Effects of Rumen-Protected Betaine on Growth Performance and Digestive Metabolism in Meat Sheep (Postprint)

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Abstract

This experiment aimed to investigate the effects of rumen-protected betaine on growth performance, nutrient apparent digestibility, and blood biochemical indices in meat sheep. Sixty 4-month-old Hu ram lambs were selected and randomly divided into 5 groups: blank control (Con) group, 2.2 g/d betaine group (B-2.2), 2 g/d rumen-protected betaine group (RPB-2), 4 g/d rumen-protected betaine group (RPB-4), and 6 g/d rumen-protected betaine group (RPB-6). The pre-trial period was 15 days, and the formal trial period was 60 days. The results showed: 1) Compared with the Con group, the average daily feed intake, average daily gain, and feed-to-gain ratio of meat sheep in the B-2.2 group showed no significant differences ($P > 0.05$); the apparent digestibility of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber showed no significant changes ($P > 0.05$); the contents of serum total protein, urea nitrogen, total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein showed no significant changes ($P > 0.05$). 2) Compared with the Con and B-2.2 groups, the RPB-2, RPB-4, and RPB-6 groups showed no significant differences in average daily feed intake ($P > 0.05$), but average daily gain was significantly increased ($P < 0.05$) and feed-to-gain ratio was significantly decreased ($P < 0.05$); the apparent digestibility of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber showed no significant changes ($P > 0.05$); the contents of urea nitrogen, total cholesterol, and triglycerides in serum were significantly decreased ($P < 0.05$), the content of low-density lipoprotein was extremely significantly decreased ($P < 0.01$), the content of total protein was extremely significantly increased ($P < 0.05$), and the content of high-density lipoprotein showed no significant difference ($P > 0.05$). Therefore, in this experiment, dietary supplementation with rumen-protected betaine could improve the growth performance of meat sheep and ameliorate protein and lipid metabolism, with an appropriate supplementation level of 4 g/d.

Full Text

Manipulation of Rumen-Protected Betaine on Growth Performance and Digestion and Metabolism of Lambs

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Abstract: This study was conducted to investigate the effects of betaine and rumen-protected betaine on growth performance, nutrient apparent digestibility, and blood biochemical indices of lambs. Sixty 4-month-old male Hu lambs were randomly allocated to five groups: control (Con) group, 2.2 g/d betaine group (B-2.2), 2 g/d rumen-protected betaine group (RPB-2), 4 g/d rumen-protected betaine group (RPB-4), and 6 g/d rumen-protected betaine group (RPB-6). The experiment consisted of a 15-day pre-trial period and a 60-day formal trial period. The results showed: 1) Compared with the Con group, the B-2.2 group showed no significant differences in average daily feed intake, average daily gain, or feed-to-gain ratio ($P>0.05$); no significant changes in apparent digestibility of dry matter, crude protein, neutral detergent fiber, or acid detergent fiber ($P>0.05$); and no significant changes in serum contents of total protein, urea nitrogen, total cholesterol, triglycerides, high-density lipoprotein, or low-density lipoprotein ($P>0.05$). 2) Compared with the Con and B-2.2 groups, the RPB-2, RPB-4, and RPB-6 groups showed no significant differences in average daily feed intake ($P>0.05$), but average daily gain was significantly increased ($P<0.05$) and feed-to-gain ratio was significantly decreased ($P<0.05$). Apparent digestibility of dry matter, crude protein, neutral detergent fiber, and acid detergent fiber was not significantly affected ($P>0.05$). Serum contents of urea nitrogen, total cholesterol, and triglycerides were significantly reduced ($P<0.05$), low-density lipoprotein content was extremely significantly reduced ($P<0.01$), total protein content was extremely significantly increased ($P<0.01$), while high-density lipoprotein content showed no significant difference ($P>0.05$). In conclusion, dietary supplementation with rumen-protected betaine effectively improved lamb growth performance and promoted protein and fat metabolism, with an optimal addition level of 4 g/d.

Keywords: betaine; rumen-protected betaine; lamb; growth performance; serum biochemical indices

Betaine, also known as trimethylglycine, is a non-toxic substance naturally present in animals that was first isolated from beet molasses. As an efficient methyl donor, betaine participates in important physiological regulatory processes in the body [1]. Numerous studies have shown that betaine is involved in protein and fat metabolism [2-3] and regulates normal osmotic pressure in animals [4-5]. In monogastric animal production, betaine can be used as a feed additive to promote animal growth and development, increase feed intake, and

reduce feed-to-meat ratio [6-9]. However, in ruminant production, direct feeding of betaine can be degraded by rumen microorganisms into trimethylamine [10], resulting in unsatisfactory effects. Therefore, rumen-protected betaine (RPB) is commonly used in ruminant production. Studies by Fan Xiaojing et al. [11] and Lian Hong et al. [12] found that dietary supplementation with RPB could effectively improve lactation performance in dairy cows. Wang et al. [13] reported that betaine increased the digestibility of dietary dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in lactating cows. Additionally, Löest et al. [14] found that adding betaine to beef cattle diets could increase feed intake, improve daily gain, and reduce feed-to-meat ratio. Research on RPB has gradually increased, with corresponding product development becoming a focus for manufacturers and research institutions. However, due to variations in protection technology, coating materials, and protection degree, the application effects of related products need to be evaluated. With the rise of the lamb fattening industry, additives that regulate growth rate and meat quality have been gradually applied, and the use of betaine in lamb production is just beginning. To date, domestic RPB products have been developed and marketed, but their application and effects in lamb fattening need to be evaluated. This study aimed to investigate the regulatory effects of betaine and RPB supplementation on growth performance and digestion-metabolism in Hu lambs, providing a scientific basis for the efficient utilization of betaine in lamb production.

1.1 Experimental Materials

Betaine and rumen-protected betaine were provided by Yixing Tianshi Feed Co., Ltd. The RPB contained 55% betaine with a rumen bypass rate of 60%.

1.2 Experimental Design and Management

The experiment was conducted from September 2014 to January 2015 at Taicang Ecological Breeding Cooperative in Jiangsu Province. Sixty 4-month-old male Hu lambs with an average body weight of (30.47$±$2.04) kg were selected. A single-factor experimental design was used, and the lambs were randomly divided into five groups with 12 animals per group. The basal diet was formulated according to NRC (2007), and its composition and nutrient levels are shown in Table 1. Unprotected betaine and RPB were added to the basal diet, resulting in five treatments: control (Con) group, 2 g rumen-protected betaine group (RPB-2), 2.2 g betaine group (B-2.2), 4 g rumen-protected betaine group (RPB-4), and 6 g rumen-protected betaine group (RPB-6). Both betaine and RPB were premixed with concentrate before addition. The diets were fed as total mixed rations, with peanut vines and soybean stalks chopped to approximately 2 cm length. The lambs were fed twice daily at 07:00 and 17:00, with free access to water, and the pens were regularly disinfected. The pre-trial period was 10 days, and the formal trial period was 60 days.

Basal diet composition and nutrient levels (DM basis)

1.3.1 Growth Performance Measurement

The body weight on day 1 of the formal trial period was recorded as initial body weight. Subsequently, body weight was measured every 15 days before morning feeding, with the weight on day 60 recorded as final body weight. Feed intake and refusals were recorded for each group to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G).

1.3.2 Apparent Digestibility Measurement

During the experiment, six lambs from each group were selected every 15 days for digestion-metabolism trials using fecal collection bags for total fecal collection. The metabolism trial included a 3-day adaptation period followed by 3 consecutive days of sampling, with feces collected and weighed every 12 hours. Ten percent sulfuric acid (10% of fecal weight) was added to the feces for nitrogen fixation. The 3-day samples were mixed and frozen for storage. Daily feed intake and refusals were recorded. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents of diets, refusals, and feces were determined according to Zhang Liying [16].

Nutrient apparent digestibility (%) = [(nutrient intake - nutrient excretion) / nutrient intake] × 100.

1.3.3 Blood Biochemical Indices Measurement

During the trial, jugular blood samples were collected every 15 days before morning feeding. After standing at room temperature for 30 minutes, serum was separated by centrifugation at 2,000×g for 10 minutes. Serum contents of total protein (TP), urea nitrogen (UN), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined using a DH-364 automatic biochemical analyzer. Reagent kits were purchased from Zhejiang Dongou Diagnostic Products Co., Ltd.

1.4 Statistical Analysis

All results are expressed as means and standard errors. Data were analyzed using one-way ANOVA and Duncan' s multiple comparison test with SPSS 18.0 software. Differences were considered significant at $P<0.05$, extremely significant at $P<0.01$, and not significant at $P>0.05$.

2.1 Effects of Dietary Betaine and RPB Supplementation on Lamb Growth Performance

As shown in Table 2 , compared with the Con group, the B-2.2 group showed no significant differences in ADG and F/G ($P>0.05$). However, the RPB groups

significantly increased ADG and decreased F/G ($P<0.05$). Among all treatment groups, the RPB-4 group showed the best results, with ADG and F/G improved by 30.03% and reduced by 24.02%, respectively, compared with the Con group. There were no significant differences in ADFI among all groups ($P>0.05$).

Table 2. Effects of betaine and rumen-protected betaine on growth performance of lambs

In the same row, values with no letter or the same letter superscripts mean no significant difference ($P>0.05$), while different letter superscripts mean significant difference ($P<0.05$) or extremely significant difference ($P<0.01$) depending on the P-value. The same notation applies to subsequent tables.

2.2 Effects of Dietary Betaine and RPB Supplementation on Nutrient Apparent Digestibility in Lambs

As shown in Table 3 , there were no significant differences among groups in apparent digestibility of DM, NDF, ADF, and CP ($P>0.05$). The RPB-6 group showed the highest apparent digestibility of DM, NDF, and ADF, which increased by 3.74%, 3.09%, and 6.27%, respectively, compared with the Con group.

Table 3. Effects of betaine and rumen-protected betaine on nutrient apparent digestibility of lambs (%)

2.3 Effects of Dietary Betaine and RPB Supplementation on Blood Biochemical Indices in Lambs

As shown in Table 4 , compared with the Con group, the B-2.2 group showed no significant differences in serum TC, TG, LDL, UN, and TP contents ($P>0.05$). Compared with the Con group, the RPB groups significantly reduced serum TC, TG, and UN contents ($P<0.05$), extremely significantly reduced LDL content ($P<0.01$), and extremely significantly increased TP content ($P<0.01$). There were no significant differences in HDL content among groups ($P>0.05$).

Table 4. Effects of betaine and rumen-protected betaine on serum biochemical indices of lambs

3.1 Effects of Dietary Betaine and RPB Supplementation on Growth Performance

Betaine can replace methionine as a methyl donor, reducing the requirement for methionine and choline as methyl donors, and participates in other important physiological processes in animals, thereby promoting animal growth [2]. This study showed that dietary supplementation with betaine and RPB had no significant effect on ADFI in lambs, but RPB increased ADG in lambs, showing an increasing trend with higher RPB supplementation levels, with the best effect

observed at 4 g/d addition. Meanwhile, the RPB groups reduced F/G. Huang et al. [17] reported that dietary betaine supplementation could increase growth hormone and insulin-like growth factor-I levels, promote protein synthesis through different pathways, and maintain positive nitrogen balance, thereby improving animal growth performance. Studies have found that under heat stress conditions, dietary supplementation with coated betaine can also improve daily gain and feed conversion ratio in heat-stressed beef cattle [18]. Due to the special rumen environment of ruminants, dietary betaine is easily degraded by rumen microorganisms into trimethylamine, resulting in insignificant application effects. In this study, the B-2.2 group showed no significant difference in growth performance compared with the Con group, mainly because betaine is easily degraded in large quantities by rumen microorganisms. In addition to the form of betaine affecting its application effect, dietary protein level also influences betaine efficacy. Fernández et al. [19] reported that dietary supplementation with betaine (2 g/kg) and RPB (2 g/kg) had no significant effect on lamb growth performance, which was speculated to be due to high dietary protein levels.

3.2 Effects of Dietary Betaine and RPB Supplementation on Nutrient Apparent Digestibility

Betaine supplementation can improve animal digestibility to some extent because betaine has high osmotic pressure properties. Its accumulation in cells can enhance intestinal water retention capacity, promote proliferation of intestinal epithelial cells, and increase intestinal tension and length, thereby enlarging surface area and improving nutrient digestibility [20-21]. Wang et al. [13] reported that adding different levels of betaine to Holstein dairy cow diets could significantly improve digestibility of dietary DM, CP, NDF, and ADF. Li Huawei et al. [22] found that dietary RPB choline supplementation could improve apparent digestibility of DM, organic matter, and ADF in Dorper × Hu crossbred male lambs. However, there are also reports showing no significant effect of RPB supplementation on animal apparent digestibility. Tian Xingzhou et al. [23] reported that RPB choline supplementation in Qianbei Ma goats had no significant effect on apparent digestibility of DM, CP, EE, and NDF, but could significantly improve apparent digestibility of ADF and crude ash. In this study, dietary supplementation with betaine and RPB had no significant effect on apparent digestibility of DM, CP, NDF, and ADF in lambs, but both showed an increasing trend with higher RPB supplementation levels. The inconsistent results may be related to animal breed, diet composition, and feeding conditions.

3.3 Effects of Dietary Betaine and RPB Supplementation on Blood Biochemical Indices

Numerous studies have shown that betaine can participate in fat metabolism and protein metabolism in animals. TG and TC contents are important indicators reflecting fat metabolism. Betaine supplementation contributes to lecithin synthesis, thereby promoting synthesis and secretion of lipoproteins in the liver.

TG combined with lipoproteins is transported to extrahepatic tissues for utilization, reducing the risk of fatty liver in animals [24]. This study showed that dietary RPB supplementation reduced serum TC and TG contents, with the lowest serum TC and TG contents observed at 2 g/d RPB supplementation, which is consistent with the findings of Zhang Dongmei et al. [25] and Zhu Dinggui et al. [7]. Betaine can be converted to carnitine, promoting fatty acid oxidation and thereby reducing serum TG content. Additionally, HDL can combine with cholesterol and transport blood cholesterol to the liver for metabolism, reducing blood cholesterol content. The results of this study indicate that dietary betaine supplementation can effectively reduce cholesterol and TG deposition in fattening lambs, promote fat decomposition, reduce body fat deposition, and decrease the risk of fatty liver.

The results of this study showed that dietary betaine supplementation had no significant effect on serum TP content, while RPB supplementation significantly increased serum TP content. Serum TP content is extremely significantly positively correlated with muscle percentage; when blood TP content increases, protein anabolism is enhanced. Furthermore, serum UN is the end product of protein amino acid metabolism; when UN content decreases, amino acid catabolism in animals is weakened. These results indicate that dietary supplementation with appropriate amounts of betaine is beneficial for enhancing protein synthesis metabolism and increasing protein deposition. This is related to betaine providing methyl donors required for protein synthesis, further meeting the growth needs of lambs. This is consistent with the findings of Dong Guan [8], which showed that dietary supplementation with appropriate levels of RPB could improve protein digestion and absorption.

Dietary supplementation with rumen-protected betaine can improve lamb growth performance and promote protein and fat metabolism, with an appropriate addition level of 4 g/d.

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