology and the improvement of detection methods, feeding standards are constantly updated and improved. In this study, we hypothesise that appropriate dietary energy levels may improve fattening performance and meat quality of female Hu lamb. Basing on the NRC recommendation, we designed diets with 5 different energy levels to investigate their effects on growth performance, serum biochemical indices, carcass characteristics, and meat quality, aiming at providing technical guidance for the realisation of accurate nutrition supply, growth performance, meat quality improvement, and ecological culture of Hu sheep.

2. Materials and methods

The experimental design and procedures used in the study were reviewed and approved by the Animal Care and Use Committee of Hunan Normal University, Changsha, Hunan, China.

2.1. Animals and experimental treatments

Seventy female Hu lambs aged 4 months with similar initial body weights ($BW = 18.43 \pm 0.34$ kg) were obtained from Agriculture and Animal Husbandry Co., Ltd., Zhiqing, Hubei, China. The lambs were renumbered and treated with insect repellent after arrival. The lambs were grouped (14 per group) by body weight and lambs of each group were housed in individual pens (5.0 m long × 2.5 m wide × 1.0 m high) equipped with feeding and automatic drinking devices. The grouped lambs were then randomly allotted to 1 of 5 diets with metabolizable energy (ME) of 9.17 (E1), 9.59 (E2), 10.00 (E3), 10.41 (E4), and 10.82 (E5) MJ/kg, respectively corresponding to 91.06%, 95.23%, 99.30%, 103.38%, 107.45% of the energy level ratios recommended by the Nutrient Requirements of Small Ruminants (NRC, 2007). The ME of each raw material was calculated according to the Feeding Standard of Meat-Producing Sheep and Goats of Chinese Agricultural Industry Standards (HB, NY/T 816-2004). Composition and nutrient chemical composition of experimental diets are shown in Table 1. The lambs were given 10 d to adapt to the new environment and experimental

diets, and the experimental period lasted for 60 d. During the study, diets were offered in the form of total mixed ration (TMR) twice a day at 07:00 and 16:00 and the feed offered were adjusted in the mornings to ensure approximately 5% feed refusal. The feed offered and refused by group were strictly recorded daily and used to calculate the DM intake (DMI) throughout the experiment.

2.2. Sample collection and measurements

2.2.1. Chemical analysis

Samples of feed ingredients and diets were dried at 55 °C for 48 h in a forced air-drying oven and were grounded to pass through a 1-mm screen (Yang et al., 2018). Analytical DM content was measured after the samples were dried in a forced air-drying oven at 105 °C for 24 h (method 934.01; AOAC, 2006). Nitrogen was analysed by the combustion method, thus crude protein was calculated by $6.25 \times$ nitrogen content. The contents of crude fat (method 978.10; AOAC, 2006), neutral-detergent fibre (Van Soest et al., 1991), acid detergent fibre (method 973.18; AOAC, 2006), and ash (method 942.05; AOAC, 2006) were also determined.

2.2.2. Growth performance

All the lambs were weighed on 2 consecutive days before morning feeding at the end of adaptation period and the end of experimental period to obtain the initial and final BW, respectively. ADG was calculated by dividing the BW gained, i.e. final BW – initial BW, by the number of days. The DMI was calculated according to the difference of feed offered and refused divided by the number of lambs of each group. Feed conversion ratio (FCR) was also calculated according to the method described by Yin et al. (2001).

2.2.3. Serum biochemical indices

At the end of the experiment, blood samples were collected from 5 lambs of each group via the jugular vein into a 5-mL vacuum tube without anticoagulant (Changsha Yiqun Medical Equipment Co., Ltd., Hunan, China) before the morning feeding. The obtained samples were kept at room temperature for 2 to 3 h, then centrifuged at $3,000 \times g$ for 15 min at 4 °C. The serum samples were taken and stored at –20 °C for serum index determination (Yang

Table 1
Ingredients and chemical compositions of experimental diets (g/kg, DM basis).

Item	Treatments				
	E1	E2	E3	E4	E5
Ingredients					
Corn silage	400	320	250	170	100
Peanut seedling	300	300	300	300	300
Corn	54	136	223	303	391
Wheat bran	70	75	61	67	52
Soybean meal	146	139	137	130	128
Premix ¹	30	30	30	30	30
Chemical composition ²					
DM	884	887	890	894	897
Crude protein	132	131	130	129	129
Crude fat	20	21	22	22	23
Neutral detergent fibre	453	426	399	373	345
Acid detergent fibre	332	306	280	254	229
Ash	67	64	61	57	54
Metabolizable energy, MJ/kg	9.17	9.59	10.00	10.41	10.82

¹ Premix provided the following per kilogram of diet: vitamin A 120,000 IU, vitamin D₃ 60,000 IU, vitamin E 200 mg, Cu 0.15 g, Fe 1 g, Zn 1 g, Mn 0.5 g, I 15 mg, Se 5 mg, Co 2.5 mg, Ca 20 g, NaCl 100 to 250 g, P 10 g.

² Metabolizable energy levels were predicted and the rest nutrient levels were measured.

et al., 2012). During analysis, serum samples were dissolved on ice, centrifuged at $3,000 \times g$ for 10 min at 4 °C, and analysed for total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), blood urea nitrogen (BUN), glucose (GLU), triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDLC), lactic acid, and ammonia (NH₃) using commercial kits in accordance with manufacturer instructions (Jiancheng Bioengineering Institute, Nanjing, China) on the TBA 120FR Automatic Biochemistry Radiometer (Hitachi Co., Tokyo, Japan) according to the methods described by Chen et al. (2019) and Yin et al. (2018).

2.2.4. Carcass characteristics and meat quality routine indices

At the end of the experiment, from each group of E1 and E4, 5 lambs with BW close to their group's average BW were selected for slaughter to measure carcass characteristics and meat quality. In brief, live weights before slaughter (LWBS) were recorded after 12 h of fasting and then the lambs were slaughtered. After removing the hairs, viscera, head, forelimb knee joints, and hind limb toe joints, the carcass weight was recorded. The mesenteric and perirenal fat was collected and weighed, and furtherly used for calculating the non-carcass fat percentage. The viscera indices and fat ratio were calculated as percentages of LWBS. The dressing percentage was calculated using the following formula: Dressing percentage (%) = Carcass weight/LWBS \times 100%.

A cross-section of longissimus dorsi (LD) muscle was taken for the measurement of rib-eye area using the method described by Li et al. (2015). Meat samples of fresh LD and biceps femoris (BF) muscles (30 g) were collected from each lamb within 1 h of slaughter and bagged in valve bags and foil paper, respectively. Meat samples in valve bags were stored at 4 °C and used for routine meat quality analysis, and meat samples in foil paper were stored at –80 °C and used for nutrient determination. The pH values of LD muscle were measured using a pH meter (Russell CD700; Russell pH Limited, Germany) at 45 min after slaughter. The average value of the 3 measurements was recorded as the pH value. Meat colorimetric characteristics (brightness [*L**], redness [*a**], and yellowness [*b**]) were measured by using a fully automated colorimeter (Minolta CR-300, Minolta Camera Co., Osaka, Japan) from 3 different locations. The average was calculated and indicated as the meat colour value.

2.2.5. Muscle AA profile

Approximately 0.5 g of the freeze-dried LD and BF muscle samples were weighed and hydrolysed in a HCl solution for measuring AA profiles as described by Liu et al. (2019). Briefly, muscle samples were hydrolysed in 10 mL of 6 mol/L HCl solution at 110 °C for 22 h, and then the obtained solutions were adjusted to a volume of 100 mL, and then after 2 times dilution, 1 mL of the settled solution was subsampled for further analysis. The solution was analysed for AA profile using an ion-exchange AA analyser after filtered through a 0.22- μ m membrane (Hitachi, Tokyo, Japan; Kong et al., 2009).

2.2.6. Muscle fatty acid profile

Lipids were extracted from the freeze-dried LD and BF muscles via the benzene-petroleum ether (1:1, vol/vol) procedure. Fatty acid methyl esters were prepared by using KOH/methanol (0.4 mol/L) (Wang et al., 2020), and analysed using an Agilent 7890A gas chromatographer (Agilent Technologies, Santa Clara, California, United States) and an SP-2560-fused silica open-tubular capillary column (100 m \times 0.25 nm; CHROMPACK

Scientific Instruments Ltd.). The oven temperature was initially set at 140 °C for 5 min and then raised at 3 °C/min to 220 °C and finally held at 220 °C for 40 min. Injector and detector temperatures were set at 280 °C. The flow rate of carrier gas (hydrogen) was 30 mL/min. The identification of individual fatty acid methyl esters was accomplished by the retention time of an authentic standard. Individual fatty acid contents were quantified according to the peak area and expressed as percentages of total fatty acids (Duan et al., 2019; Yin et al., 2000).

2.2.7. Muscle fibre characteristics and myosin heavy chain (MyHC) isoform genes expression

Within 2 h of slaughter, LD and BF muscle samples were gently cut (3 cm \times 1.5 cm \times 0.5 cm) in the vertical direction of the muscle fibres and immediately placed in 4% formalin solution. After 24 h, the formalin solution was replaced and stored at 4 °C. The specimens in the formalin solution were washed, made transparent, dipped in wax, embedded, and cut into 4- μ m sections at room temperature using a microtome (RM2235; Leica, Germany). The sections were then stained with hematoxylin-eosin (H & E) and mounted for light microscopic (DM3000; Leica, Wetzlar, Germany) examination. Thirty typical fields of muscle tissue sections were selected. The diameter, area, and density of muscle fibres were measured using Image-Pro Plus 6.0 (Media Cybernetics, San Diego, CA, USA) software (Wang et al., 2019).

Approximately 100 mg of tissue from LD and BF muscles were pulverised under the protection of liquid nitrogen. Total RNA was extracted from the homogenate using TRIZOL reagent (Invitrogen, Carlsbad, CA; Yang et al., 2013). RNA quality and quantity were examined by ultraviolet spectroscopy with a spectrophotometer (NanoDrop ND-1000; Thermo Fisher Scientific, DE, USA). The selected gene primer sequences are shown in Table 2. Real-time quantitative PCR analyses (ABI 7900HT Fast Real-Time PCR System; Applied Biosystems, Carlsbad, CA) were performed with a total volume of 10 μ L. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a reference gene. The expression of target genes was calculated (Fu et al., 2006) as follows: Gene expression = $2^{-\Delta\Delta Ct}$ (sample-control), where $-\Delta\Delta Ct$ (sample - control) = (Ct of target gene - Ct of *GAPDH*)_{sample} - (Ct of target gene - Ct of *GAPDH*)_{control}, and Ct is cycle threshold.

2.3. Statistical analysis

The experimental data were analysed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA), and linear and quadratic contrasts were used to examine the effects of energy levels on the growth performance and serum biochemical indices of Hu female lamb. The data of carcass characteristics, muscle AA and fatty acid contents, the diameter, area and density of muscle fibres, and gene expression were subjected to the *t*-test. The results were expressed as the mean \pm SEM. A statistical significance was declared at $P < 0.05$ and trends were defined as $0.05 \leq P < 0.10$.

3. Results

3.1. Growth performance

Growth performance results are shown in Table 3. There was no significant difference in initial BW among treatments. Although the final BW did not differ among treatments, it was linearly increased ($P = 0.041$) with increasing dietary ME level. Similarly, the DMI and ADG were linearly increased ($P < 0.001$) with the increasing dietary

Table 2
Real-time quantitative PCR primer sequences.

Item	Primer sequence (5' to 3')	Size, bp	GenBank accession No.
<i>MyHC-I</i>	Forward: GAGCTCACGTACCAGACAGAG Reversed: CAGACCAAGAAGACGTGGCA	287	XM_012129251.1
<i>MyHC-IIa</i>	Forward: TTTGGGGAGGCTGCTCCTTA Reversed: AAAGATTCTTGGGCTCGGC	113	XM_012122422.2
<i>MyHC-IIx</i>	Forward: ACTGAGGAGGACCGAAGAAC Reversed: AGGCTCTTCCCACTCAACAGATT	136	XM_024979592.1
<i>MyHC-IIb</i>	Forward: TACCAGACTGAGGAGGACCG Reversed: CTGTGCATTCTTTGGTCACT	294	XM_012122419.2
<i>GAPDH</i>	Forward: TGAGGACCAGTTGTCTCTCT Reversed: TGAAATGTATGGAGGTCGGG	296	NM_001190390.1

MyHC-I = myosin heavy chain 7; *MyHC-IIa* = myosin heavy chain 2; *MyHC-IIx* = myosin heavy chain 1; *MyHC-IIb* = myosin heavy chain 4; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

ME level, and significantly greater ($P < 0.001$) DMI and ADG were observed in E4 and E5 than in E1. Meanwhile, the FCR linearly decreased ($P < 0.001$) with the increasing dietary ME level, and a lower ($P < 0.001$) FCR was in E4 and E5 than in E1. Notably, all the above growth performance indexes were not different between E4 and E5 treatments.

3.2. Serum biochemical indices

The increase of dietary ME level linearly increased the concentrations of serum TP ($P < 0.001$), ALB ($P = 0.017$), GLU ($P = 0.004$), and LDLC ($P = 0.006$) and quadratically affected NH_3 ($P = 0.013$) and TG concentrations, which were firstly decreased and then increased (Table 4). Concentrations of serum ALT, AST, LDH, CHOL, and HDL did not differ among treatments.

3.3. Carcass characteristics and meat quality

The growth performance of lambs in E4 and E5 groups were similar, and the high-energy diet will increase production costs, suggesting that E4 treatment was closer to the actual needs of lamb production. Therefore, E1 and E4 groups were selected to slaughter for further analysis.

Live weights before slaughter (25.36 vs. 27.44 kg; $P = 0.001$) and carcass weight (10.74 vs. 11.82 kg; $P < 0.05$) significantly differed between E1 and E4 treatments. However, slaughter ratio was not different between these 2 treatments (Table 5). Non-carcass and mesenteric fat ratios of E4 treatment was significantly higher ($P < 0.05$) than that of E1 treatment. Whereas, perirenal fat ratio was not different between E1 and E4 treatments. Lambs had greater ($P = 0.006$) loin muscle area in E4 treatment group than in E1 treatment group, and the $\text{pH}_{45\text{min}}$ was similar between treatments. The meat colour values of L^* ($P = 0.085$), a^* ($P = 0.008$) and b^* ($P = 0.025$) in E4 treatment were lower than in E1 treatment.

Table 3
Effects of dietary metabolizable energy (ME) level on growth performance of female Hu lambs ($n = 5$).

Item	Treatments ¹					SEM	Model P -value	Contrast P -value	
	E1	E2	E3	E4	E5			Linear	Quadratic
Initial BW, kg	18.51	18.33	18.64	18.64	18.04	0.34	0.979	0.791	0.693
Final BW, kg	25.00	25.30	26.59	27.51	27.20	0.45	0.298	0.041	0.673
ADG, g/d	108.10 ^c	116.19 ^{bc}	132.50 ^{ab}	147.86 ^a	152.74 ^a	3.77	<0.001	<0.001	0.787
DMI, g/d	703.05 ^c	710.88 ^c	776.75 ^b	803.64 ^a	803.06 ^a	23.81	<0.001	<0.001	0.597
FCR	6.50 ^a	6.12 ^{ab}	5.86 ^b	5.44 ^{bc}	5.26 ^c	0.26	<0.001	<0.001	0.604

BW = body weight; ADG = average daily gain; DMI = DM intake; FCR = feed conversion ratio.

^{a, b, c} Means within rows with different letter superscripts differ ($P < 0.05$).

¹ Dietary ME levels of E1, E2, E3, E4, and E5 groups were 9.17, 9.59, 10.00, 10.41, and 10.82 MJ/kg, respectively.

3.4. Amino acid composition

There were no differences of the contents of all of the AA determined in LD and BF muscles between E1 and E4 treatments, except that the E4 treatment tended to have greater Ser ($P = 0.088$) and Cys ($P = 0.092$) in LD muscle than the E1 treatment (Table 6). The contents of essential amino acid (EAA), flavour amino acid (FAA), and total amino acid (TAA) of LD muscle in E4 group were 17.60%, 14.58%, and 15.33%, respectively, higher than those in E1 group.

3.5. Fatty acid composition

The fatty acid compositions of LD and BF muscles of E1 and E4 treatments were almost the same, except that percentages of C17:0 and C18:3n-3 in LD and BF muscles were greater ($P < 0.05$) in E1 than in E4, as well as greater percentages of C18:1n-9t and C20:0 in LD muscle in E1 than those in E4 (Table 7). Thus, percentages of saturated fatty acids (SFA), unsaturated fatty acids (UFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and n-6 PUFA in LD and BF muscle were not different between E1 and E4 treatments, as well as the ratio of PUFA: SFA. Notably, the percentage of n-3 PUFA in both LD and BF muscle was greater ($P < 0.05$) in E1 than in E4 treatment. Therefore, a greater n-6:n-3 ratio was observed in E4 than in E1 treatment.

3.6. Muscle fibres characteristics and MyHC expression

There were no differences in the diameter, area or density of muscle fibers in LD or BF muscle between E1 and E4 treatments (Table 8). The expression of *MyHC* isoform genes was analysed using real-time PCR, and the expressions of all detected genes in LD or BF muscle were not different between E1 and E4 treatments (Table 9).

Table 4
Effects of dietary metabolizable energy (ME) level on serum biochemical indices of female Hu lambs (n = 5).

Item	Treatments ¹					SEM	Model P-value	Contrast P-value	
	E1	E2	E3	E4	E5			Linear	Quadratic
TP, g/L	61.32 ^b	61.20 ^b	67.06 ^a	70.08 ^a	68.58 ^a	1.01	0.002	<0.001	0.369
ALB, g/L	29.66	28.66	31.22	31.32	31.54	0.39	0.066	0.017	0.995
ALT, U/L	21.54	23.22	22.20	22.00	20.02	0.85	0.852	0.513	0.401
AST, U/L	109.40	103.20	107.40	101.80	100.60	2.53	0.807	0.325	0.993
LDH, U/L	542.60	583.00	593.00	602.20	580.40	10.71	0.490	0.229	0.181
BUN, mmol/L	6.38	6.14	6.32	7.26	6.28	0.16	0.180	0.400	0.576
GLU, mmol/L	4.68 ^{bc}	4.46 ^c	5.22 ^{ab}	5.12 ^{abc}	5.50 ^a	0.12	0.027	0.004	0.693
TG, mmol/L	0.46 ^a	0.26 ^b	0.31 ^b	0.33 ^b	0.33 ^b	0.02	0.002	0.063	0.002
CHOL, mmol/L	1.88	1.67	1.81	1.97	1.93	0.05	0.411	0.289	0.428
HDL, mmol/L	1.30	1.18	1.18	1.23	1.14	0.03	0.570	0.252	0.717
LDLC, mmol/L	0.49	0.43	0.55	0.66	0.70 ^a	0.03	0.050	0.006	0.460
Lactic acid, mmol/L	6.84	5.08	5.74	6.75	7.88	0.37	0.142	0.142	0.047
NH ₃ , μmol/L	238.98	190.18	198.24	226.68	251.80	8.28	0.072	0.248	0.013

TP = total protein; ALB = albumin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LDH = lactic dehydrogenase; BUN = blood urea nitrogen; GLU = glucose; TG = triglyceride; CHOL = cholesterol; HDL = high-density lipoprotein; LDLC = low density lipoprotein cholesterol.

^{a, b, c} Means within rows with different letter superscripts differ (P < 0.05).

¹ Dietary ME levels of E1, E2, E3, E4, and E5 groups were 9.17, 9.59, 10.00, 10.41, and 10.82 MJ/kg, respectively.

Table 5
Effects of dietary metabolizable energy (ME) level on carcass characteristics of female Hu lambs (n = 5).

Item	Treatments ¹		SEM	P-value
	E1	E4		
Slaughter performance				
LWBS, kg	25.36	27.44	0.40	0.001
Carcass weight, kg	10.74	11.82	0.50	0.027
Slaughter ratio, %	42.38	43.08	1.40	0.679
Non-carcass fat ratio, %	1.99	2.68	0.18	0.041
Mesenteric fat ratio, %	1.31	1.84	0.11	0.007
Perirenal fat ratio, %	0.68	0.85	0.07	0.289
Meat quality				
Loin muscle area, mm ²	766.51	929.04	34.05	0.006
pH _{45 min}	6.42	6.43	0.06	0.962
L*	38.27	36.16	0.62	0.085
a*	20.15	16.91	0.70	0.008
b*	4.19	2.68	0.36	0.025

LWBS = live weight before slaughter; L* = brightness; a* = redness; b* = yellowness.

¹ Dietary ME levels of E1 and E4 groups were 9.17 and 10.41 MJ/kg, respectively.

4. Discussion

Dietary energy is an important factor affecting nutrient intake, digestion, metabolic efficiency, and production performance (Rong et al., 2010). In the current study, the DMI of lambs was significantly increased as the dietary ME increased from 9.17 to 10.41 MJ/kg. However, no increase of DMI was observed when the dietary ME level continued to increase to 10.82 MJ/kg. Lu and Potchoiba (1990) observed a curvilinear response when comparing 3 levels of energy (ME = 2.46, 2.77, and 3.05 Mcal/kg, DM basis) in diets. The maximal DMI was observed in the medium-energy diet (ME = 2.77 Mcal/kg), which was higher than the energy levels in the present study. Ríos-Rincón et al. (2014) found that DMI decreased as dietary energy level increased, i.e. ME from 2.83 to 3.05 Mcal/kg, which was similar to the energy level (ME = 2.77 Mcal/kg) tested in the study of Lu (1990). All these studies suggested that DMI would increase with increasing dietary ME level, but an extremely high dietary ME level has negative effects on DMI. In the present study, ADG of female Hu lambs significantly increased as dietary energy levels increased, which was in line with previous studies, which reported that increasing dietary energy levels can lead to improved ADG (Kabir et al., 2014; Yerradoddi et al., 2015). The increase of ADG was due to the increased DMI. Attention should be paid to that after

Table 6
Effects of dietary metabolizable energy (ME) level on muscle amino acid (AA) content of female Hu lambs (% , n = 5).

Item	LD muscle ¹				BF muscle ¹			
	E1	E4	SEM	P-value	E1	E4	SEM	P-value
Asp ²	5.38	6.25	0.247	0.425	6.44	6.33	0.149	0.625
Thr ³	2.60	3.00	0.121	0.859	2.83	2.71	0.064	0.594
Ser ²	2.54	2.97	0.126	0.088	3.18	3.14	0.071	0.586
Glu ²	9.53	10.83	0.405	0.198	11.06	10.81	0.204	0.651
Gly ²	2.99	3.21	0.118	0.407	3.59	3.54	0.085	0.571
Ala ²	3.44	3.92	0.150	0.575	4.30	4.15	0.104	0.760
Cys ²	0.47	0.55	0.037	0.092	0.65	0.64	0.020	0.782
Val ^{2,3}	2.75	3.22	0.131	0.408	3.53	3.44	0.089	0.760
Met ²	1.55	1.83	0.080	0.536	2.09	2.01	0.056	0.731
Ile ^{2,3}	2.94	3.48	0.151	0.560	3.80	3.66	0.105	0.785
Leu ^{2,3}	4.67	5.48	0.228	0.443	6.04	5.81	0.160	0.751
Tyr ³	1.84	2.16	0.095	0.290	2.24	2.16	0.068	0.656
Phe ³	2.56	3.05	0.131	0.727	3.22	3.12	0.089	0.769
Lys ³	4.98	5.75	0.223	0.342	6.14	5.93	0.145	0.662
His ³	1.93	2.39	0.106	0.182	2.36	2.31	0.075	0.684
Arg ²	3.80	4.32	0.163	0.499	4.98	4.81	0.125	0.719
Pro ²	2.47	2.77	0.102	0.562	3.11	3.03	0.074	0.740
EAA	24.26	28.53	1.175	0.589	30.15	29.14	0.786	0.776
FAA	42.53	48.73	1.890	0.357	52.77	51.36	1.208	0.689
TAA	56.44	65.09	2.549	0.423	69.55	67.60	1.639	0.729

LD = longissimus dorsi; BF = biceps femoris; EAA = essential AA; FAA = flavored AA; TAA = total AA.

¹ Dietary ME levels of E1 and E4 groups were 9.17 and 10.41 MJ/kg, respectively.

² Flavored AA.

³ Essential AA.

exceeding a certain energy level (ME = 10.41 MJ/kg), the ADG of lambs tended to be stable. This was supported by the fact that the ADG of E4 group was 36.78% higher than that of E1 group, whereas, the ADG of E5 group was only 3.3% higher than that of E4 group. Our results indicated that higher feed rewards can be obtained by increasing dietary energy properly, and the optimal dietary ME level for 120- to 180-d-old female Hu lambs during fattening was recommended not exceeding 10.41 MJ/kg to achieve the best growth performance.

Blood biochemical indices can reflect the changes of metabolic, growth and development status of livestock (Joshp et al., 2002). Changes in dietary ME levels will undoubtedly lead to changes in nutrient metabolism, which could be reflected by the changes of blood biochemistry. Previous studies showed that dietary energy levels affected serum GLU concentration by affecting metabolism

Table 7
Effects of dietary metabolizable energy (ME) level on muscle fatty acid content of female Hu lambs (n = 5).

Item	LD muscle ¹				BF muscle ¹			
	E1	E4	SEM	P-value	E1	E4	SEM	P-value
Fatty acid composition, %								
C14:0	3.86	3.35	0.32	0.458	4.35	4.76	0.38	0.617
C16:0	28.82	29.13	0.65	0.826	27.68	28.69	0.57	0.410
C16:1n-7	1.96	1.91	0.10	0.830	2.37	2.32	0.14	0.871
C17:0	2.64	1.81	0.15	<0.001	2.49	2.11	0.08	0.008
C18:0	12.21	11.61	0.28	0.310	9.77	10.21	0.28	0.470
C18:1n-9t	2.31	1.11	0.27	0.013	1.91	1.54	0.23	0.466
C18:1n-9c	43.14	45.29	0.83	0.215	43.92	44.09	0.71	0.913
C18:2n-6c	3.30	3.68	0.22	0.409	4.72	4.06	0.26	0.213
C20:0	0.20	0.16	0.01	0.030	0.16	0.15	0.01	0.435
C18:3n-6	0.07	0.09	0.00	0.014	0.08	0.08	0.00	0.659
C20:1	0.10	0.09	0.00	0.332	0.10	0.09	0.00	0.207
C18:3n-3	0.30	0.22	0.02	0.003	0.35	0.25	0.02	0.001
C20:3n-6	0.09	0.11	0.01	0.390	0.14	0.11	0.01	0.123
C20:4n-6	0.95	1.39	0.17	0.203	1.88	1.49	0.14	0.194
C22:6n-3	0.05	0.05	0.00	0.854	0.08	0.06	0.01	0.025
Fatty acid partial sums								
SFA, %	47.73	46.06	0.89	0.379	44.46	45.91	0.68	0.315
UFA, %	52.27	53.94	0.89	0.378	55.54	54.09	0.68	0.315
MUFA, %	47.50	48.40	0.58	0.472	48.30	48.05	0.45	0.799
PUFA, %	4.77	5.54	0.38	0.339	7.24	6.04	0.42	0.163
PUFA:SFA ratio	0.10	0.11	0.01	0.517	0.15	0.13	0.01	0.139
n-3 PUFA, %	0.35	0.28	0.02	0.025	0.43	0.31	0.02	0.002
n-6 PUFA, %	4.41	5.27	0.38	0.287	6.82	5.74	0.40	0.197
n-6:n-3 ratio	12.55	18.98	1.35	0.006	16.01	18.76	0.99	0.161

LD = longissimus dorsi; BF = biceps femoris; C14:0 = myristic acid; C16:0 = palmitic acid; C16:1n-7 = palmitoleic acid; C17:0 = margaric acid; C18:0 = stearic acid; C18:1n-9t = trans-9-elaidic acid; C18:1n-9c = cis-9-elaidic acid; C18:2n-6c = cis-linoleate acid; C20:0 = arachidic acid; C18:3n-6 = γ -linolenic acid (GLA); C20:1 = cis-11-eicosenoate acid; C18:3n-3 = α -linolenic acid (ALA); C20:3n-6 = eicosatrienoate acid; C20:4n-6 = arachidonic acid; C22:6n-3 = docosahexaenoic acid (DHA). SFA = saturated fatty acids, C14:0 + C16:0 + C17:0 + C18:0 + C20:0; UFA = unsaturated fatty acids, C16:1n-7 + C18:1n-9t + C18:1n-9c + C18:2n-6c + C18:3n-6 + C20:1 + C18:3n-3 + C20:3n-6 + C20:4n-6 + C22:6n-3; MUFA = monounsaturated fatty acids, C16:1n-7 + C18:1n-9t + C18:1n-9c + C20:1; PUFA = polyunsaturated fatty acids, C18:2n-6c + C18:3n-6 + C18:3n-3 + C20:3n-6 + C20:4n-6 + C22:6n-3; n-3 PUFA = C18:3n-3 + C22:6n-3; n-6 PUFA = C18:3n-6 + C20:3n-6 + C20:4n-6.

¹ Dietary ME levels of E1 and E4 groups were 9.17 and 10.41 MJ/kg, respectively.

Table 8
Effects of dietary metabolizable energy (ME) level on muscle fibre's diameter, area, and density of female Hu lambs (n = 5).

Item	Treatments ¹		SEM	P-value
	E1	E4		
BF muscle				
Diameter, μm	29.05	26.60	1.04	0.280
Area, μm^2	704.10	592.32	46.54	0.272
Density, fibers/mm ²	1,003.83	1,177.35	85.65	0.367
LD muscle				
Diameter, μm	23.19	24.70	0.65	0.275
Area, μm^2	442.60	505.92	25.53	0.235
Density, fibers/mm ²	1,409.67	1,291.04	66.21	0.402

LD = longissimus dorsi; BF = biceps femoris.

¹ Dietary ME levels of E1 and E4 groups were 9.17 and 10.41 MJ/kg, respectively.

(Graugnard et al., 2012; Oler and Glowinska, 2013). When dietary energy intake was insufficient, a decrease of serum GLU concentration was observed, reflecting reduced nutrient utilization. In the present study, the significantly higher concentration of GLU in E3, E4, and E5 treatments than in E1 and E2 treatments, suggesting an insufficient energy intake in E1 and E2 treatments. This was consistent with the significantly lower ADG observed in E1 and E2

Table 9
Effects of dietary metabolizable energy (ME) level on the expression of MyHC isoform genes in the muscles of female Hu Sheep (n = 5).

Item	Treatments ¹		SEM	P-value
	E1	E4		
BF muscle				
MyHC-I	1.26	0.92	0.262	0.550
MyHC-IIa	0.76	1.09	0.163	0.341
MyHC-IIb	0.92	1.11	0.168	0.595
MyHC-IIx	1.71	1.30	0.317	0.555
LD muscle				
MyHC-I	0.87	1.01	0.076	0.395
MyHC-IIa	1.18	1.10	0.244	0.882
MyHC-IIb	2.75	1.27	0.630	0.275
MyHC-IIx	0.64	1.09	0.262	0.433

LD = longissimus dorsi; BF = biceps femoris; MyHC-I = myosin heavy chain 7; MyHC-IIa = myosin heavy chain 2; MyHC-IIx = myosin heavy chain 1; MyHC-IIb = myosin heavy chain 4.

¹ Dietary ME levels of E1 and E4 groups were 9.17 and 10.41 MJ/kg, respectively.

treatments. Our results agreed with a study by Chelikani et al. (2003) that concentration of serum GLU increased in step with the increase of dietary energy level in cattle. Furthermore, serum BUN concentration represents the status of protein and AA metabolism, and it was reported to be negatively correlated with nitrogen deposition and protein and AA utilization (Stanley et al., 2002). In the present study, concentration of BUN was greater in E4 than in E2 treatment, and E4 had a greater dietary ME level (10.41 vs. 9.59 MJ/kg), indicating that diets with appropriate ME level improved nitrogen metabolism. Serum TG is a fat metabolite that can be decomposed by various tissues and its concentration is closely related to fat metabolism (Abdel-Sala et al., 2014). Serum concentration of TG in E1 group was the highest, indicating that the fat utilization rate of lambs was higher. Notably, Song et al. (2017) found that fat deposition, especially intramuscular fat, can be regulated by dietary energy levels. When normal life activities cannot be maintained, the body breaks down fat to get more energy. This may help explain the results that a low-energy diet increased serum TG concentrations. Serum LDLC may reflect the feed nutritional value, the level of physiology, growth, and development of animals. Serum concentration of TP and ALB roughly reflect the protein absorption, synthesis, and decomposition. To some extent, they also reflect the ability of the liver to synthesise proteins. In the present study, LDLC, TP, and ALB concentrations increased with increasing dietary ME level, indicating that dietary energy levels have a certain effect on liver function and protein metabolism.

The energy levels of diets closely relate to carcass traits, among which the dressing percentage is an important index to measure carcass traits. In the present study, although the LWBS and carcass weight of E4 group was significantly higher than those of E1 group, the slaughter ratio did not differ between treatments, which was in contrast with previous studies that slaughter ratio increased with increasing lamb LWBS (Gökdal et al., 2012; Sen et al., 2011; Hawkins et al., 1985). The differences might be due to breed and growth stage of animals used. Greater non-carcass fat and mesenteric fat ratios were observed in E4 than in E1, indicating that increasing dietary the ME level promotes fat deposition.

Meat colour is a direct factor in judging meat quality and the customers' willingness to buy (Khlijji et al., 2010). Meanwhile, meat quality is also determined by some other factors like muscle pH and muscle fibres. Muscle pH is an important index reflecting post-mortem animal muscle glycolysis rates, which are related to muscle's water-holding capacity and meat colour (Li et al., 2015).

Meanwhile, the increase of muscle fibres' diameter, area and length determined the weight gain of lambs (Joubert, 1956). Four types of muscle fibres, i.e., *MyHC-I* (slow-oxidative), *MyHC-IIa* (fast-oxidative), *MyHC-IIx* (intermediate type), and *MyHC-IIb* (fast-glycolytic), vary as animals adapt to different physiological requirements (Pette and Staron, 1997). The inherent differences between ATPase activity, glycolytic enzyme profiles, and glycogen contents that exist in different muscle fibre types affect meat quality (Ryu and Kim, 2005; Joo et al., 2013). In the present study, the loin muscle area of E1 group was significantly lower than that of E4 group, indicating that energy level improved muscle growth. The meat colour variables of L^* , a^* , and b^* decreased with increasing dietary ME level, suggesting that meat colour might be negatively correlated with dietary energy level. Our results are consistent with a previous study by Keady et al. (2017), who reported that energy compensation growth decreased the a^* value and tended to decrease L^* and b^* values of meat colour. Meat pH value of post-slaughter 45 min in both E1 and E4 groups presented weak acid and was lower than that of normal slaughtered muscle (7.0 to 7.5). This may be due to the interruption of oxygen to the muscles, which promotes the process of anaerobic glycolysis of sugar (Tang et al., 2010). The meat quality negatively correlates with muscle fibres' diameter and area but positively with density (Eddinger et al., 1985; Fahey et al., 2005). There were no significant differences in muscle fibre diameter, area, density, and gene expression of different subtypes of *MyHC* between E1 and E4 treatments. Dietary energy level did not affect the fibre type transformation and the rise in dietary energy level reduced the meat colour a^* and b^* values of Hu lambs in the present study.

There were no significant differences among the 20 AA indexes of LD and BF muscles between low and high dietary ME levels, indicating that the dietary ME had very little effect on AA composition or AA deposition in LD and BF muscles. It was found that adjusting dietary energy levels may directly affect fatty acid profiles in meat. The AA composition showed that the mean EAA-to-TAA ratio of the meat samples was from 42% to 44%, slightly higher than the recommended ratio 40% in FAO/WHO standard (Campo et al., 2003). Essential amino acid-to-non-essential amino acid (NEAA) ratio in each test group was higher than 70% and much higher than the evaluation standard of superior protein (60%) in FAO/WHO. Our results suggested that LD and BF muscles of Hu lambs is high quality meat with a high level of EAA. Dietary nutritional levels affect the contents and types of fatty acids (Wood et al., 2008; Luciano et al., 2013). Song et al. (2017) found that restricted feeding changed the fatty acid composition of meat. In the present study, dietary ME level was modulated with different contents of dietary corn, which is rich in UFA, especially C18:2 (Baldin et al., 2018). With the increase of dietary energy level, UFA-to-SFA ratio numerically increased in LD muscle but decreased in BF muscle. This change may be related to corn fatty acids. And, UFA-to-SFA ratio >1.0 in both LD and BF muscles, which indicated that the meat has a good proportion of unsaturated fatty acids and meets the standards of modern healthy green food (Scollan et al., 2006). The conclusion, based on the above results and analysis, is that increasing dietary energy level changes the fatty acid composition but does not affect meat quality and flavour.

5. Conclusions

The ADG of female Hu lambs increased as the dietary energy level increased from 9.17 to 10.41 MJ/kg due to increased DMI, but did not continue to increase as the dietary energy level increased from 10.41 to 10.82 MJ/kg due to the DMI remained stable. The serum TP, GLU, TG, and LDLC concentrations increased with increasing dietary ME level. The LWBS, carcass weight, non-carcass

fat ratios, and loin muscle area were greater in E4 than in E1. Amino acid composition, muscle fibres' diameter, area, and density, along with *MyHC* expression were not affected by dietary energy level. Some fatty acid contents were significantly affected by dietary energy level. Taken together, the dietary ME of 10.41 MJ/kg was recommended in Hu lamb production to obtain the best fattening performance without affecting the meat quality.

Author contributions

Yancan Wang: investigation, data curation, writing - original draft. Qiye Wang: investigation, supervision, software. Chunpeng Dai: visualization. Jianzhong Li: project administration. Pengfei Huang: resources. Yali Li: formal analysis. Xueqin Ding: software, validation. Jing Huang: resources. Tarique Hussain: conceptualization, supervision, writing - review & editing. Huansheng Yang: conceptualization, supervision, funding acquisition, methodology, software, writing - review & editing.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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