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Effects of Feeding Level on Nutrient Metabolism and Energy Requirements of Crossbred Male Meat Sheep (Postprint)

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Date: 2017-11-08T00:00:00+00:00

Abstract

This experiment aimed to utilize open-circuit respiration calorimetry apparatus to study nutrient metabolism and energy requirement parameters in male meat sheep. A completely randomized experimental design was employed, in which 15 Dorper × Small-tailed Han F1 crossbred male lambs with similar birth dates, good body condition, and similar body weight were randomly allocated into 3 groups (5 sheep per group), and fed diets at three feeding levels: ad libitum (AL), 70% feed restriction (IR70), and 40% feed restriction (IR40). Phase 1 and Phase 2 trials were conducted when body weight reached 22 and 35 kg, respectively. Each phase comprised a digestion and metabolism trial (3-day preliminary period and 5-day formal collection period) and a respiration metabolism trial (24-hour adaptation to respiration chambers, followed by 5 consecutive days of measurement). The results showed: 1) With decreasing feeding level, the average daily gain of meat sheep decreased extremely significantly ($P<0.01$), and the final body weight of the IR40 group was extremely significantly lower than that of the AL group ($P<0.01$). 2) With decreasing feeding level, dry matter and nitrogen intake of meat sheep decreased extremely significantly ($P<0.01$), but the apparent digestibility of these two parameters did not change significantly ($P>0.05$). 3) With decreasing feeding level, methane production, methane energy, and heat production of meat sheep all decreased, with the IR40 group being extremely significantly lower than the AL group ($P<0.01$). 4) After 60 hours of fasting, the respiratory quotient was 0.71, methane production in Phase 1 and Phase 2 was 0.86 and 1.18 L/d, respectively, fasting heat production was 0.211 and 0.260 MJ/kg W0.75, respectively, and net energy for maintenance was 0.252 and 0.312 MJ/kg W0.75, respectively. In conclusion, with decreasing feeding level, production performance, nutrient intake, average daily gain, methane production, methane energy, and heat production of meat sheep all decreased, but nutrient apparent digestibility did not change significantly. Under fasting conditions, methane production was 0.86~1.18 L/d, fasting heat production was 0.211~0.260 MJ/kg W0.75, and net energy for maintenance was 0.252~0.312 MJ/kg W0.75.

Full Text

Effects of Feeding Level on Nutrient Metabolism and Energy Requirements in Crossbred Male Sheep

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Abstract

This experiment was conducted to investigate nutrient metabolism and energy requirement parameters in crossbred male sheep using an open-circuit respiratory calorimetry system. A completely randomized design was employed, in which fifteen Dorper × Small-Tailed Han crossbred F1 male lambs with similar birth dates, good body condition, and comparable body weights were randomly divided into three groups (n=5 per group) and fed at three feeding levels: ad libitum (AL), 70% restricted intake (IR70), and 40% restricted intake (IR40). Two experimental periods were conducted when the lambs reached 22 kg and 35 kg body weight, respectively. Each period consisted of a digestion and metabolism trial (3-day preliminary period and 5-day collection period) and a respiratory metabolism trial (24-hour adaptation to the respiration chambers followed by 5 consecutive days of measurement). The results showed that: 1) With decreasing feeding level, average daily gain decreased significantly ($P<0.01$), and the final body weight of the IR40 group was significantly lower than that of the AL group ($P<0.01$). 2) Dry matter and nitrogen intake decreased significantly with reduced feeding level ($P<0.01$), while their apparent digestibility remained unchanged ($P>0.05$). 3) Methane production, methane energy, and heat production all decreased with feeding level, with the IR40 group being significantly lower than the AL group ($P<0.01$). 4) After 60 hours of fasting, the respiratory quotient was 0.71, methane production was 0.86 and 1.18 L/d in periods 1 and 2, respectively, fasting heat production was 0.211 and 0.260 MJ/kg $W^{0\cdot75}$, and net energy for maintenance was 0.252 and 0.312 MJ/kg $W^{0\cdot75}$. In conclusion, decreasing feeding level reduced growth performance, nutrient intake, average daily gain, methane production, methane energy, and heat production, but did not significantly affect nutrient apparent digestibility. Under fasting conditions, methane production was 0.86-1.18 L/d, fasting heat production was 0.211-0.260 MJ/kg $W^{0\cdot75}$, and net energy for maintenance was 0.252-0.312 MJ/kg $W^{0\cdot75}$.

Keywords: meat sheep; energy metabolism; feeding level; open-circuit respira-

tory calorimetry system

China' s meat sheep industry has developed rapidly, transforming from a country with only 40 million head into the world' s largest sheep-producing nation within just a few decades. Although China is rich in sheep and goat breed resources distributed across all provinces, municipalities, and autonomous regions, large-scale meat sheep production is still in its infancy, characterized by low breed improvement levels, extensive farming practices, low feed processing technology, and particularly late initiation of research on nutrient requirements, with no established standard system for meat sheep nutrition, feed evaluation, and production. Therefore, studying energy requirements and developing intensive or semi-intensive meat sheep production is crucial for rational feed utilization, maximizing production potential, and improving economic efficiency. Developed countries have long been leaders in animal energy metabolism research. From the mid-18th to early 19th centuries, France, Germany, the United States, Denmark, and other nations developed various types of animal respiratory calorimetry devices, achieving fruitful results in feed nutritional value assessment, energy requirement determination, and feeding standard formulation. China' s energy metabolism research began much later, starting in the 1970s, when renowned animal nutritionists such as YANG Shixing, YANG Jiashi, and FENG Yanglian developed different types of mask-type, closed-circuit, and open-circuit respiratory devices for energy metabolism studies in pigs, chickens, cattle, and sheep. In recent years, researchers including XU Guishan et al., LOU Can et al., and PENG Jinjin have investigated energy metabolism parameters in meat sheep at different physiological stages using respiration chambers and comparative slaughter methods. Despite these advances, few studies have utilized open-circuit respiratory calorimetry systems for meat sheep energy metabolism research. This study investigated nutrient metabolism and energy requirement parameters in Dorper × Small-Tailed Han crossbred F1 male sheep at two weight stages (20–35 kg and 36–50 kg) using an eight-chamber parallel multifunctional respiratory calorimetry system independently developed by the Branch Institute of Animal Husbandry, Jilin Academy of Agricultural Sciences. The research combined feeding trials, digestion and metabolism experiments, and respiratory metabolism measurements to provide data support for meat sheep production.

Received: March 17, 2017
Funding: National Special Fund for Modern Agricultural Industry Technology System (CARS-39); Open Project of Key Laboratory of Feed Biotechnology, Ministry of Agriculture; Jilin Province Meat Sheep Industry System
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1. Materials and Methods

1.1 Experimental Design

Experimental animals were Dorper × Small-Tailed Han crossbred F1 male lambs. A completely randomized design was used to allocate fifteen lambs with similar birth dates, good body condition, and comparable body weights into three groups (n=5 per group), which were fed at three feeding levels: ad libitum (AL), 70% restricted intake (IR70), and 40% restricted intake (IR40). The experimental diet was formulated according to NRC (2007) requirements for 25 kg sheep with a daily gain of 0.300 kg/d, fed as a total mixed ration with a concentrate-to-forage ratio of 45:55, using Chinese wildrye as the roughage source. Diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of the diet (air-dry basis), %

Items	Contents
Chinese wildrye	
Corn	
Soybean meal	
$CaHPO_4$	
NaCl	
Limestone	
Premix[1]	
Total	
Nutrient levels[2]	
CP	
ME/(MJ/kg)	
Ca	
NaCl	

[1] The premix provided the following per kg of diets: VA 15,000 IU, VD 5,000 IU, VE 50 mg, Fe (as ferrous sulfate) 90 mg, Cu (as copper sulfate) 12.5 mg, Mn (as manganese sulfate) 50 mg, Zn (as zinc sulfate) 100 mg, Se (as sodium selenite) 0.3 mg, I (potassium iodide) 0.8 mg, Co (cobalt chloride) 0.5.
[2] Nutrient levels were calculated values.

1.2 Feeding Management

Prior to the experiment, each lamb was administered 2.5 mL of ivermectin solution for deworming. Lambs were fed twice daily with free access to water. The AL group feeding amount was adjusted daily based on the previous day' s intake, and the intake levels for the two restricted groups were determined based on the AL group intake. The same diet was fed during both digestion and metabolism trials and respiratory metabolism trials.

1.3 Digestion and Metabolism Trial

Digestion and metabolism trials were conducted when the lambs reached 22 kg and 35 kg body weight. The trials were performed in specially designed metabolism cages, with a 3-day preliminary period followed by a 5-day collection period. Daily feed samples were collected, and feed intake and refusals were recorded accurately. Feces and urine were collected using the total collection method; daily fecal output was weighed and recorded, with 10% of fecal samples collected and pooled for each lamb and stored frozen. Urine was collected in plastic buckets containing 100 mL of 10% H_2SO_4; daily urine volume was recorded, and 10% of urine samples were pooled for each lamb and stored frozen for analysis.

1.4 Respiratory Metabolism Trial

The respiratory metabolism trial was conducted immediately after the digestion and metabolism trial using an eight-chamber parallel open-circuit animal respiratory calorimetry system independently developed by the Animal Energy Metabolism Laboratory of the Branch Institute of Animal Husbandry, Jilin Academy of Agricultural Sciences. This system features open gas pathways, controllable environmental temperature in chambers, high automation, and high analytical precision. After a 24-hour adaptation period in the respiration chambers, measurements were recorded continuously for 5 days. The same diet amounts as in the digestion and metabolism trial were fed, with no feces or urine collection during this period. The system automatically detected and recorded atmospheric and chamber concentrations of oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4) every 3 minutes.

1.5 Fasting Metabolism Measurement

Fasting metabolism was measured after the IR40 group completed the respiratory metabolism trial. Lambs were fasted for over 60 hours in digestion metabolism cages, fitted with fecal collection bags, and heat production was measured continuously for 24 hours in the respiration chambers. Fecal and urine samples were collected and processed as in the digestion and metabolism trial, and respiratory metabolism data were recorded.

1.6 Sample Analysis and Calculations

1.6.1 Determination of Routine Nutrient Contents in Feed, Feces, and Urine Feed and fecal samples were analyzed for initial moisture content and then prepared as air-dry samples for determination of gross energy (GE) and contents of dry matter (DM), nitrogen (N), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Urine samples were analyzed for GE and nitrogen content. DM, nitrogen, NDF, and ADF contents were determined according to the methods in *Feed Analysis and Feed Quality Detection Technology.* GE was measured using an oxygen bomb calorimeter (LRY-900AT, Hebi Keli Measurement and Control Technology Co., Ltd.) following the manufacturer' s instructions.

1.6.2 Calculation Formulas Apparent digestibility of a nutrient (%) = 100 × (nutrient intake -nutrient in feces) / nutrient intake
Gross energy intake (MJ/d) = daily feed intake × dietary GE
Digestible energy (MJ/d) = gross energy intake -fecal energy
Metabolizable energy (MJ/d) = gross energy intake -fecal energy -urinary energy -methane energy
Gross energy digestibility (%) = 100 × digestible energy / gross energy intake
Gross energy metabolic rate (%) = 100 × metabolizable energy / gross energy intake
Digestible energy metabolic rate (%) = 100 × metabolizable energy / digestible energy
O_2 consumption (L/min) = air intake to chamber (L/min) × O_2 concentration in outside air -air exhaust from chamber (L/min) × O_2 concentration in chamber
CO_2 production (L/min) = air exhaust from chamber (L/min) × CO_2 concentration in chamber -air intake to chamber (L/min) × CO_2 concentration in outside air
CH_4 production (L/min) = air exhaust from chamber (L/min) × CH_4 concentration in chamber -air intake to chamber (L/min) × CH_4 concentration in outside air
Respiratory quotient (R.Q.) = CO_2 production / O_2 consumption
Methane energy (KJ/d) = CH_4 production (L/d) × 39.54 KJ/L
Heat production (KJ) = 16.175 × O_2 consumption (L) + 5.0208 × CO_2 production (L) -0.958 × urinary nitrogen content (g) × 6.25 -2.167 × CH_4 production (L)

1.7 Statistical Analysis

Experimental data were initially processed using Excel 2010, and one-way ANOVA was performed using SPSS 19.0. Differences were considered significant at $P<0.05$.

2. Results

2.1 Effects of Feeding Level on Growth Performance and Nutrient Apparent Digestibility

As shown in Table 2 , initial body weights did not differ significantly among groups in either period ($P>0.05$). Final body weights in both periods were similar between AL and IR70 groups ($P>0.05$) but were significantly higher than the IR40 group ($P<0.01$). Average daily gain in both periods followed the pattern AL > IR70 > IR40, with highly significant differences among groups ($P<0.01$). In periods 1 and 2, the IR40 group achieved average daily gains of only 0.052 and 0.023 kg, respectively. Dry matter intake in both periods also followed AL > IR70 > IR40, with highly significant differences among groups ($P<0.01$). However, apparent digestibility of DM, NDF, and ADF did not differ significantly among groups ($P>0.05$). Regression analysis yielded the following equation:

$$\text{DMI (g/d)} = 229.3995 + 7.7065W\ \text{(kg)} + 3.2245\Delta W\ \text{(g/d)}\ (R^2 = 0.97,\ P = 0.014)$$

where DMI is dry matter intake, W is body weight, and ΔW is average daily gain (same for equations below).

Table 2 Effects of feeding level on growth performance and nutrient apparent digestibility of meat sheep

Items	Groups	P-value
Period 1		
Initial weight (kg)		>0.05
Final weight (kg)	27.81Aa (AL), 24.63Bb (IR70), 27.42Aa (IR40)	<0.01
ADG (kg/d)	0.182A (AL), 0.107B (IR70), 0.052C (IR40)	<0.01
DM intake (g/d)	974.78A (AL), 845.53B (IR70), 521.68C (IR40)	<0.01
Apparent digestibility (%)		
DM	40.75Aa (AL), 32.62Bb (IR70), 38.80ABab (IR40)	<0.01
NDF	0.202A (AL), 0.113B (IR70), 0.023C (IR40)	<0.01
ADF	1108.11A (AL), 932.46B (IR70), 521.68C (IR40)	<0.01
Period 2		
Initial weight (kg)		>0.05
Final weight (kg)		<0.01
ADG (kg/d)		<0.01
DM intake (g/d)		<0.01
Apparent digestibility (%)		
DM		<0.01
NDF		<0.01
ADF		<0.01

In the same row, values with no letter or the same letter superscripts mean no significant difference ($P>0.05$), while different small letter superscripts mean significant difference ($P<0.05$), and different capital letter superscripts mean highly significant difference ($P<0.01$). The same as below.

2.2 Effects of Feeding Level on Nitrogen Metabolism

As shown in Table 3 , nitrogen apparent digestibility did not differ significantly among groups in either period ($P>0.05$). In period 2, retained nitrogen/digested nitrogen in the AL group was significantly higher than in the IR40 group ($P<0.05$), while neither differed significantly from the IR70 group ($P>0.05$). All other nitrogen metabolism indices showed the pattern AL > IR70 > IR40, with significant or highly significant differences among groups ($P<0.05$ or $P<0.01$). Regression analysis of nitrogen intake and retained nitrogen against DM intake, body weight, and average daily gain yielded:

Nitrogen intake (g/d) = 1.8841 + 0.0106 DMI (g/d) + 0.06919 W (kg) + 0.04194 ΔW (g/d) ($R^2 = 0.99$, $P = 0.010$)
Retained nitrogen (g/d) = -3.4952 + 0.004741 DMI (g/d) + 0.1459 W (kg) + 0.02896 ΔW (g/d) ($R^2 = 0.99$, $P = 0.012$)

Table 3 Effects of feeding level on N metabolism of meat sheep

Items	Groups	P-value
Period 1		
Intake N (g/d)	21.62A (AL), 16.98B (IR70), 10.89C (IR40)	<0.01
Fecal N (g/d)	6.17A (AL), 4.60B (IR70), 3.21C (IR40)	<0.01
Urinary N (g/d)	5.88a (AL), 4.49b (IR70), 4.28b (IR40)	<0.01
Digested N (g/d)	15.31A (AL), 12.93B (IR70), 7.69C (IR40)	<0.01
NAD (%)	10.07A (AL), 6.95B (IR70), 3.49C (IR40)	<0.01
Retained N (g/d)	0.45A (AL), 0.33B (IR70), 0.24C (IR40)	<0.01
RN/IN	0.67A (AL), 0.56B (IR70), 0.39C (IR40)	<0.01
RN/DN		<0.01
Period 2		
Intake N (g/d)	24.50A (AL), 18.90B (IR70), 10.89C (IR40)	<0.01
Fecal N (g/d)	6.29A (AL), 4.75B (IR70), 2.90C (IR40)	<0.01
Urinary N (g/d)	6.06a (AL), 5.06ab (IR70), 4.69b (IR40)	<0.01
Digested N (g/d)	18.01A (AL), 14.15B (IR70), 7.98C (IR40)	<0.01
NAD (%)	12.62A (AL), 9.49B (IR70), 4.67C (IR40)	<0.01
Retained N (g/d)	0.51a (AL), 0.48b (IR70), 0.46b (IR40)	<0.01
RN/IN	0.67a (AL), 0.63ab (IR70), 0.56b (IR40)	<0.01
RN/DN		<0.01

2.3 Effects of Feeding Level on Respiratory Metabolism

As shown in Table 4 , respiratory quotient and CH_4 production in the IR40 group were significantly lower than in AL and IR70 groups in both periods ($P<0.01$). O_2 consumption and CO_2 production in both periods followed the pattern AL > IR70 > IR40, with highly significant differences among groups ($P<0.01$), except for CO_2 production [L/(kg·d)] in period 1, where AL and IR70 groups did not differ significantly ($P>0.05$). Regression analysis of CH_4 production against DM, NDF, and ADF intake yielded:

CH_4 production (L/d) = -4.8586 -0.2424 DMI (g/d) + 0.1986 NDFI (g/d) + 0.4424 ADFI (g/d) ($R^2 = 0.99$, $P = 0.005$)

where NDFI is NDF intake and ADFI is ADF intake (same for equations below).

Table 4 Effects of feeding level on respiratory metabolism of meat sheep

Items	Groups	P-value
Period 1		
R.Q.	0.89Aa (AL), 0.86Aa (IR70), 0.82Bb (IR40)	<0.01
O_2 consumption L/(kg·d)	287.14A (AL), 251.07B (IR70), 193.70C (IR40)	<0.01
O_2 consumption L/(W^{0}·^{75}·d)	13.37A (AL), 10.42B (IR70), 8.58C (IR40)	<0.01
CO_2 output L/(kg·d)	28.62A (AL), 23.05B (IR70), 18.66C (IR40)	<0.01
CO_2 output L/(W^{0}·^{75}·d)	251.06A (AL), 221.70B (IR70), 159.73C (IR40)	<0.01
CH_4 output L/(kg·d)	11.69Aa (AL), 9.20Aa (IR70), 7.07Bb (IR40)	<0.01
CH_4 output L/(W^{0}·^{75}·d)	25.03A (AL), 20.35B (IR70), 15.39C (IR40)	<0.01
Period 2		
R.Q.	0.85A (AL), 0.74B (IR70), 0.58C (IR40)	<0.01
O_2 consumption L/(kg·d)	386.63A (AL), 329.09B (IR70), 241.85C (IR40)	<0.01
O_2 consumption L/(W^{0}·^{75}·d)	12.87A (AL), 8.81B (IR70), 8.26C (IR40)	<0.01
CO_2 output L/(kg·d)	30.02A (AL), 21.78B (IR70), 19.21C (IR40)	<0.01
CO_2 output L/(W^{0}·^{75}·d)	334.69A (AL), 280.07B (IR70), 196.86C (IR40)	<0.01
CH_4 output L/(kg·d)	1.82A (AL), 1.63B (IR70), 1.26C (IR40)	<0.01

Items	Groups	P-value
CH_4 output L/($W^{0.75}$ • d)	25.76A (AL), 22.21B (IR70), 13.92C (IR40)	<0.01

2.4 Fasting Metabolism Test Results

As shown in Table 5 , the respiratory quotient during fasting metabolism was 0.71, significantly lower than during the growth period (0.82) ($P<0.01$). Methane production during fasting was extremely low, at only 0.86 and 1.18 L/d in periods 1 and 2, respectively. Methane production is considered a sensitive indicator of rumen fermentation status, and when it becomes negligible, the animal can be considered in a post-absorptive state. In this study, lambs were fasted for over 60 hours, meeting the criteria for a post-absorptive fasting state.

Table 5 Results of fasting metabolism test of meat sheep

Items	Period 1	Period 2
Body weight (kg)		
Metabolic weight $W^{0.75}$ (kg)		
R.Q.	0.71	
O_2 consumption L/(kg • d)		
O_2 consumption L/($W^{0.75}$ • d)		
CO_2 output L/(kg • d)		
CO_2 output L/($W^{0.75}$ • d)		
CH_4 output L/(kg • d)	0.86	1.18
CH_4 output L/($W^{0.75}$ • d)		
Urinary N g/(kg • d)		
Urinary N g/($W^{0.75}$ • d)		
Heat production MJ/(kg • d)		
Heat production MJ/$W^{0.75}$	2.64	3.29
Fecal N g/(kg • d)		
Fecal N g/($W^{0.75}$ • d)		
DM output g/(kg • d)		
DM output g/($W^{0.75}$ • d)		
Body protein decomposition (g)	26.50	38.75
Body protein HP (MJ)		
Body protein HP/HP (%)	18–22	

2.5 Effects of Feeding Level on Energy Transformation Efficiency

As shown in Table 6 , gross energy intake, fecal energy, urinary energy, methane energy, and heat production all followed the pattern AL > IR70 > IR40, with highly significant differences among groups ($P<0.01$), except for urinary energy and methane energy in period 1 and urinary energy in period 2, where AL and IR70 groups did not differ significantly ($P>0.05$). The ratio of methane energy to gross energy intake ranged from 0.041 to 0.057 across groups. Both gross energy digestibility and gross energy metabolic rate were linearly correlated with gross energy intake, as determined by regression analysis:

Gross energy digestibility = 0.5904 + 0.005237 GEI (MJ/d) ($R^2 = 0.83$, $P = 0.042$)
Gross energy metabolic rate = 0.48 + 0.007138 GEI (MJ/d) ($R^2 = 0.91$, $P = 0.011$)

where GEI is gross energy intake.

Gross energy digestibility and gross energy metabolic rate were also linearly correlated with DM, NDF, and ADF intake:

Gross energy digestibility = 0.4879 -0.00257 DMI (g/d) + 0.002183 NDFI (g/d) + 0.004245 ADFI (g/d) ($R^2 = 0.97$, $P = 0.082$)
Gross energy metabolic rate = 0.4620 + 0.000134 DMI (g/d) + 0.0000386 NDFI (g/d) ($R^2 = 0.91$, $P = 0.067$)

Table 6 Effects of feeding level on energy transformation efficiency of meat sheep

Items	Groups	P-value
Period 1		
Body weight (kg)	20.99A (AL), 15.63B (IR70), 9.65C (IR40)	<0.01
Metabolic weight $W^{0\cdot75}$ (kg)	1.73A (AL), 1.30B (IR70), 0.87C (IR40)	<0.01
Gross energy intake MJ/(d $\cdot$ $W^{0\cdot75}$)	6.89A (AL), 5.83A (IR70), 3.34C (IR40)	<0.01
Fecal energy MJ/(d)	0.54A (AL), 0.48A (IR70), 0.28B (IR40)	<0.01
Urinary energy MJ/(d)	0.73A (AL), 0.71A (IR70), 0.52B (IR40)	<0.01
Methane energy MJ/(d)	0.041C (AL), 0.048B (IR70), 0.054A (IR40)	<0.01
CH_4E/GEI	12.83A (AL), 9.10B (IR70), 5.51C (IR40)	<0.01
GE digestibility (%)	1.06A (AL), 0.76B (IR70), 0.50C (IR40)	<0.01
GE metabolic rate (%)	5.83A (AL), 5.11B (IR70), 2.88C (IR40)	<0.01
DE metabolic rate (%)	0.48A (AL), 0.43B (IR70), 0.26C (IR40)	<0.01
Metabolizable energy MJ/(d $\cdot$ $W^{0\cdot75}$)		<0.01

Items	Groups	P-value
Heat production MJ/(d · $W^{0 \cdot 75}$)		<0.01
Fasting heat production MJ/(d · $W^{0 \cdot 75}$)	0.49A (AL), 0.43B (IR70), 0.28C (IR40)	<0.01
Period 2		
Body weight (kg)	22.49A (AL), 17.25B (IR70), 9.65C (IR40)	<0.01
Metabolic weight $W^{0 \cdot 75}$ (kg)	1.39A (AL), 1.11B (IR70), 0.71C (IR40)	<0.01
Gross energy intake MJ/(d · $W^{0 \cdot 75}$)	6.02A (AL), 5.31B (IR70), 3.54C (IR40)	<0.01
Fecal energy MJ/(d)	0.62A (AL), 0.60A (IR70), 0.42B (IR40)	<0.01
Urinary energy MJ/(d)	1.02A (AL), 0.88B (IR70), 0.55C (IR40)	<0.01
Methane energy MJ/(d)	0.050B (AL), 0.048B (IR70), 0.057A (IR40)	<0.01
CH_4E/GE	73.23A (AL), 69.22A (IR70), 63.32B (IR40)	<0.01
GE digestibility (%)	1.06A (AL), 0.67B (IR70), 0.40C (IR40)	<0.01
GE metabolic rate (%)	7.84A (AL), 6.65B (IR70), 3.84C (IR40)	<0.01
DE metabolic rate (%)	0.92A (AL), 0.67B (IR70), 0.40C (IR40)	<0.01
Metabolizable energy MJ/(d · $W^{0 \cdot 75}$)	14.83A (AL), 10.46B (IR70), 5.41C (IR40)	<0.01
Heat production MJ/(d · $W^{0 \cdot 75}$)		<0.01
Fasting heat production MJ/(d · $W^{0 \cdot 75}$)		<0.01

3. Discussion

3.1 Effects of Feeding Level on Growth Performance and Nutrient Apparent Digestibility

The experimental diet was formulated according to NRC (2007) requirements for 25 kg sheep with a daily gain of 0.300 kg/d and fed at three levels: ad libitum, 70% restricted, and 40% restricted. However, the AL group achieved average daily gains of only 0.182 and 0.202 kg/d in periods 1 and 2, respectively, failing to reach the target of 0.300 kg/d. This may be attributed to environmental conditions, as the feeding trial was conducted during autumn and winter when lower barn temperatures reduced average daily gain. The IR40 group achieved average daily gains of 0.052 and 0.023 kg/d in periods 1 and 2, respectively, which aligns with the experimental design target of <50 g/d. XU Guishan reported that Dorper × Small-Tailed Han crossbred male lambs under 40% restriction had an average daily gain of 0.0376 kg/d, while WANG Peng reported 0.05714 kg/d for meat-type male lambs under 40% restriction, both consistent with our results.

The DM intake of ruminants typically accounts for 2–4% of body weight. In this study, DM intake ranged from 1.6% to 4.3% of body weight and showed a regression relationship with body weight and average daily gain. AFRC (1990, 1993) proposed the simplest estimation method for growing sheep: DMI (kg/d) = 0.028W. ZANG Yanquan reported that DM intake of growing Boer crossbred meat sheep was related to metabolic body weight and average daily gain: DMI (g/d) = $26.45W^{0\cdot7}$ (kg) + $0.99\Delta W$ (g/d) + 312.24 ($R^2 = 0.6681$, $P = 0.0008$, where $W^{0\cdot75}$ is metabolic body weight). These prediction models represent intake under experimental conditions and require adjustment for specific practical conditions.

3.2 Effects of Feeding Level on Nitrogen Metabolism

In ruminants, dietary protein is partially degraded by rumen microbes, with degraded protein (RDP) used to synthesize microbial crude protein (MCP). Undegraded dietary protein (UDP) and MCP enter the small intestine as intestinal protein for digestion, absorption, and utilization. In this study, nitrogen intake, fecal nitrogen, digested nitrogen, and retained nitrogen all decreased with feeding level, while nitrogen apparent digestibility did not differ significantly among groups.

ZANG Yanquan established prediction models for protein requirements in growing Boer crossbred meat sheep: CPI (g/d) = $28.87 + 1.91W^{0\cdot75} + 0.20\Delta W$ ($R^2 = 0.6890$, $P = 0.0008$, where CPI is crude protein intake), and DCP (g/d) = $(0.03\Delta W + 1.27)W^{0\cdot75}$ ($R^2 = 0.9869$, $P = 0.0007$, where DCP is digested crude protein). NIE Haitao et al. established a regression equation for DCP against dietary crude protein in 4–6 month old Dorper × Hu crossbred F1 female lambs: DCP = $(-4.446 \pm 1.522) + (1.099 \pm 0.1603)$ TCPC ($R^2 = 0.83$, $P = 0.07$, where TCPC is total dietary crude protein). YANG Weiren et al. reported prediction models for digested crude protein requirements in 4-month-old Dorper × Small-Tailed Han crossbred meat sheep: RDCP = $2.59W^{0\cdot75} + 374.98\Delta W$, and RCP = $3.88W^{0\cdot75} + 560\Delta W$ (where RDCP is digested crude protein requirement and RCP is crude protein requirement). These models were obtained under specific experimental conditions and require further validation and correction for practical application.

3.3 Effects of Feeding Level on Respiratory Metabolism

Due to the lack of specialized equipment for respiratory metabolism research in China, few studies have investigated meat sheep respiratory metabolism. XU Guishan used a Sable head-box system to study gas metabolism in Dorper × Small-Tailed Han crossbred lambs, reporting that O_2 consumption, CO_2 production, and CH_4 production all followed the pattern ad libitum > 70% restricted > 40% restricted, with respiratory quotients ranging from 0.68 to 0.84. LOU Can et al. used respiratory chambers to study gas metabolism characteristics of Dorper × Small-Tailed Han crossbred ewes during non-lactating and lactating periods, finding that O_2 consumption, CO_2 production, CH_4 production, and respiratory quotient were significantly correlated with feed intake. These results are consistent with our findings.

Previous studies have shown that NDF and ADF digestibility were significantly linearly correlated with CH_4 production. Additionally, digestible crude fiber, crude protein, crude fat, and nitrogen-free extract intake were significantly correlated with CH_4 production. In this study, regression analysis of CH_4 production against DM, NDF, and ADF intake yielded: CH_4 production (L/d) = -4.8586 -0.2424 DMI (g/d) + 0.1986 NDFI (g/d) + 0.4424 ADFI (g/d) ($R^2 = 0.99$, $P = 0.0049$). ZHAO Yiguang reported a regression model for CH_4 production against nutrient intake in meat sheep: CH_4 production (L/kg DOM) = 0.2260 OMI (g) + 0.15234 CPI (g) + 0.06465 NDFI -0.60549 EEI (g) + 218.23715 ($R^2 = 0.9786$, $P = 0.0077$, where DOM is digestible organic matter, OMI is organic matter intake, and EEI is ether extract intake). Compared with single-variable linear regression models, multiple regression models show higher correlation and accuracy. YOU Yubo reported significant relationships between nutrient intake and CH_4 production in beef cattle, noting that multi-factor prediction models are more accurate than single-factor models. These findings align with our CH_4 production prediction model, which can calculate CH_4 production from DM, NDF, and ADF intake. However, this model used only Chinese wildrye as the roughage source, limiting its applicability across forage types, as different diets, particularly roughage sources, affect prediction accuracy.

3.4 Fasting Metabolism in Meat Sheep

The fasting heat production (FHP) measured in this study was 2.64 and 3.29 MJ/d in periods 1 and 2, respectively. Body protein decomposition was 26.50 and 38.75 g, with body protein heat production accounting for 18-22% of total heat production. In China, FHP is commonly determined using the method of Lofgreen et al., where the logarithm of heat production in producing animals is linearly related to metabolizable energy intake: Log(HP) = a + b MEI (where HP is heat production and MEI is metabolizable energy intake). When MEI = 0, the antilogarithm of a gives FHP. ZHAO Minmeng et al. used this method to determine FHP values of 339.63, 332.89, and 259.66 KJ/kg $W^{0\cdot75}$ for Dorper rams, ewes, and Qingmian goats, respectively. YANG Zaibin et al. reported FHP values of 376 KJ/kg $W^{0\cdot75}$ for Small-Tailed Han ewes with single lambs and 391 KJ/kg $W^{0\cdot75}$ for those with twin lambs. ACU (1984) used the following models for sheep: for animals under 1 year, F (MJ/d) = C[0.25(W/1.08)$^{0\cdot75}$]; for animals over 1 year, F (MJ/d) = C[0.23(W/1.08)$^{0\cdot75}$] (where F is FHP and C is 1.15 for rams and 1.0 for ewes and wethers). WEI Bingdong et al. measured FHP of 444.40 KJ/kg $W^{0\cdot75}$ in 30 kg Northeast fine-wool rams using an open-circuit respiratory calorimetry system. In this study, FHP measured after 60 hours of fasting was 0.211 and 0.260 MJ/kg $W^{0\cdot75}$.

3.5 Effects of Feeding Level on Energy Transformation Efficiency in Crossbred Meat Sheep

Tyrrell et al. reported that increased gross energy intake leads to corresponding increases in fecal energy. In this study, gross energy intake, fecal energy, and urinary energy all decreased with reduced feed intake, with fecal energy accounting for 32.8%, 32.4%, and 34.6% of gross energy intake in period 1 and 26.8%, 30.8%, and 36.7% in period 2 for AL, IR70, and IR40 groups, respectively. Urinary energy loss in ruminants is primarily affected by feed structure, particularly dietary protein level and amino acid balance. YANG Zaibin et al. reported that urinary energy is positively correlated with urinary nitrogen. Increased dietary protein levels and amino acid imbalance in meat sheep can increase urinary nitrogen excretion, thereby increasing urinary energy loss. In this study, urinary energy did not differ significantly between AL and IR70 groups but was significantly higher in AL than IR40 group, with urinary energy averaging 3.2% of gross energy intake. This indicates that moderate feed restriction does not affect urinary energy, but severe restriction reduces it. PENG Jinjin et al. reported similar results, finding no significant difference in urinary energy between ad libitum and 60% restricted feeding groups, but significantly higher values than in 40% restricted groups. However, some studies have shown that dietary energy and protein levels do not affect urinary energy; ZOU Caixia reported that different energy and protein feeding levels did not affect urinary energy in growing buffalo.

In period 1, average gross energy digestibility, gross energy metabolic rate, and digestible energy metabolic rate were 66.73%, 59.34%, and 88.66%, respectively, with no significant differences among groups. In period 2, the IR40 group had significantly lower gross energy digestibility, gross energy metabolic rate, and digestible energy metabolic rate than AL and IR70 groups, which did not differ significantly from each other. Current results on energy utilization in sheep vary with breed and feeding system. Kamalzadeh et al. reported a gross energy metabolic rate of 54% for Baluchi sheep. ACU reported that gross energy metabolic rate for sheep under ad libitum feeding ranged from 42% to 60%. ZHAO Minmeng reported gross energy digestibility and metabolic rates of 63.07% and 51.75%, respectively, for Dorper sheep during the growing period. ZANG Yanquan reported that gross energy metabolic rate for Boer crossbred meat sheep ranged from 50.14% to 60.87% and gross energy digestibility from 60.35% to 69.24% with increasing concentrate intake. YANG Zaibin et al. reported gross energy digestibility, metabolic rate, and digestible energy metabolic rates of 59.22%, 49.41%, and 83.23% during the early growth stage and 68.60%, 56.84%, and 81.92% during the late growth stage for Large-Tailed Han sheep. Our results are generally consistent with these reports, though our digestible energy metabolic rates were slightly higher.

GAO Xiuhua et al. reported that gross energy digestibility and metabolic rate in sika deer during the antler growth period increased significantly with increasing gross energy intake, showing a positive linear correlation. The French National Institute for Agricultural Research (INRA) calculated gross energy digestibility from organic matter digestibility: for grasses, ED = 0.957 OMD -0.07 (R^2 = 0.995); for straw, ED = 0.985 OMD -2.95 (R^2 = 0.996); for corn silage, ED = 1.001 OMD -2.86 (R^2 = 0.981) (where ED is gross energy digestibility). These results correlated digestible nutrients with gross energy digestibility, whereas our study regressed gross energy digestibility and metabolic rate against DM, NDF, and ADF intake, requiring further analysis for future application.

Due to the lack of specialized equipment, most methane energy measurements in China have been estimated using regression models. ZHAO Minmeng used the Blaxter (1965) formula to estimate methane energy/gross energy ratios of 8.36% for Dorper rams and 8.20% for ewes, with rams being significantly higher than ewes. YANG Zaibin et al. suggested estimating methane energy as 9% of gross energy intake for Dorper × Small-Tailed Han crossbred F1 meat sheep. LIU Haibin used the prediction formula of Pelchen and Peters: CH_4 production (g/d) = -17.7026 + 0.0414 NEFI (g/d) + 0.03213 DCPI (%/CP) -0.0611 DEEI (g/d) (where NEFI is nitrogen excretion per unit intake, DCPI is digestible crude protein intake, and DEEI is digestible ether extract intake), then multiplied by 58.81 KJ/g (heat value of CH_4) to obtain methane energy. ACU (1984) estimated methane energy as 8% of gross energy intake at maintenance level, decreasing to 6% of maintenance-level gross energy intake as gross energy intake increased. FENG Yanglian reported that CH_4 production in fistulated cattle fed different diets was significantly linearly correlated with fermentable NDF/fermentable organic matter: CH_4 production (L/FOM kg) = 60.4562 + 0.2967 [FNDF/FOM (%)] (r = 0.9842, P<0.01, where FOM is fermentable organic matter and FNDF is fermentable neutral detergent fiber). Methane energy loss from digestible energy was also significantly linearly correlated: CH_4E/DE (%) = 8.6804 + 0.0373 [FNDF/FOM (%)] (r = 0.9845, P<0.01). GAO Xiuhua et al. measured respiratory metabolism in sika deer during the antler growth period using a respiratory calorimetry system at China Agricultural University and found that methane energy production increased with feeding level: CH_4E (KJ/d) = 0.070 GEI -101.04 (r = 0.9420, P<0.01). WEI Bingdong et al. measured respiratory metabolism in Northeast fine-wool rams using the open-circuit respiratory calorimetry system developed by Jilin Academy of Agricultural Sciences, reporting CH_4 production of 2.38 L/$W^{0 \cdot 75}$ and methane energy loss of 94.10 KJ/$W^{0 \cdot 75}$ (10.72% of metabolizable energy) at a metabolic body weight of 12.88 kg. Other methods have been used for respiratory metabolism research in China, including mask and indicator methods. YANG Zaibin et al. reported methane energy/gross energy ratios of 10.11% for Large-Tailed Han sheep during the growing period using the mask method. LI Ruili et al. reported a ratio of 7.99% for Liaoning cashmere goats during the non-pregnant period using the mask method. KONG Xiangtong used the sulfur hexafluoride (SF_6) tracer technique to determine CH_4 production and calculated methane energy, reporting a ratio of 8.1% for Shaanxi white cashmere goats. Using an open-circuit respiratory calorimetry system, we measured CH_4 production decreasing with feed intake in different weight stages, with methane energy averaging 5% of gross energy intake, slightly lower than previous reports, possibly due to differences in methodology, animal breeds, and feed composition.

Energy loss during fasting represents net energy for maintenance (NEm), including FHP, energy loss in feces and urine during fasting, and energy expended by muscle work: NEm = FHP + activity energy, with activity energy estimated as 20% of FHP, thus NEm = 120% FHP. The calculated NEm values in this study were 0.252 and 0.312 MJ/kg $W^{0 \cdot 75}$. According to AFRC (1993), the maintenance requirement for sheep is calculated as: for rams under 1 year, F (MJ/d) = $C[0.25(W/1.08)^{0 \cdot 75}] + 0.0067W$, where C = 1.15, giving an NEm of 0.289 MJ/kg $W^{0 \cdot 75}$ for 25 kg rams, slightly higher than our results. XU Guishan reported NEm of 250.61 KJ/kg $W^{0 \cdot 75}$ for 20-35 kg Dorper × Small-Tailed Han crossbred rams, consistent with our results. WANG Peng reported NEm of 240.44 KJ/kg $W^{0 \cdot 75}$ for 20-35 kg Tan × Small-Tailed Han crossbred rams, also consistent with our findings. NIE Haitao et al. reported NEm of 193.76 KJ/kg $W^{0 \cdot 75}$ for Dorper × Hu crossbred rams, slightly lower than our results. These discrepancies may be attributed to breed, sex, growth stage, body weight, and environmental factors.

4. Conclusion

Decreasing feeding level reduces growth performance, nutrient intake, average daily gain, methane production, methane energy, and heat production in meat sheep, but does not significantly affect nutrient apparent digestibility. Under fasting conditions, methane production is 0.86-1.18 L/d, fasting heat production is 0.211-0.260 MJ/kg $W^{0 \cdot 75}$, and net energy for maintenance is 0.252-0.312 MJ/kg $W^{0 \cdot 75}$.

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