

Effects of Calcium and Phosphorus Levels in Concentrate Supplement on Growth Performance, Serum Biochemical Parameters, and Bone Composition of Lambs in Pastoral Areas During Winter-Spring Season: Postprint

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Abstract

This experiment aimed to investigate the effects of supplementary feeding with concentrate supplements containing different calcium and phosphorus levels on growth performance, serum indices, and bone composition of weaned lambs under conditions of low-quality roughage supply in pastoral areas during winter and spring. Ninety-six weaned Ujimqin lambs, aged (30 ± 2) days with a body weight of (12.50 ± 1.21) kg, were selected and randomly divided into 3 groups with 32 lambs per group, half male and half female. The three groups were supplemented with three types of concentrate supplements with the same calcium to phosphorus ratio but different calcium and phosphorus levels: Group A (calcium 0.40%, phosphorus 0.38%), Group B (calcium 0.50%, phosphorus 0.48%), and Group C (calcium 0.68%, phosphorus 0.65%). The experimental period lasted 55 d, including a 5-day preliminary period and a 50-day formal experimental period. The results showed that with increasing calcium and phosphorus levels in the concentrate supplement, total feed intake and average daily gain of lambs showed no significant changes ($P > 0.05$), with average daily gain approaching 200 g/d. On day 50 of the experiment, serum calcium, parathyroid hormone content, and alkaline phosphatase activity also showed no significant differences among groups ($P > 0.05$), while serum phosphorus content in Group C was significantly higher than in Groups A and B ($P < 0.05$). Tibia calcium content in Group C was significantly higher than in Group A ($P < 0.05$), with no significant difference from Group B ($P > 0.05$), and tibia phosphorus content showed no significant differences among groups ($P > 0.05$). It can be concluded that during the withered grass period in winter and spring in pastoral areas, concentrate supplements with different calcium

and phosphorus levels did not have significant effects on growth performance, some serum indices, and tibia phosphorus content of Ujimqin lambs. Calcium at 0.40% and phosphorus at 0.38% in the concentrate supplement could meet the growth requirements of lambs, and adding excess calcium and phosphorus would waste resources and pollute the environment.

Full Text

Preamble

Effects of Calcium and Phosphorus Levels of Supplementary Concentrate on Growth Performance, Serum Biochemical Indices and Bone Composition of Lambs in Pastoral Areas during Winter and Spring Season

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Abstract: This study aimed to investigate the effects of different calcium and phosphorus levels in supplementary concentrate on growth performance, serum biochemical indices, and bone composition of weaned lambs fed low-quality roughages in pastoral areas during winter and spring. Ninety-six healthy Ujumuin weaner lambs (half male and half female) aged (30 ± 2) days with an initial body weight of (12.50 ± 1.21) kg were randomly allocated to three groups (32 lambs per group) using a single-factor experimental design. Lambs in groups A, B, and C were offered supplementary concentrate containing calcium and phosphorus at 0.40% and 0.38%, 0.50% and 0.48%, and 0.68% and 0.65%, respectively, with the same calcium-to-phosphorus ratio across treatments. The experiment lasted 55 days, including a 5-day pre-experimental period and a 50-day experimental period. The results showed that increasing calcium and phosphorus levels in the supplementary concentrate did not significantly affect total feed intake or average daily gain (ADG) ($P > 0.05$), with ADG approaching 200 g/d across all groups. On day 50, serum calcium concentration, parathyroid hormone content, and alkaline phosphatase activity showed no significant differences among groups ($P > 0.05$), while serum phosphorus concentration in group C was significantly higher than in groups A and B ($P < 0.05$). Tibia calcium content in group C was significantly higher than in group A ($P < 0.05$), but did not differ significantly from group B ($P > 0.05$). No significant differences were observed in tibia phosphorus content among groups ($P > 0.05$). These findings indicate that

different calcium and phosphorus levels in supplementary concentrate had no significant effects on growth performance, certain serum biochemical indices, or tibia phosphorus content in Ujumuin lambs during the winter-spring withered grass period in pastoral areas. Calcium and phosphorus levels of 0.40% and 0.38% in supplementary concentrate, respectively, were sufficient to meet the growth requirements of lambs, while excessive supplementation would waste resources and pollute the environment.

Keywords: lamb; calcium; phosphorus; growth performance; supplementary concentrate

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Introduction

Inner Mongolia Autonomous Region represents China's largest pastoral area, where high-quality forage constitutes the most important feed source for herbivorous animals. However, during winter and spring, pasture grasses wither and their nutritional value declines, resulting in phosphorus (P) intake from grazing that cannot meet the requirements for lactating ewes and rapidly growing lambs during the lambing season [1]. Therefore, timely supplementation of lambs represents an effective measure to improve survival rates and growth performance. Calcium (Ca) and phosphorus are essential mineral elements in animals, accounting for approximately 70-75% of total body minerals and playing crucial roles in metabolism, internal environment stability, and physiological functions. Currently, most nutrient requirements for ruminants reference the NRC (2007) standards, yet numerous studies have demonstrated that these recommended levels do not align with the nutritional needs of Chinese sheep [2-3]. Moreover, calcium and phosphorus requirements vary among different breeds and physiological states [4]. The Ujumuin sheep, produced in the Ujumuin grasslands of Xilinguole League in Inner Mongolia, is an excellent breed developed through long-term selective breeding of Mongolian sheep under local conditions. Adapted to year-round grazing on natural pastures, Ujumuin sheep exhibit rapid growth, early maturity, and tender meat quality, but their specific nutrient requirements remain undocumented. Therefore, this study employed three supplementary concentrates with different calcium and phosphorus levels to investigate their effects on growth performance, serum indices, and bone composition of Ujumuin lambs, thereby determining optimal calcium and phosphorus levels for supplementation. The objective was to improve dietary calcium and phosphorus utilization and provide theoretical reference for lamb production practices under winter-spring environmental conditions in Inner Mongolia.

1.1 Experimental Period and Location

The animal feeding trial was conducted from April 9 to May 28, 2014, at a family ranch in Xilinhot City, Inner Mongolia Autonomous Region.

1.2 Experimental Animals and Design

A single-factor experimental design was employed. Ninety-six Ujumuin lambs aged (30 ± 2) days with a body weight of (12.50 ± 1.21) kg were randomly divided into three groups of 32 lambs each (half male and half female). Groups A, B, and C received three different supplementary concentrates varying in calcium and phosphorus levels. The trial lasted 55 days, including a 5-day pre-experimental period and a 50-day experimental period. Lambs were housed in separate pens per group, each equipped with independent feed and water troughs. The pens were semi-open, sheltered from wind, south-facing, dry, and well-ventilated.

Based on lamb body weight and nutritional requirements, and referencing the “Feeding Standard of Meat-producing Sheep” (NY/T 816-2004) for growing and fattening lambs (18 kg body weight, 200 g/d daily gain, with calcium and phosphorus requirements of 2.5 and 1.7 g/d, respectively), three supplementary concentrates were formulated considering local winter-spring forage nutritional values. The composition and nutrient levels are presented in Table 1. Digestible energy and crude protein levels were determined based on previous research from our laboratory [5]. The supplementary concentrate was pelleted (6 mm diameter, 10 mm length) and produced by Inner Mongolia Youmote Animal Husbandry Technology Co., Ltd. Roughage consisted of locally harvested and sun-dried hay from autumn mowing fields, predominantly *Leymus chinensis* (Chinese wildrye), with the following nutrient levels (air-dry basis): dry matter (DM) 93.25%, crude protein (CP) 5.22%, calcium 0.49%, phosphorus 0.09%. During the trial, supplementary concentrate was fed twice daily at 07:00 and 18:00. Each group received 1,700 g/d of milk replacer (calcium 0.23%, phosphorus 0.49%) dissolved in warm water. Lambs had ad libitum access to hay and water. Residual feed and hay were weighed each evening to calculate dry matter intake.

1.3 Measurements and Methods

1.3.1 Diet Composition Weekly samples of supplementary concentrate and hay were collected and stored at 4°C. After the trial, samples were transported to the laboratory for determination of dry matter, digestible energy, crude protein, neutral detergent fiber, acid detergent fiber, calcium, and phosphorus content [6].

1.3.2 Growth Performance Individual lamb body weight was measured before morning feeding at the beginning and end of the experimental period to calculate average daily gain and feed-to-gain ratio.

1.3.3 Serum Biochemical Indices On day 1 (before the trial) and day 50 (at the end), blood samples (approximately 10 mL) were collected from the jugular vein of six lambs per group (half male and half female). Samples were centrifuged at 3,000 r/min for 10 minutes, and serum was collected and stored in 1.5 mL centrifuge tubes at -20°C until analysis. Parathyroid hormone

(PTH) concentration was determined by radioimmunoassay using kits provided by the Institute of Atomic Energy, Chinese Academy of Sciences. Serum calcium and phosphorus concentrations and alkaline phosphatase (AKP) activity were measured using an Olympus AU600 automatic biochemical analyzer.

1.3.4 Slaughter Trial Bone composition reflects the final result of calcium and phosphorus metabolism. After blood collection, four lambs per group with body weight close to the group average were selected for slaughter. Tibia bones were collected to determine calcium and phosphorus content. Tibiae were boiled for 30 minutes, drained, cooled, and stripped of muscle and tendon. The bones were crushed, wrapped in filter paper, dehydrated in anhydrous ethanol for 24 hours, and defatted in anhydrous ether for 96 hours. Processed tibiae were oven-dried at 105°C for 12 hours to obtain defatted dry weight, then ashed in a muffle furnace at 600°C for 3 hours to obtain crude ash. Tibia calcium content was determined by potassium permanganate titration (GB 6436-86), and phosphorus content by molybdenum yellow colorimetry (GB 6437-86).

1.4 Statistical Analysis

Experimental data were analyzed using SAS 9.2 statistical software. ANOVA was performed, and differences among means were tested using Duncan's multiple comparison method. Significance was declared at $P < 0.05$.

2.1 Growth Performance of Lambs

As shown in Table 2, no significant differences were observed in total feed intake among groups ($P > 0.05$). Average daily gain approached 200 g/d across all treatments without significant differences ($P > 0.05$). However, numerically, group A exhibited the highest average daily gain and optimal feed-to-gain ratio.

2.2 Serum Biochemical Indices of Lambs

Table 3 shows that on day 1, no significant differences were detected in serum calcium, phosphorus, parathyroid hormone concentrations, or alkaline phosphatase activity among groups ($P > 0.05$). On day 50, serum calcium concentration, parathyroid hormone content, and alkaline phosphatase activity remained non-significantly different among groups ($P > 0.05$), while serum phosphorus concentration in group C was significantly higher than in the other two groups ($P < 0.05$).

2.3 Bone Composition

As presented in Table 4, on day 50, tibia calcium content in group C was significantly higher than in group A ($P < 0.05$), but did not differ significantly from group B ($P > 0.05$). No significant differences were observed in tibia phosphorus content among groups ($P > 0.05$).

3.1 Effects of Calcium and Phosphorus Levels on Lamb Growth Performance

Calcium and phosphorus are essential mineral elements constituting 70-75% of total body minerals. The ideal dietary calcium-to-phosphorus ratio is generally considered to be 1:1 to 2:1, though ratios between 1:1 and 7:1 do not affect calcium and phosphorus absorption in ruminants [7]. In this study, dietary calcium-to-phosphorus ratios fell within the ideal range. Results showed that increasing calcium and phosphorus intake did not significantly affect total feed intake or average daily gain, which approached 200 g/d across groups. Calcium and phosphorus intake in group A were 2.70 and 1.87 g/d, respectively, slightly exceeding designed requirements, and lambs in all groups exhibited normal growth and development. Hutcheson et al. [8] fed crossbred wethers (average weight 25.1 kg) diets with different calcium and phosphorus levels (calcium 0.8% or 0.6%; phosphorus 0.4% or 0.3%) and obtained similar results, finding no significant effects on average daily gain, feed intake, or feed-to-gain ratio. Li et al. [9] also reported that different phosphorus levels and calcium-to-phosphorus ratios did not significantly affect average daily gain or feed-to-gain ratio in growing-finishing pigs. Similarly, Erickson et al. [10] fed calves (265.0±\$16.6 kg) diets with varying phosphorus levels (0.16%, 0.22%, 0.28%, 0.40%) at a constant calcium level (0.62%) and observed no significant effects on dry matter intake, average daily gain, or feed-to-gain ratio. These findings indicate that the calcium and phosphorus levels in group A were sufficient to meet the growth requirements of Ujumuin lambs, and higher levels did not improve average daily gain but instead showed a declining trend. Under outdoor pastoral conditions with adequate sunlight exposure, lambs generally do not suffer from calcium deficiency but may experience phosphorus deficiency. Since grains in supplementary diets are rich in phosphorus and ruminants can effectively utilize phosphorus from various sources (including phytates) [11], the calcium and phosphorus levels in group A's supplementary concentrate satisfied lamb growth requirements. Excessive dietary calcium and phosphorus increase urinary and fecal excretion, leading to resource waste and environmental pollution.

3.2 Effects of Calcium and Phosphorus Levels on Serum Biochemical Indices

Serum calcium, phosphorus, parathyroid hormone concentrations, and alkaline phosphatase activity are commonly used hematological parameters to estimate calcium and phosphorus nutritional status and requirements. According to Lu [12], normal reference values for sheep are 2.5-3.0 mmol/L for serum calcium and 1.29-5.16 mmol/L for inorganic phosphorus, while Lu [13] suggests critical levels of 2.00 and 1.45 mmol/L for diagnosing deficiencies. In this study, except for slightly low serum calcium at the trial's end, all measured values fell within reference ranges and exceeded critical levels. Increasing calcium intake did not significantly affect serum calcium concentration, consistent with Pond [14] who reported that dietary calcium supplementation did not significantly

alter serum calcium in lambs. However, Oberbauer et al. [15] found that serum calcium increased with dietary calcium levels. Serum phosphorus concentration in group C was significantly higher than in other groups with increasing phosphorus intake, similar to Hutcheson et al. [8] who reported that high calcium and phosphorus diets significantly increased serum calcium and phosphorus in growing lambs. Conversely, Cohen [16] found that dietary phosphorus level did not significantly affect serum inorganic phosphorus in beef cattle. These inconsistent reports suggest that serum calcium and phosphorus concentrations are not readily influenced by calcium and phosphorus levels in supplementary concentrate. All groups maintained serum calcium and phosphorus within normal ranges, indicating adequate calcium and phosphorus nutrition.

Parathyroid hormone, an alkaline single-chain polypeptide hormone secreted by chief cells of the parathyroid gland, primarily regulates calcium and phosphorus metabolism in vertebrates. It maintains stable blood calcium levels by increasing secretion when blood calcium decreases [17-18]. In this study, serum calcium and parathyroid hormone concentrations remained stable across groups and were not correlated with calcium intake. Serum alkaline phosphatase, secreted by osteoblasts, reflects osteoblast activity and indirectly indicates calcium and phosphorus nutritional status. Its activity is negatively correlated with blood phosphorus concentration; decreased blood phosphorus leads to increased alkaline phosphatase activity to maintain phosphorus homeostasis. In this study, stable blood phosphorus concentration and alkaline phosphatase activity across groups indicated that osteoblasts were not excessively secreting alkaline phosphatase into blood, reflecting normal calcium and phosphorus status [19]. While Hutcheson et al. [8] found that high calcium and phosphorus diets significantly increased serum parathyroid hormone, other researchers (Pond [14]; Wheeler et al. [20]) reported that serum parathyroid hormone and alkaline phosphatase activity were unaffected by dietary calcium and phosphorus levels.

3.3 Effects of Calcium and Phosphorus Levels on Bone Composition

Calcium and phosphorus are the most abundant mineral elements in animals, with 98-99% of calcium and 80% of phosphorus stored in bone and teeth [11]. Bone serves as a calcium and phosphorus reservoir, and through mobilization and deposition, animals maintain strong homeostatic control of blood calcium and phosphorus. In this study, lambs showed no signs of appetite loss or limb weakness, tibia ash calcium-to-phosphorus ratio was approximately 2:1, bones exhibited no obvious deformities, and growth was normal. Supplementary concentrate calcium and phosphorus levels significantly affected tibia calcium content, with lower levels resulting in significantly lower tibia calcium compared to higher levels, indicating that increased dietary calcium promoted tibia mineralization and calcium deposition, while phosphorus content remained unchanged. This aligns with Wang et al. [21] who reported that increasing dietary calcium and phosphorus significantly increased tibia calcium content in Beijing ducks without affecting tibia phosphorus. However, Wang et al. [22] and Li et al. [23]

found that increased dietary calcium and phosphorus significantly increased both tibia ash and phosphorus content in broiler chickens. Hutcheson et al. [8] observed no significant changes in metacarpal calcium and phosphorus content between high (calcium 0.8%, phosphorus 0.6%) and low (calcium 0.4%, phosphorus 0.3%) dietary levels in lambs. These inconsistent findings may relate to differences in experimental animals or growth stages. This study demonstrates that low calcium and phosphorus levels (calcium 0.40%, phosphorus 0.38%) in supplementary concentrate did not impair normal bone growth and met skeletal growth requirements, consistent with recommendations in China's "Feeding Standard of Meat-producing Sheep" (NY/T 816-2004).

Conclusion

Supplementing Ujumuqin lambs with different calcium and phosphorus levels during the winter-spring withered grass period in pastoral areas did not significantly affect growth performance, serum calcium concentration, parathyroid hormone content, or alkaline phosphatase activity. However, increasing calcium and phosphorus levels significantly elevated serum phosphorus and tibia calcium content. Calcium and phosphorus levels of 0.40% and 0.38% in supplementary concentrate, respectively, were sufficient to meet the growth requirements of Ujumuqin lambs, while excessive supplementation would waste resources and potentially pollute the environment.

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