Effects of Dietary Methionine Level on Nutrient Digestion, Gastrointestinal pH, and Serum Indices in Male Hu Sheep Lambs: Postprint

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Abstract

This study aimed to investigate the effects of dietary methionine level on nutrient digestion, gastrointestinal pH, and serum indices in male Hu sheep lambs. Twelve pairs of 7-day-old weaned male Hu sheep twins were selected and assigned using a paired experimental design to a control (CON) group and a low methionine (LM) group, with each twin pair split between the two groups. The experiment was conducted in two phases. In Phase 1 (8–56 days of age), lambs in the CON group were fed a basal milk replacer and basal starter feed; lambs in the LM group were fed milk replacer and starter feed with all supplemental methionine (0.70% and 0.40%, respectively) removed from the CON diet, while other nutrient levels remained consistent. In Phase 2 (57–84 days of age), lambs in both groups were weaned from milk replacer and fed only the basal starter feed. Digestion and metabolism trials were conducted on four randomly selected twin pairs before the end of Phase 1 (46–55 days of age) and before the end of Phase 2 (74–83 days of age). The results showed: 1) At 56 days of age, apparent digestibility of dietary crude protein, ether extract, and neutral detergent fiber in LM group lambs was significantly lower than in the CON group ($P<0.05$); at 84 days of age, no significant differences in nutrient apparent digestibility were observed between the two groups ($P>0.05$). 2) At 56 days of age, except for duodenal pH which was significantly lower in the LM group than in the CON group ($P<0.05$), no significant differences were observed in other gastrointestinal pH values ($P>0.05$); at 84 days of age, no significant differences in gastrointestinal pH were found between the two groups ($P>0.05$). 3) Except for growth hormone and insulin concentrations which were significantly lower in the LM group at 56 days of age ($P<0.05$), no significant differences in other serum indices were observed between the two groups at either 56 or 84 days of age ($P>0.05$). These results indicate that during 8–56 days of age, low dietary methionine level reduced nutrient apparent digestibility and inhibited the increase in duodenal pH and serum growth hormone and insulin concentrations in male Hu sheep lambs; during 57–84 days of age, after dietary methionine level was increased, nutrient apparent digestibility, gastrointestinal pH, and serum hormone indices were subsequently compensated.

Full Text

Effects of Dietary Methionine Level on Nutrient Digestion, Gastrointestinal pH, and Serum Indexes of Male Hu Lambs

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Abstract

This study investigated the effects of dietary methionine level on nutrient digestion, gastrointestinal pH, and serum indexes in male Hu lambs. Twelve pairs of 7-day-old weaned male Hu twin lambs were selected and allocated to two groups using a paired design: a control (CON) group and a low methionine (LM) group, with each twin pair split between the two groups. The experiment consisted of two stages. During Stage 1 (8–56 days of age), lambs in the CON group were fed a basal milk replacer and basal starter, while those in the LM group received the same diets with 0.70% and 0.40% of the supplemental methionine removed from the milk replacer and starter, respectively, while maintaining consistent nutrient levels otherwise. During Stage 2 (57–84 days of age), all lambs were weaned from milk replacer and fed only the basal starter.

Digestion and metabolism trials were conducted at the end of each stage, using four randomly selected twin pairs (46–55 days of age for Stage 1; 74–83 days of age for Stage 2). The results showed: (1) At 56 days of age, the apparent digestibility of crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF) in the LM group was significantly lower than in the CON group ($P<0.05$); however, at 84 days of age, no significant differences were observed between the two groups in nutrient apparent digestibility ($P>0.05$). (2) At 56 days of age, except for duodenal pH, which was significantly lower in the LM group ($P<0.05$), no significant differences were found in other gastrointestinal pH values between groups ($P>0.05$); at 84 days of age, no significant differences were observed in gastrointestinal pH between the two groups ($P>0.05$). (3) Except for growth hormone (GH) and insulin (INS) concentrations at 56 days of age, which were significantly lower in the LM group ($P<0.05$), no significant differences were observed in other serum indexes between the two groups at either 56 or 84 days of age ($P>0.05$). In conclusion, a low dietary methionine level during 8–56 days of age reduced nutrient apparent digestibility and inhibited the increase in duodenal pH and serum GH and INS concentrations in male Hu lambs; however, during 57–84 days of age, subsequent elevation of dietary methionine level resulted in compensatory recovery of nutrient apparent digestibility, gastrointestinal pH, and serum hormone indexes.

Keywords: methionine; lamb; digestion and metabolism; gastrointestinal pH; serum index

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Introduction

Methionine, as the only sulfur-containing essential amino acid, plays a crucial role in protein synthesis in animals. Since animals cannot synthesize essential amino acids, they must obtain them from the diet to meet nutritional requirements [1]. However, methionine is often deficient in common feed ingredients such as soybean meal. For newborn lambs, the underdeveloped gastrointestinal system is highly susceptible to nutritional regulation, which can affect their subsequent fattening performance. Therefore, appropriate dietary methionine levels are essential for maintaining normal physiological activities in lambs.

Previous studies have shown that early-weaned lambs are prone to significant stress responses due to dietary composition and environmental factors [2], which can impair gastrointestinal function [3-4] and ultimately reduce nutrient digestion and absorption capacity [5-9]. Abdelrahman et al. [10] reported that dietary methionine supplementation improved both nutrient utilization and growth performance in lambs. Conversely, methionine restriction can negatively affect normal growth and development. Wang et al. [11] found that limiting dietary methionine significantly reduced growth performance and slaughter characteristics in lambs. Abouheif et al. [12] observed that feed restriction significantly decreased average daily gain and nutrient digestibility in fattening sheep, ultimately affecting their growth.

Hu sheep, a world-renowned prolific breed, typically produce 2–3 lambs per litter, making it difficult for ewe milk to meet the nutritional needs of suckling lambs. This results in significant individual differences in body condition development among nursing lambs, which is detrimental to intensive and large-scale management. Current research has primarily focused on the effects of single methionine addition or deficiency on nutrient digestibility in lambs or fattening sheep [13], while studies on the impact of early-life nutritional restriction under low methionine conditions on subsequent compensatory growth remain unreported. Animal growth and development is a continuous process, and nutritional deficiency during the restriction period must have intrinsic connections with nutritional supplementation during the compensation period. Therefore, this study investigated whether the reduced nutrient digestibility caused by low dietary methionine levels in early life could be compensated by subsequent elevation of dietary methionine, thereby improving gastrointestinal absorption capacity in lambs. The findings provide a theoretical basis for the rational and scientific feeding of early-weaned Hu twin lambs in China.

1. Materials and Methods

1.1 Experimental Location and Duration The experiment was conducted from October to December 2015 at Linqing Runlin Animal Husbandry Co., Ltd. in Shandong Province, China.

1.2 Experimental Design Twelve pairs of healthy male Hu twin lambs weaned at 7 days of age, with an initial body weight of 4.93 ± 0.20 kg, were selected. Using a paired design, the lambs were divided into a control (CON) group and a low methionine (LM) group, with each twin pair split between the two groups. The experiment consisted of two stages. During Stage 1 (8–56 days of age), lambs in the CON group were fed a basal milk replacer and basal starter, while those in the LM group received the same diets with 0.70% and 0.40% of the supplemental methionine removed from the milk replacer and starter, respectively, while maintaining consistent nutrient levels otherwise. During Stage 2 (57–84 days of age), all lambs were weaned from milk replacer and fed only the basal starter.

1.3 Experimental Diets The methionine supplement used in this experiment had the following specifications: DL-methionine content \$ \$99%; weight loss on drying \$ \$0.5%; arsenic \$ \$0.002‰; heavy metals \$ \$0.02‰; sulfate \$ \$0.30%; chloride \$ \$0.20%; residue on ignition \$ \$0.5%; sodium nitroprusside test qualified; copper sulfate test qualified.

Nutrient levels of the basal starter and basal milk replacer were formulated according to China' s "Feeding Standard of Meat-Producing Sheep" (NY/T 816-2004) [14] and the national patent ZL 02128844.5 [15]. Methionine levels were set based on the findings of Patureau-Mirand et al. [16] and Wang et al. [17]. The nutrient levels of the basal milk replacer and the composition and nutrient levels of the basal starter are presented in Table 1 .

1.4 Feeding Management Before the experiment began, the entire barn was thoroughly disinfected with a strong disinfectant solution, and this procedure was repeated weekly throughout the trial. All experimental lambs received routine vaccinations according to the standard immunization program.

From birth to 7 days of age, all lambs suckled their dams. At 8 days of age, lambs were weaned and artificially fed milk replacer until 56 days of age. Starter was supplemented from 8 days of age until the end of the experiment at 84 days of age. During milk replacer feeding, lambs were fed 4 times daily from 8–14 days, 3 times daily from 15–28 days, and 2 times daily from 29–56 days. Milk replacer feeding followed the method described by Wang et al. [17], with adjustments made according to the health status of the lambs to ensure normal growth. Throughout the experimental period, the intake of milk replacer and starter was maintained at similar levels between the CON and LM groups. Fresh water was available ad libitum.

1.5 Digestion and Metabolism Trials Two digestion and metabolism trials were conducted during the experimental period, from 46–55 days and 74–83 days of age. For each trial, four lambs were randomly selected from each group, consisting of four twin pairs (one twin from each pair per group). Selected lambs were marked and transferred to individual metabolism cages, where they were fed separately with ad libitum access to water. Each digestion and metabolism trial lasted 10 days, including a 5-day adaptation period followed by a 5-day collection period, using the total feces and urine collection method.

1.6 Sample Collection and Analysis

1.6.1 Determination of Nutrient Apparent Digestibility in Lambs During the digestion and metabolism trials, fecal and urine samples were collected at 07:00 and 19:00 daily. Feed intake, feed residue, fecal output, and urine volume were recorded for each lamb daily. Fecal samples were collected at 10% of total daily output, and 10 mL of 10% sulfuric acid was added per 100 g of fresh feces for nitrogen fixation before storage at -20 °C. Prior to urine collection, 100 mL of 10% sulfuric acid was added to the collection container. Daily urine samples were manually mixed, and 1% of the total volume was sampled and stored at -20 °C.

After the trials, nutrient levels in milk replacer, starter, feces, and urine were determined as follows: amino acid content was measured using an A300 automatic amino acid analyzer; gross energy was determined using a Parr-6400 oxygen bomb calorimeter; dry matter (DM), crude protein (CP), ether extract (EE), ash, calcium, phosphorus, and neutral detergent fiber (NDF) contents were analyzed according to AOAC (1980) methods [18].

1.6.2 Determination of Gastrointestinal pH in Lambs At 56 and 84 days of age, six twin pairs (six lambs per group) were slaughtered after 16 hours of feed and water deprivation. Lambs were exsanguinated via jugular venipuncture, and after skinning, the abdominal cavity was opened and the gastrointestinal tract was dissected. Contents from each stomach compartment and intestinal segment were collected in 30 mL centrifuge tubes, and pH was immediately measured using a PHB-2 portable pH meter for rumen, abomasum, duodenum, jejunum, and ileum contents.

1.6.3 Determination of Serum Indexes in Lambs At 56 and 84 days of age, 10 mL of blood was collected from the jugular vein of three randomly selected twin pairs (three lambs per group). Blood samples were centrifuged at 3,000 rpm for 20 minutes to separate serum, which was stored at -20 °C. Serum indexes included: serum hormone indexes, serum immune indexes, and serum biochemical indexes. Serum hormone and immune indexes were determined by enzyme-linked immunosorbent assay (ELISA) using kits purchased from Beijing Huaying Biotechnology Research Institute. Serum biochemical indexes were measured using a Hitachi 7020 automatic biochemical analyzer, except for lactate concentration, which was determined by neutralization titration.

1.7 Statistical Analysis Experimental data were initially processed using Excel 2010, and paired t-tests were performed using SAS 9.2 statistical software. Differences were considered significant at $P<0.05$.

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2. Results

2.1 Effects of Dietary Methionine Level on Nutrient Apparent Digestibility in Male Hu Lambs The effects of dietary methionine level on nutrient apparent digestibility are presented in Table 2 . At 56 days of age, apparent digestibility of CP, EE, and NDF in the LM group was significantly lower than in the CON group ($P<0.05$). At 84 days of age, no significant differences were observed in nutrient apparent digestibility between the two groups ($P>0.05$).

2.2 Effects of Dietary Methionine Level on Gastrointestinal pH in Male Hu Lambs The effects of dietary methionine level on gastrointestinal pH are presented in Table 3 . At 56 days of age, no significant differences were observed in gastrointestinal pH between groups ($P>0.05$) except for duodenal pH, which was significantly lower in the LM group ($P<0.05$). At 84 days of age, no significant differences were observed in gastrointestinal pH between the two groups ($P>0.05$).

2.3 Effects of Dietary Methionine Level on Serum Indexes in Male Hu Lambs The effects of dietary methionine level on serum indexes are presented in Tables 4 , 5 , and 6 . No significant differences were observed in serum indexes between the two groups at 56 and 84 days of age ($P>0.05$), except for GH and INS concentrations at 56 days of age, which were significantly lower in the LM group ($P<0.05$).

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3. Discussion

3.1 Effects of Dietary Methionine Level on Nutrient Apparent Digestibility in Male Hu Lambs The digestive and metabolic systems of pre-weaning lambs are not fully developed and exhibit potential plasticity. The developmental status during the non-ruminant phase is critical for determining subsequent healthy growth and fattening potential. Therefore, balanced nutrient levels provide the material foundation for metabolism in pre-weaning lambs.

Methionine serves as the primary limiting amino acid for protein synthesis in ruminant animals and plays a vital role in improving growth performance and nutrient digestion and absorption [19]. Previous studies have reported that the optimal daily methionine requirement for pre-ruminant lambs is 2 g, while the most suitable methionine level for fattening sheep is 0.64% [13,20]. In this experiment, during the 8–56 day period, daily methionine intake was 0.47 g and 1.75 g for lambs in the LM and CON groups, respectively, representing a 73.14% reduction in methionine intake for the LM group. At 56 days of age, dietary methionine deficiency significantly reduced apparent digestibility of CP, EE, and NDF. Similarly, Zeng et al. [21] reported that reducing lysine intake significantly decreased apparent digestibility of gross energy, DM, CP, and phosphorus. Puchala et al. [22] found that nutritional restriction in goats significantly reduced apparent digestibility of DM, organic matter, CP, and NDF. In this study, feeding a low-methionine diet during the non-ruminant phase reduced nitrogen intake, thereby decreasing rumen ammonia nitrogen (NH3-N) concentration, reducing microbial synthesis and activity, and consequently affecting enzyme secretion and nutrient digestion [23-24]. This phenomenon may also be related to methionine being a key essential amino acid that initiates enzyme synthesis, thereby influencing digestive enzyme composition or activity [25].

After 28 days of methionine level recovery, no significant differences were observed in apparent digestibility of DM, organic matter, CP, EE, gross energy, or NDF between the two groups. Berthiaume et al. [26] reported that dietary methionine supplementation increased methionine flow to the duodenum and improved its apparent digestibility in the small intestine. This compensatory effect may be attributed to the gradual increase in rumen NH3-N concentration with elevated methionine levels in the later stage, as well as the compensatory recovery of proteins mobilized from the digestive tract and liver during the methionine deficiency period, which enhanced rumen microbial activity and digestive function [27]. Similarly, Li et al. [28] reported that nutritional compensation after a restriction period restored visceral organ weight and increased digestive enzyme secretion, leading to improved nutrient digestibility. This phenomenon may also be explained by the near-complete development of rumen microbial digestive and metabolic functions after 56 days of age, with methionine entering the small intestine primarily derived from microbial protein, rumen undegradable protein, and endogenous methionine.

3.2 Effects of Dietary Methionine Level on Gastrointestinal pH in Male Hu Lambs Appropriate gastrointestinal acidity is an indispensable factor for maintaining normal digestive function and serves as the foundation for regulating acid-base and electrolyte balance [29]. Generally, rumen pH ranges from 5.0 to 7.5, but values below 6.5 are detrimental to fiber digestion [30]. After entering the rumen, methionine is degraded by rumen microorganisms to produce ammonia and keto acids, which are further fermented to volatile fatty acids. In this study, rumen pH values at 56 days of age were 6.00 and 6.04 for the CON and LM groups, respectively, and 5.28 and 5.55 at 84 days of age, all within the normal range, with no significant differences between groups. At 56 days of age, the keto acids produced from methionine degradation in the CON group could be fermented by microorganisms to produce volatile fatty acids, but the concentration was insufficient relative to rumen contents to cause significant pH changes. Similarly, Robinson et al. [31] confirmed that dietary methionine supplementation had no significant effect on rumen pH or volatile fatty acid concentration. Additionally, compared with 56 days of age, rumen pH at 84 days of age decreased by 12.00% and 8.11% in the CON and LM groups, respectively. This may be because the rumen does not play a dominant role during the non-ruminant phase, whereas during the ruminant phase, the rumen functions as the primary digestive organ, producing more volatile fatty acids and reducing pH. Other mechanisms may also be involved and require further investigation.

The intestinal tract maintains a relatively stable internal environment with certain buffering capacity. If pH is too low, the alkaline intestinal fluid secreted by intestinal glands may be partially neutralized, and large pH fluctuations can significantly affect digestive enzyme activity. In this study, at 56 days of age, low methionine in the LM group significantly reduced duodenal pH compared with the CON group. This may be because the strongly acidic chyme from the abomasum had not been sufficiently neutralized by pancreatic juice, bile, and bicarbonate in the duodenal lumen.

3.3 Effects of Dietary Methionine Level on Serum Indexes in Male Hu Lambs Animal growth is primarily regulated by the growth axis composed of the hypothalamus-pituitary-liver axis, with GH and insulin-like growth factor-I (IGF-I) best reflecting nutritional and growth status. GH is the main regulatory factor for postnatal growth, stimulating muscle protein synthesis and promoting animal growth. IGF-I is a multifunctional cell proliferation regulatory factor and an essential active protein peptide required for GH to exert its physiological effects. INS promotes glucose uptake by cells, enhances cellular function, stimulates glycogen synthesis, and improves glucose utilization and protein synthesis. This study found that low methionine significantly reduced serum GH and INS concentrations. Similarly, Zhang [32] reported that serum GH concentration increased gradually with increasing dietary methionine levels, with the highest concentration observed in the 0.8% methionine supplementation group. Smith et al. [33] reported that INS concentration in calf serum increased significantly with increased nutrient intake. However, other studies have shown that for most species (except rodents), nutritional deficiency leading to growth arrest is often accompanied by increased rather than decreased plasma GH concentration [34-35], a finding also confirmed in pigs by Buonomo et al. [36]. The results of this study differ from these conclusions, possibly due to the pulsatile nature of GH secretion and its regulation by IGF-I concentration. This study found that low dietary methionine level had no significant effect on serum IGF-I concentration in lambs. Similarly, Carew et al. [37] found that feeding methionine-deficient diets to 8–22-day-old male broilers did not significantly alter serum IGF-I concentration.

Serum immune and biochemical indexes are important indicators of animal health. Sun et al. [38] reported that rumen-protected methionine (RPM) reduced plasma total cholesterol, low-density lipoprotein cholesterol, and very low-density lipoprotein concentrations in periparturient dairy cows, but had no significant effect on plasma triglyceride concentration. Bi et al. [39] found that RPM supplementation in dairy cows increased plasma total protein, albumin, triglyceride, glucose, and free fatty acid concentrations, though not significantly. In this study, during the methionine deficiency period, the LM group showed reductions of 10.50%, 2.81%, 1.38%, 21.95%, and 5.04% in serum glucose, triglyceride, lactate, free fatty acid concentrations, and lactate dehydrogenase activity, respectively, compared with the CON group, but these differences were not statistically significant. This phenomenon may be caused by the combined effects of methionine deficiency duration, dosage, and environmental factors, and the specific mechanisms require further investigation.

In summary, during 8–56 days of age, low dietary methionine level reduced nutrient apparent digestibility and inhibited the increase in duodenal pH and serum GH and INS concentrations in male Hu lambs. During 57–84 days of age, subsequent elevation of dietary methionine level resulted in compensatory recovery of nutrient apparent digestibility, gastrointestinal pH, and serum hormone indexes.

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