

## Effects of Dietary Methionine Level on Production Performance, Nutrient Apparent Digestibility, and Serum Biochemical Indices in Alpacas (Postprint)

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### Abstract

This experiment aimed to investigate the effects of dietary methionine levels on production performance, nutrient apparent digestibility, and serum biochemical indices in alpacas. Nine 26-month-old female alpacas with similar body weight were selected and randomly divided into 3 groups with 3 replicates per group and 1 alpaca per replicate. The dietary methionine levels for each group were 0.16%, 0.32%, and 0.64%, respectively. A feeding trial and metabolism trial were conducted using total mixed ration. The preliminary period lasted 7 days, and the formal experimental period lasted 60 days. The results showed: 1) The average daily feed intake, average daily gain, and wool yield per unit body surface area of alpacas in the 0.32% and 0.64% groups were significantly higher than those in the 0.16% group ( $P < 0.05$ ), while the 0.32% group was slightly higher than the 0.64% group ( $P > 0.05$ ). 2) The apparent digestibility of crude protein, neutral detergent fiber, crude fiber, and calcium was highest in the 0.32% group, showing significant differences compared with the 0.16% group ( $P < 0.05$ ); the apparent digestibility of acid detergent fiber in the 0.32% group was significantly higher than that in the 0.64% group ( $P < 0.05$ ); the apparent methionine digestibility in the 0.32% and 0.64% groups was extremely significantly higher than that in the 0.16% group ( $P < 0.01$ ). 3) There were no significant differences in serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase activities, and triglycerides, glucose, blood urea nitrogen, and total protein contents among all groups ( $P > 0.05$ ). In conclusion, under the conditions of this experiment, the optimal dietary methionine level for female alpacas is 0.32%.

## Full Text

### Effects of Dietary Methionine Level on Performance, Nutrient Apparent Digestibility, and Serum Biochemical Indices of Alpacas

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**Abstract:** This experiment was conducted to investigate the effects of dietary methionine level on performance, nutrient apparent digestibility, and serum biochemical indices in alpacas. Nine healthy 26-month-old female alpacas with similar body weight [(62.77±1.06) kg] were randomly allocated into three groups, each consisting of three replicates with one alpaca per replicate. The dietary methionine levels were set at 0.16%, 0.32%, and 0.64%, respectively. All groups were fed total mixed rations during both the feeding trial and metabolic trial. The pre-experimental period lasted 7 days, followed by a 60-day experimental period. The results showed: (1) The average daily feed intake, average daily gain, and wool yield per unit body surface area in the 0.32% and 0.64% groups were significantly higher than those in the 0.16% group ( $P<0.05$ ), with the 0.32% group showing slightly higher values than the 0.64% group ( $P>0.05$ ). (2) The apparent digestibility of crude protein, neutral detergent fiber, crude fiber, and calcium was highest in the 0.32% group, which was significantly different from the 0.16% group ( $P<0.05$ ). The acid detergent fiber apparent digestibility in the 0.32% group was significantly higher than that in the 0.64% group ( $P<0.05$ ). The methionine apparent digestibility in both the 0.32% and 0.64% groups was extremely significantly higher than that in the 0.16% group ( $P<0.01$ ). (3) No significant differences were observed among groups in serum alanine transaminase, aspartate aminotransferase, or alkaline phosphatase activities, nor in serum triglyceride, glucose, urea nitrogen, or total protein contents ( $P>0.05$ ). In conclusion, under the conditions of this experiment, the optimal dietary methionine level for female alpacas is 0.32%.

**Keywords:** alpacas; methionine; performance; nutrient apparent digestibility; serum biochemical indices

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## Introduction

Alpacas, introduced to China from Australia, are native to the Andes Mountains in the upper Amazon basin and exhibit strong environmental adaptability and tolerance to roughage feeding [1]. As dual-purpose livestock for both wool and meat production, alpacas produce soft, lustrous fleece that commands high prices and is internationally renowned as “soft gold” [2]. With their distinctive appearance and gentle temperament, alpacas are also popular as ornamental animals in zoos and tourist attractions [3]. In recent years, alpaca farming has gained widespread popularity in China. According to incomplete statistics, by 2015, China had approximately 3,500 alpacas, with the industry in Xinjiang, Shanxi, Henan, Shandong, and other regions entering its initial development stage [4].

However, research on alpaca feed and nutritional requirements remains scarce in China, and domestic feeding standards for alpacas are lacking, making it difficult to formulate nutritionally appropriate diets for production. Methionine (Met) is one of the essential limiting amino acids for protein synthesis in animals, and supplemental methionine is commonly added to animal diets to meet requirements [5]. Currently, reports on methionine requirements for alpacas are rare. Moore et al. [6] reported that supplementing alpaca diets with 2–4 g of methionine for 4–7 weeks significantly increased fiber diameter. Yan et al. [7] found that the optimal supplemental level of rumen-protected methionine in Small-tailed Han sheep diets was 0.485% when adding 0.243%–0.971%. Based on domestic and international literature regarding methionine nutrition in alpacas and sheep, this experiment was designed to evaluate the effects of different dietary methionine levels on performance, nutrient apparent digestibility, and serum biochemical indices in adult female alpacas, aiming to determine the appropriate methionine level for rational diet formulation.

## Materials and Methods

### Experimental Design

Nine healthy, disease-free, 26-month-old adult female alpacas with similar body weight [(62.77±1.06) kg] were randomly divided into three groups, with three replicates per group and one alpaca per replicate, housed individually. All three groups were fed total mixed rations with dietary methionine levels set at 0.16%, 0.32%, and 0.48% based on relevant literature [6–9], while other nutrient levels remained consistent. The composition and nutrient levels of the experimental diets are presented in Table 1. The pre-experimental period lasted 7 days, followed by a 60-day experimental period.

## Animal Management

The alpaca barn was an open-sided shed with good protection from wind, adequate sunlight, dryness, and ventilation. Before the experiment, the barn, feed troughs, water troughs, floors, and aisles were thoroughly cleaned and disinfected. Alpacas were numbered, vaccinated, and dewormed before entering the barn. During the trial, alpacas had free access to feed and water. The barn was cleaned daily and disinfected regularly to maintain hygiene.

## Sample Collection and Analysis

**Production Performance** From the start of the experimental period, daily feed offered and refusals were recorded accurately. Feed samples and daily refusals were sealed and stored at  $-20^{\circ}\text{C}$  for dry matter (DM) determination. Body weight was measured on the morning of day 1 and day 60 after overnight fasting to calculate average daily gain (ADG). At the start of the experimental period, wool on the upper left buttock near the back ( $15\text{ cm} \times 15\text{ cm}$  area) was clipped clean using hand shears. At the end of the period, wool samples were collected from the same area and weighed to calculate wool yield per unit body surface area ( $\text{mg}/\text{cm}^2$ ).

Daily DM intake = daily feed offered  $\times$  DM content - daily feed refusal  $\times$  DM content

Average daily gain = (final weight - initial weight) / number of experimental days

Wool yield per unit body surface area ( $\text{mg}/\text{cm}^2$ ) = wool weight produced during trial / ( $15 \times 15$ )

**Nutrient Apparent Digestibility** A metabolic trial was conducted starting on day 30 of the experimental period. Alpacas were housed in specialized metabolic cages, and total feces collection was performed over a 7-day pre-trial period followed by a 3-day collection period. Daily feed offered and refusals were recorded accurately, and refusals were collected daily, dried at  $65^{\circ}\text{C}$  to produce air-dry samples, and stored for analysis. During each collection period, all fecal and urine samples were collected continuously for 3 days and weighed accurately. Feces were preserved with 10% hydrochloric acid for nitrogen fixation and stored at  $-20^{\circ}\text{C}$  pending analysis.

Nutrient contents in feed and feces were determined as follows: fecal samples were thawed at room temperature, mixed thoroughly, dried to constant weight in a  $65^{\circ}\text{C}$  oven, and processed into air-dry samples. Dry matter content was determined using the method described in reference [10]. Crude protein (CP) content was measured by the Kjeldahl method (GB/T 6432-1994). Ether extract (EE) content was determined by the residual method (GB/T 6433-2006). Acid detergent fiber (ADF) content was measured according to NY/T 1459-2007. Crude fiber (CF) content was determined using filter bag technology with an ANKOM A200i semi-automatic fiber analyzer. Neutral detergent fiber

(NDF) content was measured by the method of GB/T 20806–2006. Calcium (Ca) content was determined by potassium permanganate titration (GB/T 6436–2002). Phosphorus (P) content was measured by spectrophotometry (GB/T 6437–2002). Methionine and lysine (Lys) contents were analyzed using a Hitachi L-8900 high-speed amino acid analyzer.

**Serum Biochemical Indices** On the final day of the experiment, 5 mL of blood was collected from the jugular vein of each alpaca before morning feeding after overnight fasting. After standing for 2 hours, serum was separated by centrifugation at 3,500 rpm for 10 minutes and stored at -20°C pending analysis. Serum alanine transaminase, aspartate aminotransferase, and alkaline phosphatase activities, as well as triglyceride, glucose, urea nitrogen, and total protein contents were measured using a Hitachi 7600 automatic biochemical analyzer.

### Statistical Analysis

Experimental data were initially processed using Excel 2016 software. Statistical analysis was performed using SPSS 20.0 software, and differences among groups were tested using Duncan's multiple comparison method. Results are expressed as "mean  $\pm$  standard deviation."

## Results

### Effects of Dietary Methionine Level on Alpaca Performance

The effects of dietary methionine level on alpaca performance are shown in Table 2. No significant differences were observed in initial or final body weight among the three groups ( $P > 0.05$ ). The average daily feed intake, average daily gain, and wool yield per unit body surface area in the 0.32% and 0.64% groups were significantly higher than those in the 0.16% group ( $P < 0.05$ ), with the 0.32% group showing slightly higher values than the 0.64% group ( $P > 0.05$ ).

### Effects of Dietary Methionine Level on Nutrient Apparent Digestibility

The effects of dietary methionine level on nutrient apparent digestibility are presented in Table 3. The 0.32% group exhibited the highest apparent digestibility of crude protein and neutral detergent fiber, which differed significantly from the other two groups ( $P < 0.05$ ). The apparent digestibility of ether extract and total phosphorus in the 0.32% group was slightly higher than in the 0.16% and 0.64% groups ( $P > 0.05$ ). Calcium apparent digestibility in the 0.32% group was significantly higher than in the 0.16% group ( $P < 0.05$ ) and slightly higher than in the 0.64% group ( $P > 0.05$ ). Acid detergent fiber apparent digestibility in the 0.32% group was significantly higher than in the 0.64% group ( $P < 0.05$ ). Crude fiber apparent digestibility in the 0.32% group was significantly higher than in the 0.16% group ( $P < 0.05$ ) and slightly higher than in the 0.64% group ( $P > 0.05$ ).

Methionine apparent digestibility in both the 0.32% and 0.64% groups was extremely significantly higher than in the 0.16% group ( $P < 0.01$ ).

### **Effects of Dietary Methionine Level on Serum Biochemical Indices**

The effects of dietary methionine level on serum biochemical indices are shown in Table 4. No significant differences were detected among the three groups in serum alanine transaminase, aspartate aminotransferase, or alkaline phosphatase activities ( $P > 0.05$ ). Similarly, no significant differences were found in serum triglyceride, glucose, or total protein contents ( $P > 0.05$ ). Serum urea nitrogen content tended to increase with dietary methionine level ( $P > 0.05$ ).

## **Discussion**

### **Effects of Dietary Methionine Level on Alpaca Performance**

Alpacas have been raised in China for only about 20 years, and research on their nutritional requirements, breeding, and disease control remains in the exploratory stage. Few studies have reported on the effects of dietary methionine on alpaca performance. Alpacas are herbivores with digestive anatomy similar to ruminants such as sheep and camels, but with unique characteristics—for example, they have only three stomach compartments [11]. Wang et al. [12] found that low dietary methionine levels significantly reduced growth and slaughter performance in lambs. Mao [13] reported that increasing dietary amino acid levels increased average daily gain and significantly reduced feed-to-gain ratio in meat sheep. Yan [14] supplemented 9-month-old Small-tailed Han sheep with rumen-protected methionine at 0%, 0.243%, 0.485%, and 0.728% and found that increasing dietary methionine significantly improved body weight gain. Mi et al. [15] studied the effects of rumen-protected methionine on meat sheep growth performance and found that weight gain in methionine-supplemented groups was higher than in the control group, though the difference was not significant. Alpacas are sheared once annually, producing 3–5 kg of fleece per year, which is 8–10 times the wool yield of adult female goats. Although alpaca fiber fineness is inferior to fine wool and cashmere, it offers advantages in staple length and clean wool yield with good overall quality [16]. The present results demonstrate that increasing dietary methionine level (0.16%–0.64%) improved feed intake, body weight gain, and wool production in alpacas, with similar effects observed at high (0.64%) and medium (0.32%) levels, indicating that an appropriate dietary methionine level can promote feed intake, weight gain, and fleece growth.

### **Effects of Dietary Methionine Level on Nutrient Apparent Digestibility**

Methionine is typically the first or second limiting amino acid for ruminants [17]. Wang et al. [12] investigated the effects of dietary methionine level on nutrient apparent digestibility in Hu sheep and found that low methionine levels reduced nutrient digestibility. Gao [18] reported that intra-ruminal administration of

N-hydroxymethyl methionine calcium (N-HMM-Ca) significantly improved digestibility of dry matter, crude protein, acid detergent fiber, and neutral detergent fiber in sheep diets. The current results show that the 0.32% methionine group exhibited higher apparent digestibility of crude protein, neutral detergent fiber, acid detergent fiber, crude fiber, and calcium compared with the 0.16% and 0.64% groups. Research indicates that increasing dietary methionine promotes nutrient digestibility in ruminants by stimulating rumen microbial growth and enhancing rumen fermentation [17-18]. However, the 0.64% methionine group showed lower nutrient apparent digestibility than the 0.32% group, suggesting that 0.64% exceeds the normal requirement for alpacas. This may be due to methionine toxicity, as excess methionine can adversely affect physiology through its metabolic intermediates or by altering enzyme activities in amino acid metabolism [7]. The exact mechanism requires further investigation.

### Effects of Dietary Methionine Level on Serum Biochemical Indices

Rulquin and Delaby [19] reported that dietary supplementation with rumen-protected methionine promoted hepatic gluconeogenesis and increased serum glucose content. In this study, serum glucose content in the 0.64% group was slightly higher than in the 0.16% and 0.32% groups, possibly related to methionine's stimulatory effect on hepatic gluconeogenesis. Sun [20] and Bi et al. [21] found that dietary methionine levels tended to increase serum alanine transaminase, aspartate aminotransferase activities and total protein, albumin, triglyceride, and glucose contents in dairy cows, though not significantly. Moore et al. [6] reported that supplementing alpaca diets with 2 or 4 g of methionine did not significantly affect serum glucose or urea nitrogen compared with the control group. The present results showing no significant effects of dietary methionine level on serum biochemical indices are consistent with these reports. Alanine transaminase and aspartate aminotransferase are important transaminases that catalyze amino acid transamination, primarily located intracellularly with low serum activity; highest activities occur in the heart and liver, and serum levels increase when tissues are damaged [21]. Alkaline phosphatase is a key enzyme in animal digestion and metabolism, mainly originating from bone, liver, and intestinal mucosa, with its activity closely related to animal growth [22]. Wang et al. [23] established normal ranges for serum biochemical indices in alpacas, and the values obtained in this study are similar, indicating that dietary methionine levels of 0.16%-0.64% did not significantly affect physiological metabolism.

### Conclusion

Under the conditions of this experiment, the optimal dietary methionine level for female alpacas is 0.32%.

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