

Effects of Methionine Restriction and Compensation on Growth Performance and Visceral Organ Development in Lambs (Postprint)

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Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of methionine restriction and compensation on growth performance and visceral organ development in lambs. Twelve pairs of 1-week-old weaned Hu sheep twin male lambs were selected using a paired experimental design, with each twin pair assigned to two groups. In phase 1 (2–8 weeks of age), the control (CON) group was supplemented with basal milk replacer + 0.70% methionine and basal starter + 0.40% methionine, while the methionine restriction (RES) group received basal milk replacer and basal starter; in phase 2 (9–12 weeks of age), lambs in both groups were fed the same diet (basal starter + 0.40% methionine). At the end of 8 weeks and 12 weeks of age, 6 pairs of twin lambs were selected from each group for slaughter, and visceral organs were isolated and weighed. The results showed that: 1) At 8 weeks of age, body weight in the RES group was significantly lower than that in the CON group ($P < 0.05$); during 2–8 weeks of age, average daily gain in the RES group was extremely significantly lower than that in the CON group ($P < 0.01$), while feed-to-gain ratio in the RES group was significantly higher than that in the CON group ($P < 0.05$); during 9–12 weeks of age, there were no significant differences in average daily gain or feed-to-gain ratio between the two groups ($P > 0.05$). 2) At 8 weeks of age, pre-slaughter live weight, empty body weight, and carcass weight in the RES group were significantly lower than those in the CON group ($P < 0.05$), but there was no significant difference in dressing percentage between the two groups ($P > 0.05$); at 12 weeks of age, there were no significant differences in pre-slaughter live weight, empty body weight, carcass weight, or dressing percentage between the RES and CON groups ($P > 0.05$). 3) At both 8 and 12 weeks of age, there were no significant differences in the weight or proportion of visceral organs relative to pre-slaughter live weight between the two groups ($P > 0.05$). 4) At 8 weeks of age, rumen weight in the RES group was

significantly lower than that in the CON group ($P < 0.05$), while no other gastrointestinal indices showed significant differences ($P > 0.05$); at 12 weeks of age, there were also no significant differences in any gastrointestinal indices between the two groups ($P > 0.05$). These results indicate that dietary methionine restriction reduced rumen weight, growth performance, and slaughter performance in lambs, and restoration of dietary methionine level was accompanied by recovery of growth performance and visceral organ development.

Full Text

Effects of Dietary Methionine Restriction and Compensation on Growth Performance and Visceral Organ Development of Lambs

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Abstract

This study aimed to investigate the effects of dietary methionine restriction and compensation on growth performance and visceral organ development in lambs. Twelve pairs of male Hu twin lambs weaned at one week of age were selected and allocated into two groups using a matched-pairs design. During Phase 1 (2 to 8 weeks of age), lambs were fed diets containing either sufficient methionine [basal milk replacer + 0.70% methionine and basal starter + 0.40% methionine, control (CON) group] or restricted methionine [basal milk replacer and basal starter, restriction (RES) group]. During Phase 2 (9 to 12 weeks of age), both groups received the same diet (basal starter + 0.40% methionine). Six pairs of twins were slaughtered at the end of 8 and 12 weeks of age for visceral organ weighing, respectively. The results showed: 1) At 8 weeks of age, body weight of RES group was significantly lower than CON group ($P < 0.05$). During 2 to 8 weeks, average daily gain was significantly lower ($P < 0.01$) while feed to gain ratio was significantly higher ($P < 0.05$) in RES group compared to CON group. No significant differences were observed in average daily gain and feed to gain ratio between groups during 9 to 12 weeks of age ($P > 0.05$). 2) At 8 weeks, live body weight before slaughter, empty body weight, and carcass weight were significantly lower in RES group ($P < 0.05$), but dressing percentage did not differ between groups ($P > 0.05$). At 12 weeks, no significant differences were found in these slaughter performance parameters between RES and CON groups ($P > 0.05$). 3) No significant differences were observed in visceral organ weights or their percentages of live body weight between groups at either 8 or 12 weeks of age ($P > 0.05$). 4) At 8 weeks, rumen weight was significantly lower in RES group ($P < 0.05$), while other gastrointestinal tract indices showed no significant differences ($P > 0.05$). At 12 weeks, no significant differences were observed

in any gastrointestinal tract indices between groups ($P>0.05$). In conclusion, dietary methionine restriction reduced rumen weight, growth performance, and slaughter performance in lambs, but subsequent compensation restored growth performance and visceral organ development.

Keywords: lamb; methionine restriction; methionine compensation; growth performance; visceral organ development

Introduction

Essential amino acids are those that cannot be synthesized by the animal body and must be obtained from the diet to meet physiological needs. As the only sulfur-containing essential amino acid, methionine, together with lysine, constitutes the first or second limiting amino acid in corn-soybean meal diets or microbial protein synthesis. With the widespread application of early weaning technology for lambs in China, milk replacer is increasingly used. Methionine often becomes the first limiting amino acid in milk replacers containing plant proteins (especially legume ingredients). Methionine deficiency or inadequate supply can affect normal metabolic processes and even lead to various diseases in lambs.

Methionine supplementation as a feed additive can improve animal production performance, enhance immunity, and prevent diseases. El-Tahawy et al. found that dietary supplementation with 0.33% methionine significantly improved lamb production performance and economic returns. Abdelrahman et al. reported that methionine supplementation not only enhanced mineral bioavailability but also increased growth performance in lambs. However, Obeidat et al. found that methionine supplementation had no significant effects on feed intake, nutrient digestibility, or growth performance in lambs. Similarly, Hussein et al. reported that methionine supplementation did not improve average daily gain in calves. These conflicting results may be related to methionine dosage, lamb age, or differences in feeding management, and require further investigation. Additionally, visceral organ development plays a crucial role in animal growth and metabolism. Although visceral organs account for only 6-10% of total body weight, they contribute to 40-50% of total protein synthesis and oxygen consumption. Therefore, dietary methionine restriction may theoretically affect visceral organ development and consequently growth performance, but this lacks experimental verification. Furthermore, whether visceral organ development and growth can recover after methionine restriction is lifted remains unclear. This study investigated the effects of dietary methionine restriction and compensation on weaned Hu twin lambs from the perspective of growth performance and visceral organ development, providing a theoretical basis for nutritional parameter research and scientific feeding of lambs.

Materials and Methods

1.1 Experimental Location and Duration

The experiment was conducted at Linqing Runlin Animal Husbandry Co., Ltd. in Shandong Province from September 25, 2015 to December 24, 2015.

1.2 Experimental Design

Twelve pairs of newborn male Hu twin lambs with normal development and initial body weight of (4.93 ± 0.20) kg were selected. All lambs were nursed by their dams from birth to one week of age. After one week, they were weaned and artificially fed milk replacer until 8 weeks of age. Starter feed was supplemented from 2 weeks of age until the end of the experiment at 12 weeks.

A matched-pairs design was used with two phases. Phase 1 (2 to 8 weeks): lambs received either sufficient methionine [basal milk replacer + 0.70% methionine and basal starter + 0.40% methionine, control (CON) group] or restricted methionine [basal milk replacer and basal starter, restriction (RES) group]. Phase 2 (9 to 12 weeks): both groups received the same diet (basal starter + 0.40% methionine).

1.3 Experimental Diets

Methionine specifications: DL-methionine content \geq ; weight loss on drying \leq ; arsenic \leq ; heavy metals \leq ; sulfate \leq ; chloride \leq ; ignition residue \leq ; sodium nitroprusside test qualified; copper sulfate test qualified.

The methionine level in CON group milk replacer was set according to Chinese invention patent ZL 02128844.5, while starter methionine level was based on Mirand et al. Nutrient levels of basal milk replacer are shown in Table 1, and composition and nutrient levels of basal starter are shown in Table 2.

Table 1 Nutrient levels of basal milk replacer (DM basis)

Nutrient levels	Content
Dry matter	
ME/(MJ/Kg)	
Crude protein	
Ether extract	
Ash	
Calcium	
Total phosphorus	
Lysine	
Methionine	
Tryptophan	
Threonine	

Due to patent application, composition of milk replacer was not given.

Table 2 Composition and nutrient levels of basal starter (DM basis)

Ingredients	Content
Corn	
Wheat bran	
Soybean meal	
Limestone	
Fat powder	
CaHPO ₄	
NaCl	
Compound amino acids ¹	
Premix ²	
Total	

Nutrient levels ³	Content
Dry matter	
Crude protein	
Ether extract	
Ash	
Calcium	
Total phosphorus	
ME/(MJ/Kg)	
Lysine	
Methionine	
Tryptophan	
Threonine	

¹Compound amino acids were composed of Lys, Try, Thr, Val, His and other amino acids. ²One kg of premix contained: Fe 4-30 g, Mn 2-25 g, Cu 0.8-2 g, Zn 4-25 g, Se 0.04-0.3 g, I 0.04-0.5 g, Co 0.03-0.05 g, VA 800,000-2,500,000 IU, VD3 200,000-400,000 IU, VE\$ \$3,000 IU. ³ME was a calculated value, while others were measured values.

1.4 Animal Management

Before the experiment, the entire barn was thoroughly disinfected with Powerful Disinfectant solution, and this disinfection was repeated weekly throughout the trial. All lambs received routine vaccination procedures at the start of the experiment.

Lambs were weaned at one week of age and began receiving milk replacer. Feeding frequency was 4 times daily at 2 weeks, 3 times daily at 3-4 weeks, and

2 times daily at 5-8 weeks. Milk replacer feeding methods followed Wang et al. Feeding amounts were adjusted according to lamb health status to ensure normal growth. Additionally, CON and RES groups received similar amounts of milk replacer supplementation, with free access to water throughout the experiment.

1.5 Measurements

1.5.1 Dietary Nutrient Levels Amino acid content was determined using an A300 automatic amino acid analyzer. Metabolizable energy was measured using a Parr-6400 oxygen bomb calorimeter. Dry matter, crude protein, ether extract, ash, calcium, and phosphorus contents were determined according to *Feed Analysis and Feed Quality Detection Technology*.

1.5.2 Growth Performance Body weight was recorded at the end of weeks 1, 8, and 12 before morning feeding to calculate average daily gain. Starter feed intake was recorded daily to calculate feed consumption, with amounts adjusted based on lamb health status to accurately record milk replacer intake. Feed to gain ratio was calculated based on average daily gain and feed intake for each phase.

1.5.3 Organ Indices and Slaughter Performance At the end of weeks 8 and 12, six pairs of twins (six lambs per group) were slaughtered after 16 hours of feed and water deprivation. Live weight before slaughter (LWBS) was recorded at 08:00 on the slaughter day. Lambs were stunned with CO₂ and exsanguinated via jugular venipuncture. After skinning and removal of head, feet, and viscera, carcass weight was recorded. Visceral organs (heart, liver, spleen, lung, kidney, small intestine, and large intestine) were separated and weighed to calculate their percentages of LWBS. Rumen, reticulum, omasum, and abomasum were separated and weighed to calculate their percentages of LWBS and total stomach weight (TCSW).

Calculations: - Empty body weight (EBW, kg) = LWBS - total gastrointestinal content weight - Carcass weight (CW, kg) = LWBS - weight of skin, head, feet, reproductive organs and surrounding fat, and viscera (kidneys and surrounding fat retained) - Dressing percentage (%) = $100 \times CW / LWBS$

1.6 Statistical Analysis Data were initially processed using Excel 2010, then analyzed using paired t-test in SAS 9.2 software. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

2.1 Effects of Methionine Restriction and Compensation on Growth Performance

The effects of methionine restriction and compensation on growth performance are shown in Table 3 . No significant difference was observed in body weight between CON and RES groups at 1 week of age ($P>0.05$). At 8 weeks, body weight of RES group was significantly lower than CON group ($P<0.05$), but no significant difference existed between groups at 12 weeks ($P>0.05$). During 2 to 8 weeks, average daily gain was significantly lower ($P<0.01$) and feed to gain ratio was significantly higher ($P<0.05$) in RES group compared to CON group. No significant differences were found in average daily gain or feed to gain ratio between groups during 9 to 12 weeks or the entire 2 to 12 week period ($P>0.05$).

Table 3 Effects of methionine restriction and compensation on growth performance of lambs

2.2 Effects of Methionine Restriction and Compensation on Slaughter Performance

The effects of methionine restriction and compensation on slaughter performance are presented in Table 4 . At 8 weeks, CON group showed significantly higher live weight before slaughter, empty body weight, and carcass weight compared to RES group ($P<0.05$), though dressing percentage did not differ between groups ($P>0.05$). At 12 weeks, no significant differences were observed in any slaughter performance parameters between groups ($P>0.05$).

Table 4 Effects of methionine restriction and compensation on slaughter performance of lambs

2.3 Effects of Methionine Restriction and Compensation on Visceral Organ Indices

The effects of methionine restriction and compensation on visceral organ indices are shown in Table 5 . No significant differences were observed in visceral organ weights or their percentages of live body weight between CON and RES groups at either 8 or 12 weeks of age ($P>0.05$).

Table 5 Effects of methionine restriction and compensation on internal organ indexes of lambs

2.4 Effects of Methionine Restriction and Compensation on Gastrointestinal Tract Indices

The effects of methionine restriction and compensation on gastrointestinal tract indices are presented in Table 6 . At 8 weeks, rumen weight was significantly lower in RES group compared to CON group ($P<0.05$), while other gastrointestinal indices showed no significant differences ($P>0.05$). At 12 weeks, no sig-

nificant differences were observed in any gastrointestinal indices between groups ($P>0.05$).

Table 6 Effects of methionine restriction and compensation on gastrointestinal tract indexes of lambs

Discussion

3.1 Effects of Methionine Restriction and Compensation on Growth Performance

Due to immature gastrointestinal development, early-weaned lambs are highly susceptible to rearing methods, dietary composition, and environmental factors. Therefore, balanced nutrient content is the material basis for metabolism in early-weaned lambs. As a primary limiting amino acid for protein synthesis in growing animals, methionine plays an important role in improving growth performance, dietary protein utilization, and reducing nitrogen emissions. Goedecken et al. found that methionine supplementation in ruminant diets improved growth performance and nitrogen utilization efficiency. Mata et al. reported that daily supplementation of 2.5 g methionine significantly improved growth performance in Merino lambs. However, Wiese et al. found that rumen-protected methionine supplementation had no significant effects on growth performance, feed conversion, or carcass weight in Merino lambs. These conflicting results may be attributed to differences in lamb breed, dietary composition, and methionine levels. Previous studies indicate that methionine supplementation affects lamb growth performance, suggesting that methionine restriction would similarly impact development. Whether growth can recover after methionine restriction is lifted requires further investigation. Studying methionine supplementation or restriction alone makes it difficult to establish the intrinsic relationship between early restriction and later compensation.

To eliminate genetic variation, twin male lambs with similar initial weights were selected. Basal dietary methionine levels were deliberately controlled at minimal levels to study the effects of methionine restriction, while supplemented levels were designed to maximally meet nutritional requirements for studying compensatory effects. At 8 weeks, RES group body weight was significantly lower than CON group. After methionine compensation during 9-12 weeks, no significant difference existed between groups at 12 weeks, indicating that methionine restriction inhibited early growth but compensation partially restored body weight. Similarly, Liu reported that nutritional restriction followed by compensation partially restored lamb growth. Additionally, with similar feed intake between groups, CON group showed significantly higher average daily gain during 2-8 weeks, confirming that methionine restriction inhibited early growth. During restriction, RES group had significantly higher feed to gain ratio, but this difference disappeared after compensation, suggesting methionine restriction impaired nutrient utilization. These results indicate that methionine restriction reduced average daily gain and growth rate while impairing feed

utilization in early-weaned Hu lambs.

3.2 Effects of Methionine Restriction and Compensation on Slaughter Performance

Before 8 weeks, RES group experienced methionine restriction, resulting in significantly lower body weight at weaning from milk replacer. Slaughter lambs were randomly selected. The results showed that except for dressing percentage, all slaughter parameters (live weight before slaughter, empty body weight, and carcass weight) were significantly lower in RES group, indicating that methionine restriction not only affected growth but also reduced slaughter performance and meat yield. After 4 weeks of methionine compensation, no significant differences existed between groups at 12 weeks, corresponding to the growth performance results. This demonstrates that methionine levels in milk replacer and starter before 8 weeks limited slaughter performance, but compensation during 9-12 weeks allowed RES group to grow faster and narrow the gap with CON group, consistent with body weight differences at 12 weeks. Galvani et al. found that nutrient deficiency in early-weaned lambs affected subsequent feed utilization and delayed slaughter weight achievement, while adequate nutrition enabled faster attainment of slaughter weight with improved economic returns and meat quality. This confirms that animals can recover lost weight to varying degrees through nutritional compensation after restriction. However, Obeidat et al. found no significant effects of methionine supplementation at different levels on growth, slaughter performance, or meat quality in Awassi lambs, possibly because supplementation effects had not manifested within the experimental timeframe or levels were insufficient for optimal growth.

3.3 Effects of Methionine Restriction and Compensation on Visceral Organ Indices

Visceral organ development is determined by both nutritional and physiological status, with organ weights and indices reflecting overall development. At 8 weeks, no significant differences were observed in visceral organ weights or their percentages of live body weight between groups, which was inconsistent with body weight differences. This suggests that methionine restriction first affected overall body weight before manifesting as differences in specific organ weights. After methionine compensation during 9-12 weeks, groups remained similar in visceral organ indices. Atti et al. reported that nutritional restriction and compensation had no significant effects on visceral organs. Li reported that Mongolian lamb visceral organs could fully recover when nutrition was restored after restriction. Our results differ, possibly because compensatory growth is influenced by age at restriction onset, restriction degree and duration, as well as breed, sex, and genetics. Therefore, methionine restriction and compensation had no significant effects on visceral organ indices in this study.

3.4 Effects of Methionine Restriction and Compensation on Gastrointestinal Tract Indices

Due to underdeveloped gastrointestinal tracts in young lambs, nutritional levels during this period are critical for gastrointestinal development. Nutrient level changes affect gastrointestinal differentiation and alter stomach compartment and intestinal weights and relative proportions. At 8 weeks, RES group showed significantly lower rumen weight, likely because the rumen was not fully developed during 2-8 weeks and low methionine levels impaired gastrointestinal development, causing weight loss. As age increased and nutrient absorption shifted primarily to rumen and small intestine, methionine compensation eliminated differences in gastrointestinal indices by 12 weeks. Liu et al. reported that early nutrient restriction significantly affected small intestinal mucosa development with slow recovery, reducing developmental rate. Our results differ, possibly because only methionine levels differed between diets, and rumen development inhibition by early methionine restriction could be recovered through later compensation.

Conclusion

1. During 2-8 weeks, methionine restriction inhibited growth and development in twin lambs, affecting growth performance and slaughter performance.
2. During 9-12 weeks, methionine compensation allowed restricted twin lambs to recover growth performance and visceral organ development.

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