

## Effects of Methionine Level on Body Condition Development, Gastrointestinal Tissue Morphology, and Serum Antioxidant Indices in Lambs: Postprint

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

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### Abstract

This experiment aimed to investigate the effects of methionine levels on body condition development, gastrointestinal tissue morphology, and serum antioxidant indices in lambs. Twelve pairs of 7-day-old weaned Hu sheep twin male lambs 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 stages: Stage 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 additionally added methionine (0.70% and 0.40%) completely removed from the CON group basis, while other nutrient levels remained consistent. Stage 2 (57–84 days of age), both groups of lambs stopped receiving milk replacer and the diet consisted of basal starter feed only. At 56 and 84 days of age, 6 pairs of twin lambs were selected for slaughter, gastrointestinal tissues were isolated, and serum samples were collected. The results showed: 1) In terms of feed intake, methionine intake of lambs in the LM group was extremely significantly lower than that in the CON group during both the 8–56 days and 8–84 days periods ( $P < 0.01$ ), while dry matter intake was significantly higher than that in the CON group ( $P < 0.05$ ); In terms of body measurement indices, at 56 days of age, except for body weight, body diagonal length, chest girth, and body length index which were significantly lower in the LM group than in the CON group ( $P < 0.05$ ), other body condition development indices showed no significant differences ( $P > 0.05$ ); At 84 days of age, no significant differences were observed in body condition development indices between the two groups ( $P > 0.05$ ). 2) At 56 days of age, rumen papilla width of lambs in the LM group was significantly lower than that in the CON group ( $P < 0.05$ ), with no significant differences observed between the two groups in other gastrointestinal morphological de-

velopment indices ( $P>0.05$ ). 3) At 56 days of age, serum superoxide dismutase activity of lambs in the LM group was extremely significantly lower than that in the CON group ( $P<0.01$ ); At 84 days of age, except for glutathione peroxidase activity which was significantly lower in the LM group than in the CON group ( $P<0.05$ ), no significant differences were observed between the two groups in other serum antioxidant indices ( $P>0.05$ ). The results suggest that low dietary methionine levels inhibited body condition development (body weight, body diagonal length, chest girth, body length index) and rumen papilla width increase in lambs, while simultaneously reducing serum superoxide dismutase activity in the body, yet increasing dry matter intake; After increasing dietary methionine levels, body condition development and gastrointestinal tissue morphological development in lambs were subsequently compensated, but the body's antioxidant defense system remained incompletely improved.

## Full Text

### Effects of Methionine Level on Development of Body Condition, Morphology of Digestive Tract, and Serum Antioxidant Indexes in Lambs

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

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

#### Abstract

This study investigated the effects of dietary methionine level on body condition development, digestive tract morphology, and serum antioxidant indexes in 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 comprised two stages. During Stage 1 (8 to 56 days of age), CON lambs received basal milk replacer and basal starter, while LM lambs received the same diets with supplemental methionine removed (0.70% from milk replacer and 0.40% from starter), maintaining all other nutrients at identical levels. During Stage 2 (57 to 84 days of age), all lambs were weaned from milk replacer and fed only the basal starter.

At 56 and 84 days of age, six pairs of twins from each group were slaughtered to collect gastrointestinal tissues and serum samples. The results showed: (1) Methionine intake in LM lambs was significantly lower than in CON lambs during both 8-56 days and 8-84 days ( $P<0.01$ ), while dry matter intake was significantly higher ( $P<0.05$ ). At 56 days, LM lambs exhibited significantly lower body weight, body length, heart girth, and body length index compared to CON lambs ( $P<0.05$ ), with no significant differences in other body condition parameters ( $P>0.05$ ). By 84 days, no significant differences in body condition

were observed between groups ( $P>0.05$ ). (2) At 56 days, ruminal papillary width in LM lambs was significantly reduced compared to CON lambs ( $P<0.05$ ), with no other significant differences in digestive tract morphology ( $P>0.05$ ). (3) At 56 days, serum superoxide dismutase (SOD) activity in LM lambs was significantly lower than in CON lambs ( $P<0.01$ ). At 84 days, only glutathione peroxidase (GSH-Px) activity remained significantly lower in LM lambs ( $P<0.05$ ), with no other differences in antioxidant indexes ( $P>0.05$ ). These findings indicate that low dietary methionine inhibited body condition development (body weight, body length, heart girth, body length index) and ruminal papillary width while reducing serum SOD activity, yet paradoxically increased dry matter intake. Although elevated dietary methionine subsequently compensated for deficits in body condition and digestive tract morphology, the antioxidant defense system remained incompletely restored.

**Keywords:** methionine; lamb; body condition development; digestive tract morphology; antioxidant indexes

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## Introduction

The pre- and postnatal development of lambs is a critical period that determines their subsequent health and fattening potential. Due to the immaturity of their digestive and metabolic systems at birth, newborn lambs exhibit substantial plasticity and are highly susceptible to environmental changes, particularly nutritional interventions, which can profoundly alter their later fattening performance. Previous research has demonstrated that early-weaned lambs are vulnerable to stress induced by nutrient availability, leading to digestive dysfunction and detrimental changes in small intestinal morphology, including reduced villus length, decreased crypt depth, and diminished absorptive surface area.

Methionine, as the sole sulfur-containing essential amino acid, plays a vital role in protein synthesis within animal tissues. Abdelrahman et al. reported that dietary methionine supplementation not only enhanced mineral bioavailability but also improved growth performance in lambs. Therefore, meeting the methionine requirements of neonatal lambs is crucial for maintaining normal growth and health.

The gastrointestinal tract serves as the primary site for digestion and absorption in ruminants, and its normal mucosal development provides the physiological foundation for adequate nutrient utilization. Although intestinal tissue represents only 5–7% of body weight, it consumes 15–20% of the body's nutrient requirements. In monogastric animals, Manzoor et al. demonstrated that low dietary methionine reduced broiler body weight and inhibited gastrointestinal morphological development. Similarly, Krutthai et al. found that methionine deficiency in weaned piglets suppressed growth and significantly decreased serum urea nitrogen and albumin levels while impairing gastrointestinal morphology. In production settings, lambs often experience methionine deficiency

before weaning due to health status, feed ingredients, and management practices, resulting in substantial individual variation in body condition that complicates intensive management. While most research has focused on single methionine supplementation effects on lamb growth, studies investigating the impact of methionine deficiency followed by compensation on pre- and post-weaning development remain scarce. This experiment was designed to manipulate dietary methionine levels before and after weaning to determine whether growth retardation caused by early methionine deficiency could be compensated through subsequent supplementation, thereby providing theoretical support for healthy sheep production.

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## Materials and Methods

### 1.1 Experimental Animals and Duration

The experiment utilized twelve pairs of healthy male Hu twin lambs weaned at 7 days of age, with an initial body weight of  $4.93 \pm 0.20$  kg. The study was conducted from October to December 2015 at Linqing Runlin Animal Husbandry Co., Ltd. in Shandong Province.

### 1.2 Experimental Diets

The DL-methionine supplement used in this study contained 99% methionine, 0.5% moisture loss, 0.002% arsenic, 0.02% heavy metals, 0.30% sulfate, 0.20% chloride, and 0.5% ignition residue, and passed both sodium nitroprusside and copper sulfate tests.

The nutritional levels of basal starter and milk replacer were formulated according to the Chinese “Feeding Standard of Meat-Producing Sheep” (NY/T 816-2004) and patent ZL 02128844.5, with methionine levels established based on studies by Patureau-Mirand et al. and Wang et al. The nutrient composition of basal milk replacer and basal starter is presented in Table 1 .

### 1.3 Experimental Design and Management

A matched-pairs design was employed, with twelve twin pairs divided into a control group (CON) and low methionine group (LM), assigning one twin from each pair to each group. The experiment consisted of two stages. During Stage 1 (8–56 days of age), CON lambs received basal milk replacer and basal starter, while LM lambs received the same diets with supplemental methionine completely removed (0.70% from milk replacer and 0.40% from starter), maintaining identical nutritional levels otherwise. During Stage 2 (57–84 days of age), all lambs were weaned from milk replacer and fed only basal starter.

Lambs were artificially fed milk replacer from day 8 to 56, with starter supplementation beginning on day 8 and continuing until the experiment concluded on

day 84. Prior to the trial, the entire facility was thoroughly disinfected with a strong disinfectant solution, with weekly pen disinfection thereafter. All lambs received standard immunization protocols. Milk replacer feeding frequency was four times daily during days 8–14, three times daily during days 15–28, and twice daily during days 29–56, following the methods described by Wang et al. Feeding amounts were adjusted according to lamb health status to ensure normal growth, with both groups receiving similar quantities of milk replacer and starter. Fresh water was available ad libitum throughout the experiment.

#### 1.4 Measurement Indicators and Methods

**1.4.1 Nutritional Levels of Milk Replacer and Starter** Amino acid content was determined using an A300 automatic amino acid analyzer. Gross energy was measured with a Parr-6400 oxygen bomb calorimeter and used to calculate metabolizable energy. Dry matter, crude protein, crude fat, ash, calcium, and phosphorus contents were analyzed according to “Feed Analysis and Feed Quality Detection Technology.”

**1.4.2 Body Weight and Body Measurement Indicators** Body weight and measurements were recorded before morning feeding on days 8, 56, and 84. Lambs were positioned on level ground in natural standing posture using measuring sticks, tapes, and circular calipers. Measurements included: (1) body height (vertical distance from highest point of scapula to ground), (2) body length (straight distance from shoulder point to ischial tuberosity), (3) heart girth (circumference behind scapula), (4) chest width (widest point behind scapula), (5) chest depth (vertical distance from withers to sternum), (6) cannon circumference (mid-upper cannon bone). Body indices were calculated as: body length index (%) = (body length/body height) × 100, heart girth index (%) = (heart girth/body height) × 100, and body index (%) = (heart girth/body length) × 100.

**1.4.3 Collection of Gastrointestinal Tissue Samples** Six twin pairs from each group were slaughtered on days 56 and 84 following a 16-hour fast. After exsanguination via jugular venipuncture, the gastrointestinal tract was dissected. Stomach compartments were separated, emptied, and weighed fresh. Rumen dorsal sac samples (1 cm × 1 cm) were fixed in 10% formalin for paraffin sectioning. Small intestine segments were washed, weighed fresh, and 3 cm sections of duodenum, jejunum, and ileum were fixed in 10% formalin.

**1.4.4 Measurement of Gastrointestinal Morphology** Ruminal morphology was assessed by measuring papillary height, papillary width, and muscular layer thickness. Small intestinal morphology was evaluated by measuring villus height, crypt depth, mucosal thickness, muscular layer thickness, and villus height/crypt depth ratio. Tissue sections were observed under an Olympus BX51 microscope, imaged using an Olympus DP70 system, and measured with

Image-Pro Plus 5.1 software. Three non-consecutive sections were examined per sample, with three fields of view per section and five measurements per field, following the methodology described by Li Hui. Mean values were calculated for each parameter.

**1.4.5 Determination of Serum Antioxidant Indexes** On days 56 and 84, blood samples (10 mL) were collected from the jugular vein of three randomly selected lambs per group, centrifuged at 3,000 r/min for 20 minutes, and serum was stored at -20°C. Antioxidant parameters included catalase (CAT), glutathione S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities. GSH-Px and GST activities were measured by colorimetric methods, SOD by pyrogallol autoxidation, and CAT by visible spectrophotometry using an automatic biochemical analyzer.

## 1.5 Data Processing

Data were initially processed using Excel 2010, then analyzed with SAS 9.2 software using paired t-tests. Significance was declared at  $P < 0.05$ , with trends noted at  $0.05 \leq P < 0.10$ .

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## Results

### 2.1 Effects of Methionine Level on Feed Intake and Body Condition Development

Feed intake and body condition development are presented in Table 2. Methionine intake in LM lambs was significantly lower than in CON lambs during both 8-56 days and 8-84 days ( $P < 0.01$ ), while dry matter intake was significantly higher ( $P < 0.05$ ). At 8 days, no significant differences in body weight or measurements existed between groups ( $P > 0.05$ ). However, at 56 days, LM lambs showed significantly lower body weight, body length, heart girth, and body length index compared to CON lambs ( $P < 0.05$ ), with no differences in other parameters ( $P > 0.05$ ). By 84 days, all body condition parameters were similar between groups ( $P > 0.05$ ).

### 2.2 Effects of Methionine Level on Digestive Tract Morphology

Digestive tract morphology results are shown in Table 3. At 56 days, LM lambs exhibited significantly reduced ruminal papillary width compared to CON lambs ( $P < 0.05$ ), with papillary height showing a decreasing trend ( $0.05 \leq P < 0.10$ ). No other significant differences in digestive tract morphology were observed between groups ( $P > 0.05$ ). At 84 days, papillary height in LM lambs remained lower than in CON lambs ( $0.05 \leq P < 0.10$ ), with no other significant morphological differences ( $P > 0.05$ ).

### 2.3 Effects of Methionine Level on Serum Antioxidant Indexes

Serum antioxidant indexes are presented in Table 4. At 56 days, SOD activity in LM lambs was significantly lower than in CON lambs ( $P < 0.01$ ), with no differences in other antioxidant parameters ( $P > 0.05$ ). At 84 days, only GSH-Px activity remained significantly lower in LM lambs ( $P < 0.05$ ), while CAT, GST, and SOD activities were similar between groups ( $P > 0.05$ ).

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## Discussion

### 3.1 Effects of Methionine Level on Body Condition Development

Methionine, as the only sulfur-containing essential amino acid, is crucial for ruminant growth and development. Research indicates that the optimal methionine requirement is approximately 2 g/day for pre-ruminant lambs and 0.64% for fattening sheep. In this study, during 8–56 days, methionine intake was 0.47 g/day and 1.75 g/day for LM and CON lambs respectively, representing a 73.14% reduction in the LM group. Previous work by Wang et al. demonstrated that during 8–56 days, LM lambs exhibited significantly lower average daily gain and feed efficiency, though these differences disappeared during 57–84 days. When nutritional intake fails to meet basic growth requirements, animals mobilize stored energy reserves, leading to weight loss and deteriorated body condition. Rooke et al. reported that feeding ewes 75% of energy requirements during early gestation significantly reduced body weight at day 90. Similarly, Gao et al. found that energy restriction during late gestation increased maternal weight loss and reduced lamb birth weight. Puchala et al. demonstrated that feed restriction in fattening goats decreased average daily gain and visceral organ weight, but subsequent refeeding produced compensatory weight recovery. In the current study, low methionine significantly suppressed weight gain, yet after 28 days of methionine repletion, body weight differences between groups were no longer significant. These results suggest that growth inhibition from methionine restriction can be partially compensated through subsequent supplementation, consistent with findings by Liu Xiaogang.

Body measurement indicators directly reflect an animal's physical size and structural development while indirectly indicating organ development status, which correlates with reproductive function, disease resistance, and environmental adaptability. Since individual measurements only describe localized development, body indices are calculated to assess proportional relationships between anatomical regions. Ma Cunshou et al. reported that among body measurements, heart girth showed the strongest phenotypic correlation and direct effect on body weight in Qinghai semi-fine wool lambs, followed by body length. In this study, at 56 days, low methionine significantly reduced heart girth, body length, and body length index. Following methionine supplementation, these differences were eliminated, aligning with the observed recovery in body weight.

These findings corroborate reports that body measurements significantly influence body weight in various livestock species.

### 3.2 Effects of Methionine Level on Digestive Tract Morphology

The nutritional value of protein is essentially the nutritional value of its constituent amino acids. As a sulfur-containing essential amino acid, methionine influences gastrointestinal structure and function, ultimately affecting organ weight. Studies show that dietary methionine modulates tight junction protein expression, altering intestinal barrier function and disease susceptibility. Riedijk et al. demonstrated that methionine serves as a crucial precursor for cysteine synthesis, and its transmethylation and transsulfuration pathways are vital for porcine gastrointestinal health. Malik et al. reported that methionine and its hydroxy analog improved intestinal morphology and nutrient utilization in piglets. For ruminants, diet is the primary factor influencing forestomach development, with composition, physical form, and nutrient levels all affecting morphological development. Inappropriate nutrition can restrict gastrointestinal growth.

Ruminal papillary height, width, and muscular layer thickness are standard indicators for evaluating rumen morphological development. Lesmeister et al. noted that experimental treatments primarily affect papillary height, followed by width and muscular thickness. Previous studies have shown that dietary ingredients and forage-to-concentrate ratios influence rumen development in lambs. Cai Jiansen confirmed that protein source (plant vs. milk-derived) significantly affected papillary number in weaned lambs. Sun Zhihong reported that nutrient restriction after weaning at 28 days significantly reduced papillary width, height, and surface area. In this study, low methionine significantly inhibited papillary width development and tended to reduce papillary height. Even after 28 days of methionine repletion, papillary height in LM lambs remained lower than in CON lambs, though other gastrointestinal morphological parameters showed no differences. These discrepancies may be attributed to variations in restriction duration, dosage, or environmental conditions.

The small intestine is the primary site for nutrient digestion, absorption, and transport, and its mucosal architecture is critical for physiological function and growth. Villus height, crypt depth, mucosal thickness, muscular layer thickness, and villus height/crypt depth ratio are key indicators of digestive capacity and intestinal function. Gu Xianhong reported that energy and protein malnutrition in piglets significantly decreased mucosal thickness, villus dimensions, and surface area. In the current study, neither methionine restriction nor subsequent repletion significantly affected small intestinal morphology in lambs. This may be because small intestinal development is influenced by multiple factors, and 49 days of methionine restriction was insufficient to induce morphological changes, or the restriction level may not have reached the threshold needed to impair intestinal development.

### 3.3 Effects of Methionine Level on Serum Antioxidant Indexes

As a limiting amino acid in soybean meal-based diets for lambs, methionine participates not only in protein synthesis but also exerts antioxidant effects. The redox system maintains dynamic equilibrium, and sulfur-containing amino acids regulate the activation and inactivation of various substances, thereby modulating physiological processes. GSH-Px activity reflects the capacity to scavenge oxygen free radicals and constitutes a major component of the antioxidant defense system, capable of replacing catalase in tissues with low CAT activity or hydrogen peroxide content. GSH-Px activity also determines the rate of lipid hydroperoxide clearance. SOD protects cell membrane structure and function, with its activity indirectly reflecting free radical scavenging capacity. Elevated SOD activity helps tissues resist oxidative damage. Methionine can enhance SOD activity, improve immune response, reduce tissue damage from pathogens and free radicals, and promote lipid metabolism, thereby accelerating animal growth. GST is a cytosolic enzyme that catalyzes glutathione conjugation with electrophilic groups on hydrophobic compounds, providing detoxification and free radical elimination. Therefore, CAT, GST, SOD, and GSH-Px activities are important indicators of antioxidant capacity.

Most reports on methionine's antioxidant effects involve poultry or rodents. Liu Xiuli et al. demonstrated that low methionine diets reduced serum GSH-Px activity in rats, weakening antioxidant defense. Ma Likun et al. reported that appropriate methionine levels increased serum SOD activity in laying hens. Lin Zhenping found that 0.66% dietary methionine significantly enhanced serum SOD activity in 28-70 day-old lion-head geese. In this study, at 56 days, LM lambs showed significantly reduced serum SOD activity, consistent with previous reports. The lack of significant effects on other antioxidant enzymes may indicate that during methionine restriction, SOD activity serves as the primary indicator of free radical scavenging capacity in lambs, while other enzymes remain less responsive. At 84 days, only GSH-Px activity remained significantly lower in LM lambs, suggesting that although early methionine deficiency did not immediately affect GSH-Px, its inhibitory effects on antioxidant defense persisted and manifested through this parameter by the experiment's conclusion. The underlying mechanism requires further investigation, as knowledge regarding methionine's role in the lamb antioxidant defense system remains limited.

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## Conclusions

1. Low dietary methionine level inhibited body condition development (body weight, body length, heart girth, body length index) and ruminal papillary width while reducing serum SOD activity, yet increased dry matter intake in lambs.
2. Elevated dietary methionine produced compensatory recovery in body con-

dition and digestive tract morphology, but the antioxidant defense system remained incompletely restored.

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