Effects of Different Dietary Protein Levels on Growth and Development of Tibetan Lambs: Postprint

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Abstract

This study aimed to investigate the effects of different dietary protein levels on the growth and development of Tibetan lambs. The experiment adopted a single-factor randomized design, in which ninety 2-month-old weaned male Tibetan lambs with a body weight of (20.80$±$2.75) kg were selected and randomly divided into three experimental groups (30 replicates per group, one lamb per replicate), and fed experimental diets with protein levels of 10.8%, 12.0%, and 13.2%, respectively. The experimental period lasted 127 d, including a 7-d preliminary period and a 120-d formal experimental period. The results showed: 1) The 12.0% protein level group exhibited the highest daily gain and average daily gain during days 60–90 and days 90–120 of the trial, along with the lowest feed-to-gain ratio. 2) The 12.0% protein level group had lower diarrhea and urinary tract infection rates, and the lowest mortality rate. 3) On day 60 of the experiment, serum total protein (TP) content in the 12.0% protein level group showed no significant difference from the 10.8% and 13.2% protein level groups (P>0.05), but the difference between the 10.8% and 13.2% protein level groups was significant (P<0.05). On days 90 and 120, serum TP content showed no significant difference between the 12.0% and 13.2% protein level groups (P>0.05), but both were significantly higher than that in the 10.8% protein level group (P<0.05). At all stages, serum GLU and TC contents did not change significantly with increasing dietary protein level (P>0.05). 4) The 12.0% protein level group achieved the highest weight gain per unit cost, reaching 73.05 kg/yuan, as well as the highest total income and net income, with net income reaching 7,800.51 yuan. Under the conditions of this experiment, dietary protein level affected the growth and development of early-weaned Tibetan lambs; the 12.0% protein level improved daily gain, reduced feed-to-gain ratio and mortality, improved serum biochemical indices, enhanced growth performance, and reduced feeding costs.

Full Text

Effects of Diets with Different Protein Levels on Growth and Development of Tibetan Lambs

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Abstract

This experiment was conducted to evaluate the effects of diets with different protein levels on the growth and development of Tibetan lambs. Using a single-factor randomized design, ninety 2-month-old weaned Tibetan male lambs with an initial body weight of (20.80$±$2.75) kg were randomly allocated to three experimental groups (30 replicates per group, 1 lamb per replicate). The lambs were fed experimental diets containing 10.8%, 12.0%, and 13.2% crude protein, respectively. The trial lasted 127 days, including a 7-day pre-trial period and a 120-day formal experimental period. The results showed: 1) The 12.0% protein group achieved the highest daily gain during days 60–90 and 90–120, as well as the highest average daily gain, with the lowest feed-to-gain ratio. 2) The 12.0% protein group exhibited lower diarrhea and urethral infection rates, and the lowest mortality. 3) On day 60, serum total protein (TP) content in the 12.0% protein group showed no significant difference compared with the 10.8% and 13.2% protein groups (P>0.05), but the difference between the 10.8% and 13.2% protein groups was significant (P<0.05). On days 90 and 120, serum TP content showed no significant difference between the 12.0% and 13.2% protein groups (P>0.05), but both were significantly higher than the 10.8% protein group (P<0.05). At all stages, serum glucose (GLU) and total cholesterol (TC) contents did not change significantly with increasing dietary protein level (P>0.05). 4) The 12.0% protein group achieved the highest weight gain per unit cost at 73.05 kg/RMB, along with the highest total income and net income, reaching 7,800.51 RMB in net income. Under these experimental conditions, dietary protein level significantly affected the growth and development of early-weaned Tibetan lambs. A 12.0% protein level improved daily gain, reduced feed-to-gain ratio and mortality, improved serum biochemical parameters, enhanced growth performance, and reduced feeding costs.

Keywords: protein level; Tibetan lambs; daily gain; feed-to-gain ratio; serum biochemical parameters

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Introduction

Protein is an essential nutrient for ruminants, and animals primarily obtain nutrients through feed. Dietary protein level and protein source directly affect protein digestion, metabolism, and utilization, thereby constraining animal growth

and development [1-2]. For rapidly growing Tibetan lambs, dietary protein level is a critical factor influencing growth rate [3]. Insufficient dietary protein hampers growth, while excessive protein may induce metabolic diseases in lambs. Although numerous studies have investigated dietary protein levels in animals, research on protein requirements for lambs remains limited with inconsistent results, and no existing feeding standards are available for Tibetan lambs. Based on these considerations, this experiment was conducted under confined feeding conditions using 2-month-old early-weaned Tibetan lambs to investigate the effects of different dietary protein levels on daily gain, feed-to-gain ratio, serum biochemical parameters, and economic performance. The objective was to determine the optimal dietary protein level for early-weaned Tibetan lambs and provide a scientific basis for protein requirement research and economical lamb production.

1.1 Experimental Time and Location

This experiment was conducted from January 11, 2016, to May 18, 2016, at the Highland Animal Production Internship Base in Haibei Prefecture, Qinghai Province.

1.2 Experimental Animals and Design

Ninety 2-month-old weaned Tibetan male lambs in good health with a body weight of (20.80$±$2.75) kg were selected and randomly divided into three groups according to a single-factor randomized design and the principle of balanced body weight. Each group comprised 30 replicates with one lamb per replicate. The three groups were fed diets containing 10.8% (LG group), 12.0% (MG group), and 13.2% (HG group) crude protein. The experimental period lasted 127 days, including a 7-day pre-trial period and a 120-day formal experimental period.

1.3 Experimental Diets

Based on lamb body weight and nutritional requirements, and referring to the NRC (2007) [7] nutrient requirements for sheep and China' s "Feeding Standard of Meat Sheep" [8], early-weaning diet formulations for Tibetan lambs were designed. The composition and nutrient levels of the experimental diets are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis), %

Note: 1) Each kilogram of premix provided: Fe 2,248 mg, Zn 1,200 mg, Cu 850 mg, Mn 1,100 mg, Se 7.5 mg, I 20 mg, Co 10 mg, VA 200,000 IU, VD 35,000 IU, VE 3,000 IU. 2) DE was a calculated value, while the others were measured values.

1.4 Feeding Management

Experimental lambs were housed in a semi-open barn that was windproof, sunny, dry, and well-ventilated. The barn was disinfected before the lambs entered and was cleaned daily and disinfected every other day thereafter. All experimental lambs were immunized with sheep quadruple dry inactivated vaccine via intramuscular injection (1 mL per lamb) within 30 days, and dewormed with praziquantel on day 30 (1 tablet per lamb). Feeding time, method, and amount were identical across groups, with feeding conducted daily at 08:30, 12:00, and 17:00, and free access to water. Feed allowance was adjusted weekly based on the maximum feed intake of the MG group. Feed and water troughs were cleaned once daily.

1.5 Blood Sample Collection

Blood samples were collected on days 30, 60, 90, and 120 of the formal experimental period. On collection days, lambs were restrained in lateral recumbency before morning feeding. The collection site was shaved and disinfected with 75% alcohol. The jugular vein was compressed with fingers until engorged, and approximately 5 mL of blood was collected using a disposable syringe. After collection, tubes were placed upright to avoid hemolysis from shaking. After standing at room temperature for 30–60 minutes to allow serum separation, samples were centrifuged at 3,500 r/min for 15 minutes. Serum was collected and stored at -20 °C.

1.6.1 Growth Performance

Body weight was measured on days 0 (before the experiment), 30, 60, 90, and 120 of the formal experimental period (all weighing was conducted before the first feeding in the morning). Feed intake of concentrate supplements (CSFI) and oat hay (OHFI) were recorded to calculate total feed intake (TFI), stage daily gain (SDG), average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

Calculation formulas:
Average daily feed intake = Total feed intake / (Total experimental days × Number of lambs)
Feed-to-gain ratio = Average daily feed intake / Average daily gain

1.6.2 Diarrhea and Urethral Infection Rates

The number of lambs with diarrhea and urethral infection was recorded to calculate diarrhea rate and urethral infection rate.

1.6.3 Serum Biochemical Parameters

Serum biochemical parameters included total protein (TP), glucose (GLU), and total cholesterol (TC). TP content was determined by the Affiliated Hospital of

Qinghai University using an Olymyus 640 automatic biochemical analyzer. GLU and TC contents were measured using kits purchased from Nanjing Biological Construction Engineering Research Institute.

1.7 Data Processing

Data were initially processed using Excel 2003. SAS 9.1 statistical software was used for one-way ANOVA and significance testing. Duncan' s multiple range test was used for intergroup comparisons. Results were expressed as "mean ± standard deviation," with $P<0.05$ considered statistically significant.

2.1 Effects of Different Dietary Protein Levels on Growth Performance of Tibetan Lambs

As shown in Table 2 , body weight did not differ significantly among the three groups on days 0, 30, and 60 ($P>0.05$). On day 90, body weight in the MG and HG groups was not significantly different ($P>0.05$) but was 7.65% higher than the LG group ($P<0.05$). On day 120, body weight was highest in the MG group, followed by the HG group, with no significant difference between them ($P>0.05$), but both were significantly higher than the LG group by 11.98% and 10.37%, respectively ($P<0.05$).

During days 0-30 and 30-60, daily gain was highest in the HG group, followed by the MG group, and lowest in the LG group, with significant differences among groups ($P<0.05$). During days 60-90, daily gain followed the pattern LG group < HG group < MG group, with significant differences among groups ($P<0.05$). The LG and HG groups were 21.02% and 16.52% lower than the MG group, respectively. During days 90-120, daily gain in the MG group was significantly higher than in the LG and HG groups ($P<0.05$), with the LG group slightly lower than the HG group but no significant difference between them ($P>0.05$).

Over the entire experimental period (days 0-120), average daily gain followed the pattern MG group < LG group < HG group, with significant differences among groups ($P<0.05$). The LG and HG groups were 19.39% and 6.39% lower than the MG group, respectively.

Table 2 Body weight and daily gain at each stage of Tibetan lambs

Note: In the same row, values with no letter or the same letter superscripts indicate no significant difference ($P>0.05$), while different lowercase letters indicate significant difference ($P<0.05$). The same applies to Table 5 .

As shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper], body weight increased linearly in all three groups throughout the experiment, with the MG and HG groups following nearly identical trajectories slightly above the LG group. Daily gain peaked during days 60-90 in all groups before declining, with the MG group maintaining higher values than the HG and LG groups.

As shown in Table 3 , concentrate supplement intake, oat hay intake, and total feed intake were highest in the MG group, followed by the LG group, and lowest in the HG group. Average daily gain was highest in the MG group, with the LG and HG groups being 19.39% and 6.39% lower, respectively. Feed-to-gain ratio was lowest in the MG group at 6.91, highest in the LG group, with the HG group being 6.80% higher than the MG group but 13.99% lower than the LG group.

Table 3 Feed intake and feed-to-gain ratio of Tibetan lambs

Note: In this experiment, the average daily feed intake was identical across the three groups, as were the intakes of concentrate supplement and oat hay. The differences in concentrate supplement intake and oat hay intake among groups were due to different mortality rates.

2.2 Effects of Different Dietary Protein Levels on Diarrhea and Urethral Infection Rates of Tibetan Lambs

As shown in Table 4 , diarrhea rate decreased with increasing dietary protein level, while urethral infection rate gradually increased. Mortality was high in both the LG and HG groups but lowest in the MG group. The LG group experienced mortality only from diarrhea, with no urethral infection-related deaths, while the HG group showed the opposite pattern. Only the HG group exhibited cases of urinary calculi, specifically bladder stones.

Table 4 Conditions of urethral infection and diarrhea of Tibetan lambs

Note: In the mortality data, the figure before "+" represents deaths from diarrhea, while the figure after "+" represents deaths from urethral infection.

2.3 Effects of Different Dietary Protein Levels on Serum Biochemical Parameters of Tibetan Lambs

As shown in Table 5 , serum TP content showed an increasing trend with rising dietary protein level at all stages. On day 30, no significant difference in serum TP content was observed among the three groups ($P>0.05$). On day 60, serum TP content in the MG group did not differ significantly from the LG or HG groups ($P>0.05$), but a significant difference existed between the LG and HG groups ($P<0.05$). On days 90 and 120, serum TP content showed no significant difference between the MG and HG groups ($P>0.05$), but both were significantly higher than the LG group ($P<0.05$).

At all stages, serum GLU and TC contents did not change significantly with increasing dietary protein level ($P>0.05$). Serum GLU content was relatively higher in the MG and HG groups, while serum TC content was relatively higher in the LG and MG groups.

Table 5 Serum biochemical parameters at each stage of Tibetan lambs

2.4 Economic Benefit Analysis

As shown in Table 6 , concentrate unit price, concentrate cost, and total feed cost increased gradually with rising dietary protein level. Total weight gain and weight gain per unit cost were highest in the MG group, reaching 73.05 kg/RMB. Both total income and net income were highest in the MG group, with net income reaching 7,800.51 RMB. The net incomes of the LG and HG groups were 36.77% and 25.50% lower than the MG group, respectively.

Table 6 Analysis of economic benefits

Note: The price of experimental early-weaning Tibetan lambs was 350 RMB/lamb, and the price of live weight at the end of the experiment was 23 RMB/kg. Feed consumption of dead lambs has been removed from this table.

3.1 Effects of Different Dietary Protein Levels on Body Weight and Weight Gain of Tibetan Lambs

Numerous studies have demonstrated that, within a certain range, increasing dietary protein level promotes growth and development in early-weaned lambs. This study found that during days 0–60, body weight and daily gain increased gradually with rising dietary protein level, but differences among groups were not significant, indicating that dietary protein level had no obvious effect on lamb growth during this stage. However, during days 90–120, body weight and daily gain showed a trend of increasing then decreasing with rising protein level, peaking in the 12.0% protein group with significant differences from the 10.8% and 13.2% protein groups. Furthermore, average daily gain throughout the entire experimental period was also highest in the 12.0% protein group. The possible reason is that the 10.8% protein level not only failed to meet the growth requirements of Tibetan lambs at this stage but may also have affected the synthesis of key enzymes in the digestion and metabolism of other nutrients, resulting in insufficient nutrient supply and slow growth. Conversely, the 13.2% protein level may have been excessive, potentially reducing the absorption and utilization of other nutrients through the tricarboxylic acid cycle and causing nutritional imbalance, thereby decreasing daily gain and inhibiting growth. These findings are consistent with previous research, indicating that a 12.0% protein diet is more conducive to the growth of early-weaned Tibetan lambs. Additionally, this study found that body weight increased linearly in all three groups with advancing days, with the MG and HG groups following nearly identical trajectories slightly above the LG group. Daily gain peaked during days 60–90 in all groups before declining, with the MG group maintaining higher values than the HG and LG groups, suggesting that appropriately increasing dietary protein level during days 60–90 could better promote growth.

3.2 Effects of Different Dietary Protein Levels on Feed Intake and Feed-to-Gain Ratio of Tibetan Lambs

Feed intake in lambs can be influenced by numerous factors including diet composition and concentrate-to-forage ratio, palatability (anti-nutritional factors and soluble sugar content), nutritional level (energy concentration, protein and amino acid content, fat, mineral elements), feeding technology (feed form, feeding method and timing), lamb sex, age and health status, housing environment, and water availability [15]. In this experiment, average daily feed intake decreased with increasing dietary protein level, while feed-to-gain ratio followed the pattern LG group > HG group > MG group, and average daily gain followed LG group < HG group < MG group. These results are generally consistent with findings from Walz et al. [16], Lü Kai [17], and Liu Ligang et al. [18]. This indicates that excessively high dietary protein levels do not promote growth and may even inhibit it, thereby increasing feed-to-gain ratio, while insufficient protein levels fail to meet nutritional requirements and are also detrimental to growth. Therefore, a 12.0% protein diet not only promotes growth and development in Tibetan lambs but also improves feed utilization efficiency, reduces feed-to-gain ratio, lowers feed costs, and enhances production efficiency.

3.3 Effects of Different Dietary Protein Levels on Diarrhea and Urethral Infection Rates of Tibetan Lambs

Lamb diarrhea is primarily caused by stress or improper diet leading to digestive system dysfunction, resulting in loose stools, dehydration, and potentially death in severe cases. Lü Kai [17] found that within a dietary protein range of 15%–21%, higher protein levels resulted in lower diarrhea rates. However, Yu Kang et al. [19] observed that high-protein diets easily induced diarrhea, with higher rates in female lambs than in males. This study found that diarrhea rate in Tibetan lambs decreased gradually with increasing dietary protein level, possibly because high-protein diets increased serum immunoglobulin content and enhanced immune function.

Urethral infection results from unreasonable diet formulation causing tissue damage and even calculus formation, leading to dysuria, hematuria, urinary retention, and potentially death. This study found that urethral infection rate increased gradually with rising dietary protein level, with urinary calculi observed only in the HG group. This may be because the excessively high protein level (13.2%) increased matrix protein content, a major component of urinary stone matrix, thereby increasing urethral infection risk. Additionally, high-protein diets may increase serum ammonia content through deamination pathways, increasing kidney burden and raising urine concentration and pH, leading to supersaturation and crystal formation. In summary, Tibetan lambs fed the 12.0% protein diet exhibited the lowest mortality and best health status.

3.4 Effects of Different Dietary Protein Levels on Serum Biochemical Parameters of Tibetan Lambs

Serum TP content reflects dietary protein nutritional level and protein digestion and absorption to some extent. Higher blood TP content indicates enhanced protein synthesis [20]. This study found that different dietary protein levels significantly affected serum TP content. During days 0–90, serum TP content increased gradually with rising dietary protein level, though differences among groups were not significant. On day 120, serum TP content was comparable between the MG and HG groups, both significantly higher than the LG group. This indicates that high protein levels can accelerate amino acid decarboxylation, promote protein digestion and metabolism, and benefit growth and development. Additionally, serum TP content in all three groups showed a trend of increasing then decreasing with advancing days, peaking on day 90, indicating that protein assimilation was strongest and growth rate was fastest on day 90 [3].

Serum GLU, as an important nutritional monosaccharide, is the primary energy source for metabolism in the brain, nervous system, and muscles [21]. Higher serum GLU content indicates more vigorous metabolism and faster growth. Serum TC content reflects lipid utilization, with lower lipid absorption indicating higher lean meat percentage. This study found that serum GLU and TC contents did not change significantly with increasing dietary protein level, indicating that different protein levels did not significantly affect these parameters. This suggests that the body can achieve nutritional balance through the tricarboxylic acid cycle and self-regulation. These findings are consistent with Wu Min et al. [22] and Si Bingwen et al. [23] but differ from Sahlu et al. [24] and Gao Qing et al. [25], possibly due to differences in experimental animal species and duration. Although serum GLU and TC contents did not differ significantly among groups, serum GLU was relatively higher in the MG and HG groups, while serum TC was relatively higher in the LG and MG groups. Therefore, lambs fed the 12.0% protein diet utilized serum GLU and TC more effectively, benefiting growth and development while improving lean meat percentage.

Conclusion

1. Tibetan lambs fed the 12.0% protein diet exhibited higher stage daily gain and average daily gain, with the lowest feed-to-gain ratio, indicating that 12.0% dietary protein promotes growth and improves feed utilization efficiency.
2. Tibetan lambs fed the 12.0% protein diet showed lower diarrhea and urethral infection rates with the lowest mortality, indicating that 12.0% dietary protein promotes metabolism, improves digestive system function, reduces urinary system disease incidence, and enhances overall health.
3. Serum TP, GLU, and TC contents were optimal in the 12.0% protein group, indicating that 12.0% dietary protein improves nutrient digestion and utilization, thereby promoting lamb growth and development.

4. The 12.0% protein group achieved the highest weight gain per unit cost at 73.05 kg/RMB, with the highest total income and net income reaching 7,800.51 RMB.

5. Under these experimental conditions, dietary protein level significantly affected the growth and development of early-weaned Tibetan lambs. A 12.0% protein level improved daily gain, reduced feed-to-gain ratio and mortality, improved serum biochemical parameters, enhanced growth performance, and reduced feeding costs.

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Note: Figure translations are in progress. See original paper for figures.