

Effects of Dietary Energy and Protein Levels on Growth Performance, Nitrogen Metabolism, and Serum Biochemical Indices in Hu Sheep Lambs Aged 61-120 Days: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary energy and protein levels on growth performance, nitrogen metabolism, and serum biochemical indices of weaned Hu sheep lambs aged 61 to 120 days. A 2×2 two-factor two-level experimental design was adopted, and 64 purebred Hu sheep lambs with similar body weight at 61 days of age were selected and randomly divided into 4 groups, fed diets with different energy and protein levels, namely high-energy [metabolizable energy (ME) 10.92 MJ/kg] high-protein [crude protein (CP) 15.74%] group, high-energy (ME 10.92 MJ/kg) low-protein (CP 11.78%) group, low-energy (ME 8.64 MJ/kg) high-protein (CP 15.72%) group, and low-energy (ME 8.64 MJ/kg) low-protein (CP 11.82%) group, with 4 replicates per group, 4 lambs per replicate, and half male and half female. The experimental period lasted 60 days. The results showed that: 1) There was no interaction between dietary energy and protein levels on lamb growth performance indices ($P>0.05$); dietary high-energy high-protein level significantly increased the average daily gain of lambs aged 61 to 90 days ($P<0.05$) and significantly decreased the feed conversion ratio ($P<0.05$). 2) Dietary high-protein level significantly increased nitrogen intake of lambs aged 81 to 90 days and 111 to 120 days ($P<0.05$); dietary low-energy high-protein level significantly increased urinary nitrogen excretion of lambs aged 81 to 90 days and 111 to 120 days ($P<0.05$), with the low-energy high-protein group having the highest urinary nitrogen excretion; for lambs aged 81 to 90 days, dietary high-energy high-protein level significantly increased nitrogen retention ($P<0.05$), while dietary low-energy or high-protein levels significantly decreased nitrogen biological value ($P<0.05$). 3) At 120 days of age, dietary low-energy level significantly decreased serum glucose content ($P<0.05$) and significantly increased serum urea nitrogen content ($P<0.05$); di-

etary low-protein level significantly increased serum insulin-like growth factor I content ($P < 0.05$). In conclusion, during the 61 to 90 days of age stage, dietary high-energy high-protein level could promote lamb growth; during the 91 to 120 days of age stage, dietary protein level could be appropriately reduced to save production costs without adversely affecting growth performance.

Full Text

Effects of Dietary Energy and Protein Levels on Growth Performance, Nitrogen Metabolism and Serum Biochemical Indices of Hu Lambs at 61 to 120 Days of Age

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Abstract

This experiment was conducted to investigate the effects of dietary energy and protein levels on growth performance, nitrogen metabolism, and serum biochemical indices of Hu lambs aged 61 to 120 days. Using a 2×2 factorial design with two levels for each factor, sixty-four purebred Hu lambs at 61 days of age with similar body weight were randomly allocated into four groups fed diets with different energy and protein levels: high energy [metabolizable energy (ME) = 10.92 MJ/kg] with high protein [crude protein (CP) = 15.74%], high energy (ME = 10.92 MJ/kg) with low protein (CP = 11.78%), low energy (ME = 8.64 MJ/kg) with high protein (CP = 15.72%), and low energy (ME = 8.64 MJ/kg) with low protein (CP = 11.82%). Each group consisted of four replicates with four lambs per replicate, with equal numbers of males and females. The experimental period lasted 60 days. The results showed: 1) No interaction between dietary energy and protein levels was observed for lamb growth performance indicators ($P > 0.05$). High dietary energy and protein levels significantly increased average daily gain of lambs at 61-90 days of age ($P < 0.05$) and significantly decreased feed-to-gain ratio ($P < 0.05$). 2) High dietary protein level significantly increased nitrogen intake in lambs at 81-90 days and 111-120 days of age ($P < 0.05$). Low energy combined with high protein significantly increased urinary nitrogen excretion in lambs at 81-90 days and 111-120 days of age ($P < 0.05$), with the low-energy high-protein group showing the highest urinary nitrogen output. For lambs at 81-90 days of age, high energy combined with high protein significantly increased nitrogen retention ($P < 0.05$), whereas low energy or high protein levels significantly decreased nitrogen biological value ($P < 0.05$). 3) At 120 days of age, low dietary energy level significantly decreased serum glucose content ($P < 0.05$) and significantly increased serum urea nitrogen content ($P < 0.05$), while low dietary protein level significantly increased

serum insulin-like growth factor I content ($P < 0.05$). In conclusion, high energy and protein levels promoted lamb growth during the 61-90 day period. During the 91-120 day period, dietary protein level can be appropriately reduced to save production costs without adversely affecting growth performance.

Keywords: lambs; energy level; protein level; growth performance; nitrogen metabolism; serum biochemical parameter

Introduction

The lamb stage is a critical phase in mutton sheep production that directly affects not only lamb growth and development but also lifetime performance. Lambs aged 3-4 months exhibit rapid growth yet have immature digestive system function. Research on their nutritional requirements, particularly energy and protein parameters, lags far behind that of adult sheep, severely impacting lamb development and the sustainable development of the sheep industry. Previous studies have shown that dietary energy and protein levels significantly affect lamb growth performance. Chen et al. and Li et al. found that energy and protein deficiency significantly reduced growth performance and organ development in lambs. Wang et al. reported that low-protein diets significantly decreased lamb body weight while substantially improving nitrogen metabolism efficiency. Lu et al. observed that nutritional deficiency caused a significant decline in serum triglyceride (TG) content, as lambs mobilized TG for oxidative energy supply to meet their energy needs. Nutritional deficiency also affects serum hormones in lambs. Currently, most research on how nutritional levels affect lamb growth performance, nitrogen metabolism, and serum biochemical indices has focused on grazing cattle and sheep, with few studies conducted under intensive feeding conditions, limiting the sustainable development of China's large-scale sheep farming industry. Therefore, this study used Hu lambs to explore the effects of energy and protein levels on growth performance, nitrogen metabolism, and serum biochemical indices, aiming to provide nutritional regulation strategies for lamb rearing under intensive feeding conditions.

1.1 Experimental Location and Duration

The animal experiment was conducted from January to May 2015 at Hailun Sheep Industry Co., Ltd. in Taizhou City, Jiangsu Province, China.

1.2 Experimental Design and Animals

A 2×2 factorial design was employed with two factors (energy and protein), each at two levels (high and low). Sixty-four weaned Hu lambs at 61 days of age with similar body weight $[(14.95 \pm 0.56) \text{ kg}]$ were randomly divided into four groups: high energy high protein (HEHP), high energy low protein (HELP), low energy high protein (LEHP), and low energy low protein (LELP). Each group comprised four replicates with four lambs per replicate, with equal numbers of males and females. The HEHP group received the basal diet, the HELP group

received the basal diet with 20% reduced crude protein level, the LEHP group received the basal diet with 20% reduced metabolizable energy level, and the LELP group received the basal diet with both crude protein and metabolizable energy levels reduced by 20%.

The experimental period lasted 60 days. Lambs in the HEHP group were fed ad libitum, while feeding amounts for the HELP, LEHP, and LELP groups were adjusted based on the feed intake of the HEHP group to maintain similar intake across all experimental lambs.

1.3 Experimental Diets

Nutrient levels for the HELP group diet were formulated according to the nutritional requirements for 25 kg Dorper × Han crossbred lambs with a daily gain of 300 g/d, with corresponding adjustments for the HELP, LEHP, and LELP groups. Premix was provided by Beijing Precision Animal Research Center. Experimental diets were self-formulated and pelleted (diameter 4 mm, length 10 mm). The composition and nutrient levels of experimental diets are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (DM basis) %

Note: The premix provided the following per kg of diets: VA 12,000 IU, VD 2,000 IU, VE 30 IU, Cu 12 mg, Fe 64 mg, Mn 56 mg, Zn 60 mg, I 1.2 mg, Se 0.4 mg, Co 0.4 mg, NaCl 6.4 g. Nutrient levels were all measured values except ME.

1.4 Feeding Management

Before 17 days of age, lambs were nursed by their dams, then gradually transitioned to milk replacer while receiving supplemental pellet feed ad libitum. At 20 days of age, lambs were fully weaned and fed only milk replacer. At 60 days of age, milk replacer feeding ceased and lambs were fed pellets exclusively. Milk replacer was provided by Beijing Precision Animal Research Center. During the experimental period, lambs were housed in semi-open pens with good ventilation and fed at 08:00 and 16:00 daily. Pens were disinfected every two weeks (using 0.5% Baeduer and 0.1% bromo-geramine), and lambs were vaccinated for peste des petits ruminants at 80 days of age.

1.5 Measurement Indicators and Methods

1.5.1 Dietary Nutrient Levels Conventional nutrient analysis followed the methods of Zhang Liying, and metabolizable energy calculation followed the methods of Liu Jie.

1.5.2 Growth Performance Daily feed intake and refusals were recorded accurately. Lambs were weighed before morning feeding at 61, 90, and 120 days of age to calculate average daily gain and feed-to-gain ratio.

1.5.3 Nitrogen Metabolism One male lamb per replicate was randomly selected at 81-90 days and 111-120 days of age for digestion and metabolism trials using the total feces collection method. The trial lasted 10 days, including a 5-day preliminary period and a 5-day formal collection period. During the formal period, daily urine volume and fecal output were recorded accurately. Fecal samples were collected and treated with 10 mL of 10% dilute hydrochloric acid per 100 g fresh feces for nitrogen fixation, then stored at -20°C. Daily urine was collected in containers with 100 mL of 10% dilute hydrochloric acid, and 1% of the total daily urine volume was sampled into dedicated bottles and stored at -20°C until analysis. Conventional nutrient analysis followed the methods of Zhang Liying.

1.5.4 Serum Biochemical Indices Blood samples were collected from the jugular vein before morning feeding (09:00) at 61, 90, and 120 days of age. Blood was placed in silica-containing coagulation-promoting tubes, left to stand for 30 minutes, then centrifuged at 3,000 r/min to collect plasma, which was stored at -20°C until analysis. Serum glucose (Glu), urea nitrogen (UN), and TG content were measured using an automatic biochemical analyzer (Hitachi 7600, Japan) and radioimmunoassay kits. Serum insulin-like growth factor I (IGF-I) and growth hormone (GH) content were measured using enzyme-linked immunosorbent assay kits purchased from Kmaels (Shanghai) Co., Ltd.

Experimental data were analyzed using SAS 9.4 with the GLM model, and Duncan's multiple comparison test was used. $P < 0.05$ was considered statistically significant.

Results

2.1 Effects of Dietary Energy and Protein Levels on Lamb Growth Performance

As shown in Table 2, no interaction between dietary energy and protein levels was observed for lamb growth performance indicators ($P > 0.05$). Increasing dietary energy and protein levels significantly increased body weight of lambs at 90 and 120 days of age ($P < 0.05$). Dietary energy and protein levels had no significant effect on dry matter intake of lambs at 61-90, 91-120, and 61-120 days of age ($P > 0.05$). Low dietary energy and protein levels significantly decreased average daily gain of lambs at 61-90 days of age ($P < 0.05$) and significantly increased feed-to-gain ratio ($P < 0.05$), but had no significant effect on average daily gain at 91-120 and 61-120 days of age ($P > 0.05$).

2.2 Effects of Dietary Energy and Protein Levels on Lamb Nitrogen Metabolism

As shown in Table 3, at 81-90 days of age, dietary energy level had no significant effect on nitrogen intake ($P > 0.05$), while low protein level significantly decreased nitrogen intake ($P < 0.05$). Dietary energy and protein levels had no

significant effect on fecal nitrogen output ($P > 0.05$) but significantly affected urinary nitrogen output and nitrogen retention, with a significant interaction effect ($P < 0.05$). Decreasing energy level and increasing protein level both significantly increased urinary nitrogen output ($P < 0.05$), with the low-energy high-protein group showing the highest value. The high-energy high-protein group had significantly higher nitrogen retention than other groups ($P < 0.05$), while adjusting protein level under low energy conditions or adjusting energy level under low protein conditions did not affect nitrogen retention ($P > 0.05$). Dietary protein level had no significant effect on nitrogen deposition rate ($P > 0.05$), but increasing energy level significantly increased nitrogen deposition rate ($P < 0.05$). Energy level only affected nitrogen deposition rate under high protein conditions, where decreasing energy level reduced nitrogen deposition rate ($P < 0.05$), but had no significant effect under low protein conditions ($P > 0.05$). Low dietary energy level or high protein level significantly decreased biological value of nitrogen ($P < 0.05$).

At 111-120 days of age, no interaction between dietary energy and protein levels was observed for lamb nitrogen metabolism ($P > 0.05$). Dietary energy level had no significant effect on nitrogen intake ($P > 0.05$), while low protein level significantly decreased nitrogen intake ($P < 0.05$). Dietary energy and protein levels had no significant effect on fecal nitrogen, nitrogen retention, nitrogen deposition rate, or biological value of nitrogen ($P > 0.05$), but significantly affected urinary nitrogen output ($P < 0.05$). Decreasing energy level or increasing protein level both significantly increased urinary nitrogen output ($P < 0.05$).

2.3 Effects of Dietary Energy and Protein Levels on Lamb Serum Biochemical Indices

As shown in Table 4, no interaction between dietary energy and protein levels was observed for lamb serum biochemical indices ($P > 0.05$). Dietary energy and protein levels had no significant effect on serum Glu content at 61 and 90 days of age ($P > 0.05$), but decreasing energy level significantly decreased serum GLU content at 120 days of age ($P < 0.05$). Dietary energy and protein levels had no significant effect on serum UN content at 61 days of age ($P > 0.05$), but low protein level significantly decreased serum UN content at 90 days of age ($P < 0.05$), and low energy level significantly increased serum UN content at 120 days of age ($P < 0.05$). Dietary energy and protein levels had no significant effect on serum TG and GH content at 61, 90, and 120 days of age ($P > 0.05$). Dietary energy level had no significant effect on serum IGF-I content at 61 and 90 days of age ($P > 0.05$), but low protein level significantly increased serum IGF-I content at 120 days of age ($P < 0.05$).

Discussion

3.1 Effects of Dietary Energy and Protein Levels on Lamb Growth Performance

Dietary energy level affects lamb feed intake; therefore, this experiment adjusted feed amounts for the HELP, LEHP, and LELP groups based on the HEHP group intake to observe the effects of dietary energy and protein levels while eliminating interference from different intake levels. The results showed no significant difference in dry matter intake among the four groups, which met experimental design requirements. In this study, increasing dietary energy level significantly increased average daily gain of lambs at 61–90 days of age, consistent with findings by Kabir et al. and Yerradoddi et al. However, Ríos-Rincón et al. found no significant effect of dietary energy level on average daily gain, possibly because the regulatory effect of energy level only becomes apparent at lower energy levels. Dietary energy level had no significant effect on average daily gain at 91–120 and 61–120 days of age, similar to results reported by Zhang et al., which may be related to muscle growth being predominant at 61–90 days while fat deposition increases at 91–120 days, requiring more energy. The effect of dietary protein level on average daily gain was consistent with that of energy level. Cui et al. also reported that increasing dietary protein level improved average daily gain in lambs at 61–90 days, likely because lambs at this stage are undergoing rapid development of bones, muscles, and organs, and increased protein promotes this development. In this study, increasing dietary energy and protein levels significantly decreased feed-to-gain ratio at 61–90 days, and increasing energy level significantly decreased feed-to-gain ratio at 91–120 days, though protein level had no significant effect. Considering these results, dietary protein level can be reduced during the 91–120 day period to save feed costs without adversely affecting growth performance.

3.2 Effects of Dietary Energy and Protein Levels on Lamb Nitrogen Metabolism

Nitrogen is an essential element in the body, and its utilization efficiency reflects protein utilization. In this study, increasing dietary protein level significantly increased nitrogen intake at both 81–90 and 111–120 days, as expected. Dietary energy and protein levels had no significant effect on fecal nitrogen output at either stage, consistent with Guo. Increasing dietary energy level significantly decreased urinary nitrogen output, while increasing protein level significantly increased urinary nitrogen output in lambs at both 81–90 and 111–120 days. At 81–90 days, increasing dietary energy level significantly increased nitrogen retention, deposition rate, and biological value, possibly because higher energy promoted rumen microbial growth and microbial protein synthesis. Increasing dietary protein level significantly increased nitrogen retention and biological value, consistent with Yun et al., but had no significant effect on nitrogen deposition rate, likely because excess protein beyond requirements was excreted in urine, resulting in no significant difference in deposition rate. However, at

111-120 days, increasing dietary energy and protein levels had no significant effect on nitrogen retention, deposition rate, or biological value, indicating that nitrogen metabolism changes with developmental stage.

3.3 Effects of Dietary Energy and Protein Levels on Lamb Serum Biochemical Indices

Changes in serum biochemical indices reflect metabolic alterations in the body. Serum GLU content indicates energy metabolism status. Studies have shown that dietary energy level affects serum GLU content. In this study, dietary energy and protein levels had no significant effect on serum GLU content at 61 and 90 days, consistent with Wang et al. and Mellado et al. However, at 120 days, decreasing energy level significantly reduced serum GLU content, similar to results reported by Gong et al. Serum UN content is an important indicator of nitrogen metabolism; low serum UN content indicates high nitrogen metabolism efficiency. In this study, dietary energy and protein levels had no significant effect on serum UN content at 61 days, but increasing protein level significantly increased serum UN content at 90 days, indicating that excess protein reduced nitrogen metabolism efficiency and increased liver and kidney burden. At 120 days, increasing energy level significantly decreased serum UN content, possibly because higher energy promoted microbial protein synthesis and improved protein utilization. Serum TG is closely related to energy metabolism and can be decomposed by various tissues, reflecting fat utilization. In this study, dietary energy and protein levels had no significant effect on serum TG content at any age, differing from Song et al., possibly because serum TG content is related to lamb age.

GH controls animal growth and nutrient metabolism, primarily promoting fat decomposition and protein synthesis. Insulin-like growth factors (IGF) include IGF-I and IGF-II, with IGF-I being the main factor promoting animal growth and development. Studies have shown that serum GH content may be related to average daily gain. Dietary energy and protein levels had no significant effect on serum GH content at any age, possibly because differences in average daily gain among the four groups were not significant, and lambs had similar development levels. In this study, dietary energy and protein levels had no significant effect on serum IGF-I content at 61 and 90 days, consistent with Sun et al., possibly because the reduction in dietary energy and protein levels was insufficient to cause changes in serum IGF-I, or because serum GH content did not differ significantly among groups, and IGF-I is regulated by GH. However, at 120 days, increasing dietary protein level decreased serum IGF-I content, contrary to Yan et al. and Li et al., who found a positive correlation between serum IGF-I and dietary protein level. This discrepancy may be due to individual lamb differences or age-related effects. In conclusion, dietary protein level can be appropriately reduced during the 91-120 day period to save production costs and improve economic efficiency.

Conclusions

1. During the 61-90 day period, increasing dietary energy and protein levels enhanced lamb growth performance and nitrogen metabolism at 81-90 days, but had relatively minor effects on growth performance at 91-120 days and nitrogen metabolism at 111-120 days.
2. High energy and protein levels promoted lamb growth during the 61-90 day period. During the 91-120 day period, dietary protein level can be appropriately reduced to save production costs without adversely affecting growth performance.

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