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

Authors: Jie Wang, Cui Kai, Wang Shiqin, Diao Qiyu, Zhang Naifeng

Date: 2017-11-07T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary methionine level on nutrient digestibility, gastrointestinal pH, and serum indices in Hu sheep male lambs. Twelve pairs of 7-day-old weaned Hu sheep twin male lambs were selected and assigned using a paired experimental design into 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: Phase 1 (8-56 days of age), where 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) completely removed from the CON diet, while other nutrient levels remained consistent. Phase 2 (57-84 days of age), both groups of lambs stopped receiving milk replacer and were fed only the basal starter feed. Digestion and metabolism trials were conducted on 4 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, the apparent digestibility of dietary crude protein, crude fat, and neutral detergent fiber in LM group lambs was significantly lower than that in the CON group ($P<0.05$); at 84 days of age, there were no significant differences in nutrient apparent digestibility between the two groups ($P>0.05$). 2) At 56 days of age, except for duodenal pH which was significantly lower in LM group lambs compared to the CON group ($P<0.05$), there were no significant differences in other gastrointestinal pH values ($P>0.05$); at 84 days of age, there were no significant differences in gastrointestinal pH between the two groups ($P>0.05$). 3) Except for growth hormone and insulin concentrations at 56 days of age which were significantly lower in LM group lambs compared to the CON group ($P<0.05$), there were no significant differences in other serum indices 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 of duodenal pH and serum growth hormone and insulin concentrations in Hu sheep male lambs; during 57-84 days of age, after dietary methionine level was increased, nutrient apparent digestibility, gastrointestinal pH, and serum hormone indices in Hu sheep male lambs were subsequently compensated.

Full Text

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

WANG Jie, CUI Kai, WANG Shiqin, DIAO Qiyu, ZHANG Naifeng*

(Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute of Chinese Academy of Agricultural Sciences, Beijing 100081, China)

Abstract: This study aimed to investigate the effects of dietary methionine level on nutrient digestion, gastrointestinal pH, and serum indexes in male Hu lambs. Twelve pairs of male Hu twin lambs weaned at 7 days of age were selected and allocated to two groups using a matched-pairs design: a control group (CON) and a low methionine group (LM), with each twin pair split between the two groups. The experiment consisted of two stages. During Stage 1 (8 to 56 days of age), CON lambs were fed basal milk replacer and basal starter feed, while LM lambs received the same diets with 0.70% and 0.40% supplemental methionine deducted from the milk replacer and starter, respectively, while maintaining consistent nutrient levels otherwise. During Stage 2 (57 to 84 days of age), all lambs were weaned from milk replacer and fed only basal starter feed. Digestion and metabolism trials were conducted at the end of each stage (46-55 days and 74-83 days of age) using four randomly selected twin pairs per stage. The results showed: (1) At 56 days of age, apparent digestibility of crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF) in LM lambs was significantly lower than in CON lambs ($P<0.05$); however, at 84 days of age, no significant differences were observed between groups in nutrient apparent digestibility ($P>0.05$). (2) At 56 days of age, duodenal pH in LM lambs was significantly lower than in CON lambs ($P<0.05$), while other gastrointestinal pH values showed no significant differences ($P>0.05$); at 84 days of age, no significant differences were found in gastrointestinal pH between groups ($P>0.05$). (3) Except for significantly lower growth hormone (GH) and insulin (INS) concentrations in LM lambs at 56 days of age ($P<0.05$), no significant differences were observed in other serum indexes between groups at either 56 or 84 days of age ($P>0.05$). In conclusion, low dietary methionine level during 8-56 days of age reduced nutrient apparent digestibility and inhibited increases in duodenal pH and serum GH and INS concentrations in male Hu lambs; however, after increasing dietary methionine level during 57-84 days of age, these parameters showed compensatory recovery.

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

serum index

Introduction

Methionine, as the only sulfur-containing amino acid among essential amino acids, 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 [?]. However, methionine is often deficient in common feed ingredients such as soybean meal. For newborn lambs, the underdeveloped gastrointestinal system makes them particularly 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 [?], which can impair gastrointestinal function [?, ?] and ultimately reduce nutrient digestion and absorption capacity [?, ?, ?, ?, ?]. Abdelrahman et al. [?] reported that dietary methionine supplementation improved both nutrient utilization and growth performance in lambs. Conversely, methionine restriction negatively affects normal growth and development. Wang et al. [?] found that restricting dietary methionine significantly reduced growth performance and slaughter characteristics in lambs. Abouheif et al. [?] demonstrated that feed restriction significantly decreased average daily gain and nutrient digestibility in fattening sheep, ultimately affecting growth performance.

Hu sheep, as one of the world' s most prolific sheep breeds, 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 variation in lamb development and poses challenges for intensive and large-scale management. While most research has focused on the effects of single methionine addition or deficiency on nutrient digestibility in lambs or fattening sheep [?], no studies have investigated the impact of early-life nutritional restriction on subsequent compensatory growth under low methionine conditions. Animal growth and development constitute a continuous process, and nutritional deficiency during the restriction period must have intrinsic connections with nutritional supplementation during the compensation period. Therefore, this study examined whether reduced nutrient digestibility caused by low dietary methionine in early life could be compensated by increased methionine levels later, thereby improving gastrointestinal absorption capacity. The findings provide a theoretical basis for the rational and scientific feeding of early-weaned Hu twin lambs in China.

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 male Hu twin lambs weaned at 7 days of age, with an initial body weight of (4.93±0.20) kg and normal development, were selected. Using a matched-pairs design, the lambs were divided into a control group (CON) and a low methionine group (LM), with each twin pair split between the two groups. The experiment comprised two stages. During Stage 1 (8–56 days of age), CON lambs were fed basal milk replacer and basal starter feed, while LM lambs received the same diets with 0.70% and 0.40% supplemental methionine deducted 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 basal starter feed.

1.3 Experimental Diets The methionine used in the 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%; ignition residue 0.5%; sodium nitroprusside test qualified; copper sulfate test qualified.

Nutrient levels of the basal starter and milk replacer were formulated according to China' s "Feeding Standard of Meat-Producing Sheep" (NY/T 816-2004) [?] and the national invention patent ZL 02128844.5 [?]. Methionine levels were established based on the findings of Patureau-Mirand et al. [?] and Wang et al. [?]. 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 Animal Management Before the experiment began, the entire pen was thoroughly disinfected with a strong disinfectant solution, and this procedure was repeated weekly throughout the trial. All experimental lambs underwent routine immunization protocols. From birth to 7 days of age, lambs suckled their dams. At 8 days of age, lambs were weaned and artificially fed milk replacer until 56 days of age. Starter feed 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. [?] and was adjusted according to lamb health status to ensure normal growth. Throughout the experiment, feed intake of milk replacer and starter was maintained similar between 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 at 46–55 days and 74–83 days of age. For each trial, four Hu lambs were randomly selected from each group, with the four pairs in both groups being twin siblings. Selected lambs were marked and transferred to individual metabolism cages, fed separately, and provided with free access to water. Each digestion trial lasted 10 days, consisting of a 5-day preliminary period followed by a 5-day collection period, using the total feces and urine collection method.

1.6 Measurements

1.6.1 Determination of Nutrient Apparent Digestibility in Lambs During the digestion trials, fecal and urine samples were collected at 07:00 and 19:00 daily. Feed intake, orts, fecal output, and urine volume were recorded for each lamb daily. Fecal samples were collected at 10% of total weight and preserved by adding 10 mL of 10% sulfuric acid per 100 g of fresh feces for nitrogen fixation, then stored at -20°C for later analysis. Before urine collection, 100 mL of 10% sulfuric acid was added to collection containers. Daily urine from each lamb was manually mixed, and 1% of the total daily volume was sampled into urine bottles and stored at -20°C.

After the trial, 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) were analyzed according to AOAC (1980) methods [**?**].

1.6.2 Determination of Gastrointestinal pH in Lambs At 56 and 84 days of age, six pairs of twin lambs (six lambs per group) were slaughtered. After 16 hours of fasting and water restriction, lambs were exsanguinated via jugular venipuncture. Following evisceration, the gastrointestinal tract was dissected, and digesta samples from each stomach compartment and intestinal segment were collected in 30 mL centrifuge tubes. The pH of rumen, abomasum, duodenum, jejunum, and ileum contents was immediately measured using a PHB-2 portable pH meter.

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

1.7 Data Processing Experimental data were initially processed using Excel 2010 and then analyzed using SAS 9.2 statistical software with paired t-tests. Differences were considered significant at $P<0.05$.

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 dietary CP, EE, and NDF in LM lambs was significantly lower than in CON lambs ($P<0.05$). 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 shown in Table 3 . At 56 days of age, duodenal pH in LM lambs was significantly lower than in CON lambs ($P<0.05$), while no significant differences were observed in other gastrointestinal pH values ($P>0.05$). At 84 days of age, no significant differences were found 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 growth hormone and insulin concentrations at 56 days of age, which were significantly lower in LM lambs than in CON lambs ($P<0.05$).

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, and the developmental status during the non-ruminant phase is critical for determining subsequent healthy growth and fattening potential. Therefore, balanced nutrient levels constitute the material foundation for metabolic processes in pre-weaning lambs. As the primary limiting amino acid for protein synthesis in ruminants, methionine plays a vital role in improving animal growth performance and nutrient digestion and absorption [?]. 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% [?, ?]. In this experiment, during the 8–56 day period, daily methionine intake was 0.47 g and 1.75 g for LM and CON lambs, respectively, representing a 73.14% reduction in methionine intake for LM lambs compared to CON lambs. At 56 days of age, dietary methionine deficiency significantly reduced apparent digestibility of CP, EE, and NDF in lambs. Similarly, Zeng et al. [?] reported that reducing lysine intake significantly decreased apparent digestibility of gross energy, DM, CP, and phosphorus. Puchala et al. [?] found that nutritional restriction in goats significantly reduced apparent digestibility of DM, organic matter, CP, and NDF. In this study, feeding low-methionine diets during the non-ruminant phase reduced nitrogen intake, thereby decreasing rumen ammonia nitrogen (NH3-N) concentration and reducing microbial synthesis and activity, which subsequently affected enzyme secretion and ultimately impaired nutrient digestion [?, ?]. Additionally, this phenomenon may be related to methionine being a key essential amino acid that initiates enzyme synthesis, thereby influencing digestive enzyme composition or activity [?].

After 28 days of methionine level recovery, no significant differences were observed between groups in apparent digestibility of DM, organic matter, CP, EE, gross energy, or NDF. Berthiaume et al. [?] reported that dietary methionine supplementation increased methionine flow to the duodenum and improved its apparent digestibility in the small intestine. This phenomenon may be attributed to the gradual increase in rumen NH3-N concentration with restored methionine levels, along with compensatory recovery of digestive tract and liver proteins that were mobilized during methionine deficiency, leading to enhanced rumen microbial activity and digestive function [?]. Similarly, Li et al. [?] reported that nutrient-restricted lambs could recover visceral organ weight through nutritional compensation, thereby increasing digestive enzyme secretion and ultimately improving nutrient digestibility. This may also be due to 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 [?]. Generally, rumen pH ranges from 5.0 to 7.5, but values below 6.5 are detrimental to cellulose digestion [?]. After entering the rumen, methionine is degraded by rumen microorganisms to produce ammonia and keto acids, which are further fermented into volatile fatty acids. In this study, rumen pH values at 56 days of age were 6.00 and 6.04 for CON and LM lambs, 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 CON lambs could be fermented by microorganisms to produce volatile fatty acids, but their concentration relative to rumen contents was insufficient to cause significant pH changes. Similarly, Robinson et al. [?] 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 CON and LM lambs, 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 thereby decreasing pH. Other mechanisms may also be involved and require further investigation.

The intestinal tract generally 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 LM lambs significantly reduced duodenal pH compared to CON lambs. This may be because the strongly acidic chyme from the abomasum had not been sufficiently neutralized by pancreatic juice, bile, and bicarbonate in the duodenum.

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, with growth hormone (GH) and insulin-like growth factor-I (IGF-I) best reflecting nutritional and growth status. GH is the main regulatory factor for postnatal growth and development, 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. Insulin (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 [?] reported that serum GH concentration increased gradually with increasing dietary methionine levels, with the highest GH concentration observed at 0.8% methionine supplementation. Smith et al. [?] 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 [?, ?], a finding also confirmed in pigs by Buonomo et al. [?]. 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, consistent with Carew et al. [?], who reported no significant change in serum IGF-I concentration in 8–22-day-old male broilers fed methionine-deficient diets.

Serum immune and biochemical indexes are important indicators of animal health. Sun et al. [?] reported that rumen-protected methionine (RPM) reduced 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. [?] found that RPM supplementation in dairy cows increased plasma total protein, albumin, triglyceride, glucose, and free fatty acid concentrations, but these differences were not significant. In this study, during the methionine deficiency period, LM lambs 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 to CON lambs, but these differences were also not significant. This phenomenon may be caused by combined factors including duration of methionine deficiency, supplementation dosage, and environmental conditions, and the specific mechanisms require further investigation.

In summary, low dietary methionine level during 8–56 days of age reduced nutrient apparent digestibility and inhibited increases in duodenal pH and serum GH and INS concentrations in male Hu lambs. After increasing dietary methionine level during 57–84 days of age, nutrient apparent digestibility, gastrointestinal pH, and serum hormone indexes showed compensatory recovery.

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