Effects of Rumen-Protected Leucine on Reproductive Performance, Digestive Performance, and Colostrum Quality in Tan Sheep Ewes (Postprint)

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Abstract

This experiment aimed to investigate the effects of rumen-protected leucine (RP-Leu) on reproductive performance, digestive performance, and colostrum quality of Tan sheep ewes. Forty-eight multiparous Tan sheep ewes with a body weight of (42.18$±$2.62) kg were selected and randomly divided into 4 groups, with 3 replicates per group and 4 ewes per replicate. The control group was fed a basal diet, while the experimental groups were supplemented with 0.10%, 0.20%, and 0.30% RP-Leu on top of the basal diet, respectively. The experimental period lasted from day 35 of gestation to day 60 postpartum (weaning). The results showed that compared with the control group, dietary supplementation of 0.20% RP-Leu to ewes during gestation significantly increased lamb birth weight and weaning weight ($P<0.05$), significantly improved the apparent digestibility of crude protein in gestating ewes ($P<0.05$), significantly increased the contents of milk fat, milk protein, and lactose in colostrum ($P<0.05$), and simultaneously significantly reduced serum urea nitrogen content in ewes on day 15 postpartum ($P<0.05$). In conclusion, dietary supplementation of 0.20% RP-Leu can improve nitrogen deposition and colostrum quality in pregnant Tan sheep, thereby promoting lamb growth and development.

Full Text

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Abstract: This experiment was conducted to investigate the effects of rumen-protected leucine (RP-Leu) on reproductive performance, digestive performance, and colostrum quality of Tan sheep ewes. Forty-eight multiparous Tan sheep ewes with an average body weight of (42.18$±$2.62) kg were randomly assigned to four groups, with three replicates per group and four ewes per replicate. The control group received a basal diet, while the experimental groups were supplemented with 0.10%, 0.20%, and 0.30% RP-Leu, respectively. The trial period spanned from day 35 of gestation to day 60 postpartum (weaning). The results showed that, compared with the control group, dietary supplementation with 0.20% RP-Leu during gestation significantly increased lamb birth weight and weaning weight ($P<0.05$), significantly improved the apparent digestibility of crude protein in gestating ewes ($P<0.05$), significantly elevated colostrum concentrations of milk fat, milk protein, and lactose ($P<0.05$), and significantly reduced serum urea nitrogen content in ewes on day 15 postpartum ($P<0.05$). In conclusion, dietary supplementation with 0.20% RP-Leu can improve nitrogen deposition and colostrum quality in pregnant Tan sheep ewes while promoting lamb growth and development.

Keywords: leucine; Tan sheep; reproductive performance; colostrum quality; apparent digestibility

Maternal nutritional status during gestation is closely associated with offspring growth, meat quality, and health status, producing lasting effects that persist into later life. In animal production, the influence of maternal nutrition during gestation on offspring development has become a prominent research focus. With advancing research on amino acid nutrition in ruminants, increasing attention has been directed toward the nutritional regulation of functional amino acids during gestation. Functional amino acids refer to those that serve physiological functions beyond protein synthesis, such as synthesizing various bioactive substances and modulating immune function. Branched-chain amino acids (BCAAs) constitute an important component of functional amino acids, and leucine (Leu), as one of the BCAAs, is an essential amino acid for mammals that must be obtained from the diet since it cannot be synthesized endogenously. Moreover, Leu is the most abundant essential amino acid in feedstuffs. Numerous studies have demonstrated that Leu plays crucial regulatory roles in mammals, primarily manifested in modulating immune function, providing oxidative energy, and regulating protein metabolism.

Tan sheep represent an excellent local breed in the Ningxia region, characterized by tender meat, uniform fat distribution, and delicious flavor, making them the dominant breed for producing distinctive local mutton. Currently, Tan sheep production commonly emphasizes fattening while neglecting maternal nutrition,

and few studies have investigated how gestational feeding strategies affect production performance. This experiment was designed to explore the effects of rumen-protected leucine (RP-Leu) on reproductive performance, digestive performance, and colostrum quality of Tan sheep ewes, thereby providing a basis and reference for improving production levels in Tan sheep breeding ewes.

1.1 Experimental Animals and Diets

The experiment was conducted from July 20, 2015, to March 15, 2016, at Hongsipu District Tianyuan Well-bred Sheep Breeding Co., Ltd. in Ningxia. Seventy healthy multiparous Tan sheep ewes of similar age and body weight were selected and subjected to estrus synchronization. After breeding, 48 ewes were randomly allocated to four groups, with three replicates (pens) per group and four ewes per replicate. The adaptation period lasted 7 days, and the formal trial commenced on day 35 of gestation. After natural parturition, all ewes were fed diets meeting the NRC (2007) nutrient recommendations for lactating sheep. The experiment concluded on day 60 postpartum (weaning).

The basal diet was formulated according to NRC (2007) standards, with composition and nutrient levels presented in Table 1 . Control group ewes received the basal diet, while experimental groups received the basal diet supplemented with 0.10%, 0.20%, and 0.30% RP-Leu, respectively, substituting an equal amount of wheat bran. Leucine was a white powder with 99.0% active content, provided by Evonik. The preparation of RP-Leu followed the method described by Sang et al. [11], with sodium carboxymethyl cellulose used as the coating material.

1.2 Feeding Management

All experimental ewes were individually numbered and housed in semi-open pens with good ventilation. They were fed twice daily at 08:00 and 16:00 with free access to water. Feed allowance was adjusted daily based on the previous day' s refusals to ensure a small amount of leftover feed (<5%). Routine disinfection, deworming, and vaccination procedures were followed according to standard farm protocols.

1.3 Measurements

1.3.1 Reproductive Performance The number of lambs born and their health status were recorded, and lamb birth weight and weaning weight were measured and documented.

1.3.2 Nutrient Apparent Digestibility On day 50 of the experiment, two ewes per replicate were randomly selected for a digestion-metabolism trial. Feed and refusal samples were collected using the total fecal collection method. After a 3-day adaptation period, feces were continuously collected for 4 days, weighed every 12 hours, and 10% sulfuric acid (10% concentration) was added at 10% of fecal weight for nitrogen fixation. The 4-day samples were pooled and stored

at -20 °C. The contents of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract (EE), dry matter (DM), and organic matter (OM) in feed, refusals, and feces were determined according to methods described in "Feed Analysis and Feed Quality Detection Technology" edited by Zhang Liying [13]. Nutrient apparent digestibility was calculated using the following formula:

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

1.3.3 Colostrum Collection and Quality Determination Colostrum samples (10 mL) were collected from Tan sheep ewes on the day of parturition, placed in centrifuge tubes, and transported to the laboratory under refrigeration. Colostrum concentrations of milk protein (MP), milk fat (MF), lactose, total milk solids (MTS), and milk solids-not-fat (MSNF) were determined using a Milkoscan FT-1 multifunctional milk analyzer (FOSS, Sweden).

1.3.4 Serum Biochemical Indicators On day 15 postpartum, 5 mL of blood was collected from the jugular vein of each ewe, placed in centrifuge tubes, and centrifuged at 1,980×g for 15 min at 4 °C to harvest serum, which was stored at -20 °C. Serum concentrations of total protein (TP), albumin (ALB), total cholesterol (TC), glucose (GLU), and urea nitrogen (UN) were determined using an automatic biochemical analyzer (TBA-40FR, Toshiba, Japan) via colorimetric methods. Assay kits were purchased from Nanjing Jiancheng Bioengineering Institute, and procedures were performed according to manufacturer instructions.

1.4 Statistical Analysis

Experimental data were initially processed using Excel software and subsequently analyzed using the GLM procedure of SAS 8.2. Differences among groups were evaluated using Duncan' s multiple comparison test, with $P<0.05$ considered statistically significant.

2.1 Effects of RP-Leu on Reproductive Performance of Tan Sheep Ewes

All ewes in this experiment gave birth to single lambs. The effects of RP-Leu on reproductive performance are presented in Table 2 . Compared with the control group, dietary supplementation with 0.20% or 0.30% RP-Leu during gestation significantly increased lamb birth weight and weaning weight ($P<0.05$), whereas supplementation with 0.10% RP-Leu had no significant effect on these parameters ($P>0.05$).

2.2 Effects of RP-Leu on Nutrient Apparent Digestibility of Tan Sheep Ewes

As shown in Table 4 , dietary supplementation with 0.20% RP-Leu significantly improved the apparent digestibility of crude protein (CP) in gestating Tan sheep ewes compared with the control group ($P<0.05$), while other experimental groups showed no significant differences ($P>0.05$). Supplementation with RP-Leu did not significantly affect the apparent digestibility of dry matter (DM), ether extract (EE), organic matter (OM), neutral detergent fiber (NDF), or acid detergent fiber (ADF) ($P>0.05$).

2.3 Effects of RP-Leu on Colostrum Quality of Tan Sheep Ewes

As shown in Table 3 , dietary supplementation with 0.20% or 0.30% RP-Leu during gestation significantly increased colostrum milk fat (MF) and lactose concentrations ($P<0.05$), though no significant differences were observed among the various supplemented groups ($P>0.05$). Supplementation with 0.20% RP-Leu significantly elevated colostrum milk protein (MP) content ($P<0.05$), while other experimental groups did not differ significantly from the control ($P>0.05$). Dietary RP-Leu supplementation had no significant effect on colostrum total milk solids (MTS) or milk solids-not-fat (MSNF) concentrations ($P>0.05$).

2.4 Effects of RP-Leu on Serum Biochemical Indicators of Tan Sheep Ewes

As shown in Table 5 , no significant differences were observed among groups in serum glucose (GLU), globulin (GLB), or total cholesterol (TC) concentrations ($P>0.05$). However, compared with the control group, dietary supplementation with 0.20% RP-Leu during gestation significantly increased serum total protein (TP) and albumin (ALB) concentrations ($P<0.05$) while significantly decreasing serum urea nitrogen (UN) content ($P<0.05$).

The maternal nutritional environment during embryonic development constitutes a critical factor influencing postnatal metabolic patterns in offspring. Beyond providing nutrients for embryonic development, maternal nutrition during gestation must also satisfy the dam' s own basic metabolic requirements. Nutritional imbalances in pregnant ewes can reduce lamb birth weight and affect long-term offspring health, while gestational nutrition is also closely associated with the production performance of both ewes and their offspring.

With deepening research on functional amino acids in regulating livestock physiological functions, increasing attention has focused on their role in improving animal production performance. As an essential amino acid for mammals, leucine cannot be synthesized endogenously and must be obtained from the diet. Leucine promotes protein synthesis by activating the mammalian target of rapamycin (mTOR) signaling pathway, thereby maintaining muscle mass and increasing average daily gain. The present study found that supplementing gestating Tan sheep ewes with 0.20% RP-Leu significantly increased lamb birth weight,

likely by promoting embryonic protein synthesis. Sang et al. [20] reported that dietary RP-Leu supplementation in sheep enhanced phosphorylation of key factors in the skeletal muscle mTOR signaling pathway, thereby promoting muscle protein synthesis. Similar protein synthesis-promoting effects of leucine have been demonstrated in other mammals. In weaned piglets, leucine supplementation to low-protein diets significantly increased skeletal muscle protein synthesis, while long-term leucine provision via drinking water also enhanced skeletal muscle protein synthesis in rats. These findings further support the inference that gestational RP-Leu supplementation promotes embryonic protein synthesis.

Research on leucine' s effects on nutrient apparent digestibility in herbivores is limited. Liu et al. [23] found that duodenal leucine infusion stimulated α-amylase production and pancreatic exocrine function, thereby improving starch digestibility in heifers. Similarly, leucine enhanced α-amylase activity and increased intestinal starch digestibility in dairy goats. In contrast, the present results showed that RP-Leu supplementation did not significantly affect apparent digestibility of OM, DM, EE, NDF, or ADF in gestating ewes but significantly improved CP apparent digestibility. This may be attributed to the fact that approximately 85% of leucine in RP-Leu theoretically bypasses rumen microbial degradation and becomes available for intestinal digestion and utilization, thereby balancing dietary amino acids and improving CP digestion and utilization. The concurrent increase in colostrum MP content with RP-Leu supplementation further corroborates this mechanism.

Beyond protein synthesis, leucine participates in energy metabolism and is closely associated with glucose uptake and fatty acid oxidation in animals. Fan et al. [25] reported that dietary leucine supplementation in weaned piglets inhibited oxidative phosphorylation and fatty acid β-oxidation while enhancing glycolysis, thereby altering energy metabolism status. The present study demonstrated that RP-Leu supplementation significantly increased colostrum milk fat and lactose concentrations, indicating that leucine also participates in mammary energy metabolism processes in gestating Tan sheep. Since ewe milk quality is closely related to lamb production performance, the improved postnatal lamb performance observed in this study suggests that gestational leucine exerts positive regulatory effects on milk quality in ruminants.

Serum total protein and albumin concentrations reflect protein absorption, synthesis, and catabolism status in animals and can also indicate immune function. In this study, dietary RP-Leu supplementation significantly increased serum TP and ALB concentrations in gestating ewes, suggesting that RP-Leu enhanced protein digestion and utilization and potentially improved immune function. Serum urea nitrogen content serves as an important indicator of protein metabolism and amino acid balance; elevated serum UN occurs when one or more amino acids are deficient or excessive in the diet. The significant reduction in serum UN content observed with RP-Leu supplementation indicates improved dietary nitrogen utilization and enhanced nitrogen deposition in gestating Tan sheep ewes, consistent with the observed improvements in CP appar-

ent digestibility and serum biochemical parameters.

In conclusion, dietary supplementation with 0.20% RP-Leu in gestating Tan sheep ewes improved dietary crude protein apparent digestibility and colostrum quality, increased lamb birth weight, and promoted lamb growth and development while enhancing dietary protein utilization efficiency.

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