Effects of Dietary Supplementation with Different Biological Preparations on Nutrient Digestion and Utilization in Duhan Hybrid Mutton Sheep (Postprint)

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Abstract

This experiment aimed to compare the effects of different biological preparations added to the diet on apparent nutrient digestibility, nitrogen and energy metabolism in Duhan crossbred mutton sheep. A single-factor experimental design was adopted, selecting 160 Duhan crossbred F1 mutton sheep with a body weight of approximately 32 kg, which were randomly divided into 5 groups with 4 replicates per group and 8 sheep per replicate. The control group was fed a basal diet without any biological preparation, while the experimental groups were fed the basal diet supplemented with 21 mg/kg monensin, 4$×109*CFU/kgBacilluslicheniformis*, 3.2×$109 CFU/kg Saccharomyces cerevisiae, and 1.1 g/kg enzyme-microbe preparation (containing Bacillus licheniformis, Saccharomyces cerevisiae, and alkaline protease), respectively. The preliminary period was 10 d, and the formal experimental period was 56 d. When the body weight of sheep in the enzyme-microbe preparation group reached approximately 37 kg, 4 sheep from each group were selected for a digestion and metabolism trial, with a 7 d preliminary period and a 5 d formal collection period. The results showed that: 1) The apparent dry matter digestibility and apparent acid detergent fiber digestibility of all experimental groups were significantly higher than those of the control group ($P<0.05$), and the enzyme-microbe preparation group had the highest apparent dry matter digestibility and apparent organic matter digestibility, which were significantly higher than those of the monensin group and Bacillus licheniformis group ($P<0.05$). 2) The apparent gross energy digestibility and gross energy metabolic rate of all experimental groups were significantly higher than those of the control group ($P<0.05$). 3) The apparent nitrogen digestibility of all experimental groups was significantly higher than that of the control group ($P<0.05$), and the apparent digestible nitrogen and apparent nitrogen digestibility of the enzyme-microbe preparation group were significantly higher than those of the control group, monensin group, and Bacillus licheniformis group (P<0.05). The results suggest that, based on the analysis of the effects as feed additives on nutrient digestion and utilization, energy metabolism, and nitrogen metabolism in Duhan crossbred mutton sheep, Bacillus licheniformis, Saccharomyces cerevisiae, and enzyme-microbe preparation are all superior to monensin, with the enzyme-microbe preparation being the best.

Full Text

Effects of Dietary Supplementation of Different Biological Agents on Nutrient Digestion and Utilization in Dorper×Thin-Tailed Han Crossbred Mutton Sheep

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Abstract

This study was conducted to compare the effects of dietary supplementation of different biological agents on apparent digestibility of nutrients, nitrogen metabolism, and energy metabolism in Dorper×thin-tailed Han crossbred mutton sheep. Using a single-factor experimental design, 160 Dorper×thin-tailed Han crossbred F1 lambs (approximately 32 kg body weight) were randomly allocated into 5 groups with 4 replicates per group and 8 lambs per replicate. The control group received a basal diet without any biological agents, while experimental groups were fed the basal diet supplemented with 21 mg/kg monensin, 4$×10^{9}$ CFU/kg *Bacillus licheniformis*, 3.2$×10^{9}$ CFU/kg *Saccharomyces cerevisiae*, or 1.1 g/kg enzyme-bacteria agent (containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline protease). The pre-trial period lasted 10 days, followed by a 56-day formal trial. When the lambs in the enzyme-bacteria agent group reached approximately 37 kg body weight, 4 lambs from each group were selected for a digestion and metabolism trial consisting of a 7-day adaptation period and a 5-day collection period. The results showed: (1) The apparent digestibility of dry matter (DM) and acid detergent fiber (ADF) in all experimental groups was significantly higher than that in the control group ($P<0.05$). The enzyme-bacteria agent group exhibited the highest apparent digestibility of DM and organic matter (OM), which was significantly higher than that in the monensin and *Bacillus licheniformis* groups ($P<0.05$). (2) The apparent digestibility of gross energy (GE) and metabolizability of GE in all experimental groups were significantly higher than those in the control group ($P<0.05$). (3) The apparent digestibility of nitrogen in all experimental groups was significantly higher than that in the control group ($P<0.05$). The enzyme-bacteria agent group showed significantly higher apparent digestible nitrogen and nitrogen apparent digestibility compared to the control, monensin, and *Bacillus licheniformis* groups ($P<0.05$). These results indicate that, in terms of effects on nutrient digestion and utilization, energy metabolism, and nitrogen metabolism in Dorper×thin-tailed Han crossbred lambs, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and enzyme-bacteria agents are superior to monensin, with the enzyme-bacteria agent showing the best overall performance.

Keywords: mutton sheep; nutrients; apparent digestibility; monensin; *Bacillus licheniformis*; *Saccharomyces cerevisiae*; enzyme-bacteria agent

Introduction

Antibiotics have been widely used as feed additives in animal production to significantly improve production efficiency, making them one of the most extensively applied feed additives. Statistics indicate that approximately 50% of global antibiotic production is used in livestock and aquaculture industries [1]. Monensin, an ionophore antibiotic, represents the most widely used class of antibiotic growth promoters. Feeding monensin to ruminants can alter rumen fermentation, improve carbohydrate and protein utilization, reduce methane production, and promote animal growth [2-3]. However, as concerns regarding the impacts of antibiotics on meat product safety and ecological environments have grown, antibiotic use in animal production is being subjected to increasingly strict control, necessitating the development of novel alternatives.

Probiotics, characterized by their pollution-free and residue-free properties, have become a research hotspot in recent years. According to the Ministry of Agriculture Announcement No. 2045, "Catalogue of Feed Additive Varieties (2013)," 33 probiotic strains are approved for use as feed additives [4]. Among these, *Bacillus licheniformis* and *Saccharomyces cerevisiae* have been extensively studied and demonstrate favorable application effects. *Bacillus licheniformis*, belonging to the family Bacillaceae, order Bacillales, class Bacilli, phylum Firmicutes, can promote the growth of beneficial bacteria in the animal gastrointestinal tract, secrete various digestive enzymes, and enhance endogenous digestive enzyme activity. Yeasts, unicellular fungi belonging to Ascomycota and Basidiomycota, contain abundant nutrients and possess fermentation capabilities that promote beneficial bacterial growth in the gastrointestinal tract while providing rich protein sources for the organism [5-10]. Direct feeding of *Bacillus licheniformis* and *Saccharomyces cerevisiae* as feed additives can improve production efficiency, but the efficacy of enzyme-bacteria agents combining these microorganisms with protease remains unclear. This experiment investigated the effects of dietary supplementation of monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and enzyme-bacteria agents on nutrient apparent digestibility, energy metabolism, and nitrogen metabolism in Dorper×thin-tailed Han crossbred lambs through digestion and metabolism trials, aiming to provide theoretical data for probiotic alternatives to antibiotics and promote efficient, healthy, and ecological development of China' s sheep industry.

Materials and Methods

1.1 Time and Location

The experiment was conducted from September to November 2016 at the modern mutton sheep farm of Inner Mongolia Fuchuan Feed Science and Technology Co., Ltd., with a total duration of 66 days.

1.2 Experimental Design

This study employed a single-factor experimental design using Dorper×thin-tailed Han crossbred lambs as experimental animals. One hundred and sixty healthy male lambs aged 4-6 months with similar body weight (approximately 32 kg) were randomly divided into 5 groups with 4 replicates per group and 8 lambs per replicate. The control group received a basal diet without any biological agents, while experimental groups were fed the basal diet supplemented with monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, or enzyme-bacteria agent. When the lambs in the enzyme-bacteria agent group reached approximately 37 kg body weight, 4 lambs close to the average weight were selected from each group for a digestion and metabolism trial. The digestion and metabolism trial consisted of a 7-day adaptation period followed by a 5-day collection period using the total collection method.

1.3 Additive Sources and Characteristics

Monensin (90% active ingredient content) was added at 21 mg/kg based on the study by Mirzaei-Alamouti et al. [11] (per kg air-dry basal diet, the same below) and purchased from Shandong Wudi Ruilikang Bioengineering Co., Ltd. *Bacillus licheniformis* (2×10^{11} CFU/g viable count) was added at 4×10^{9} CFU/kg; *Saccharomyces cerevisiae* (1×10^{10} CFU/g viable count) was added at 3.2×10^{9} CFU/kg; enzyme-bacteria agent (containing *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and alkaline protease, with 1×10^{10} CFU/g viable count) was added at 1.1 g/kg. The

Bacillus licheniformis, *Saccharomyces cerevisiae*, and enzyme-bacteria agent were provided by Beijing Xindayang Technology Development Co., Ltd., with addition rates following the manufacturer' s recommendations.

1.4 Basal Diet

The basal diet was formulated according to the nutritional requirements for 30-40 kg mutton sheep with a daily gain of 300 g proposed by our research team [12-16]. The feeding regimen consisted of 80% total mixed pellet feed + 20% Leymus chinensis (Chinese wildrye). Biological agents were added to the pellet feed ingredients, mixed thoroughly, and then pelleted. The diet was self-formulated, with premix provided by Beijing Precision Animal Nutrition Research Center. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients	
Corn	
Wheat bran	
Soybean meal	
Cottonseed meal	
Alfalfa hay	
DDGS	
Bean straw	
Leymus chinensis	
Sunflower meal	
NaCl	
Limestone	
$CaHPO_4$	
Premix[1])	
Total	
Nutrient levels[2])	
GE/(MJ/kg)	
DM	
CP	
EE	
Ash	
NDF	
ADF	

[1]) The premix provided the following per kg of diets: VA 15,000 IU, VD 2,200 IU, VE 50 IU, Fe 55 mg, Cu 12.5 mg, Mn 47 mg, Zn 24 mg, Se 0.5 mg, I 0.5

mg, Co 0.1 mg.
2) Nutrient levels were measured values.

1.5 Feeding Management

All experimental lambs were ear-tagged, vaccinated with a triple vaccine, and dewormed with 2.5 mL of ivermectin solution per animal. Lambs were housed individually, with each lamb occupying approximately 2.6 m^2. They were fed twice daily at 08:00 and 18:00 with free access to water. The barn temperature was maintained at 5-15 °C during the trial period under clear weather conditions. Feed intake was determined based on preliminary feeding trials, with all groups receiving the same amount of feed on an ad libitum basis. During the formal trial period, residual feed in the trough was maintained at approximately 10% of the feed offered. Leymus chinensis was fed first, followed by pellet feed 1 hour later to prevent selective feeding.

1.6 Sample Collection and Processing

During the collection period of the digestion and metabolism trial, feed offered to each lamb was accurately recorded and feed samples were collected before daily feeding. Daily feed samples from each group were pooled as the representative feed sample for the entire collection period. Residual feed was collected daily, pooled by group, and stored for analysis. Feces and urine were collected using the total collection method. Daily fecal output was weighed and recorded, thoroughly mixed, and subsampled at 10% of the total weight. Fecal samples from each lamb over the 5-day period were pooled and stored at -20 °C. After the trial, fecal samples were dried at 65 °C for 48 hours, equilibrated at room temperature for 48 hours, weighed to calculate initial moisture content, ground, and filtered through a 40-mesh sieve for nutrient analysis. Urine was collected in plastic buckets containing 100 mL of 10% H_2SO_4 to prevent uric acid precipitation during storage, diluted to 5 L, thoroughly mixed, filtered through 4 layers of gauze, and sampled at 30 mL daily. Urine samples from each lamb over the 5-day period were pooled and stored at -20 °C for subsequent determination of urinary energy (UE) and urinary nitrogen.

1.7.1 Nutrient Apparent Digestibility

Representative feed samples collected during the digestion and metabolism trial were analyzed for nutrient content according to AOAC (2000) [17] methods. Gross energy (GE) was determined using a PARR-6400 automatic oxygen bomb calorimeter, and crude protein (CP) content was measured using a KDY-9830 automatic Kjeldahl nitrogen analyzer. Nutrient apparent digestibility was calculated using the formula from Adeola [18]:

Apparent digestibility of a nutrient in diet (%) = 100 × (Feed intake × Nutrient content in feed - Fecal output × Nutrient content in feces) / (Feed intake × Nutrient content in feed)

1.7.2 Energy Metabolism Indicators

Fecal energy (FE) and UE were determined using a Parr-6400 automatic oxygen bomb calorimeter according to AOAC (2000) [17]. For UE determination, five quantitative filter papers were measured to calculate the average energy value of filter paper. Ten milliliters of urine were dropped onto filter paper in multiple aliquots, dried at 65 °C, and measured to obtain the energy value of filter paper with urine. The UE was obtained by subtracting the filter paper energy value from the total value. Digestible energy (DE), UE, metabolizable energy (ME), apparent digestibility of GE, metabolizability of GE, and metabolizability of DE were calculated as follows:

- DE (MJ/kg) = GE intake - FE
- UE (MJ/kg) = Energy value of filter paper with urine - Energy value of filter paper
- ME (MJ/kg) = GE intake - FE - UE - Methane energy
- Apparent digestibility of GE (%) = DE / GE intake
- Metabolizability of GE (%) = ME / GE intake
- Metabolizability of DE (%) = ME / DE

Methane energy values were based on previous research from our team, which indicated that methane energy accounted for 8% of GE in fattening Dorper×thin-tailed Han crossbred lambs [19].

1.7.3 Nitrogen Metabolism Indicators

Intake nitrogen, fecal nitrogen, and urinary nitrogen were measured to calculate total nitrogen excretion, apparent digestible nitrogen, retained nitrogen, nitrogen apparent digestibility, nitrogen utilization efficiency, and biological value of nitrogen using the following formulas:

- Total nitrogen excretion (g/d) = Fecal nitrogen + Urinary nitrogen
- Apparent digestible nitrogen (g/d) = Intake nitrogen - Fecal nitrogen
- Retained nitrogen (g/d) = Intake nitrogen - (Fecal nitrogen + Urinary nitrogen)
- Nitrogen apparent digestibility (%) = 100 × Apparent digestible nitrogen / Intake nitrogen
- Nitrogen utilization efficiency (%) = 100 × Retained nitrogen / Intake nitrogen
- Biological value of nitrogen (%) = 100 × [Intake nitrogen - (Fecal nitrogen + Urinary nitrogen)] / (Intake nitrogen - Fecal nitrogen)

1.8 Data Processing and Analysis

Experimental data were organized using Excel 2010 and analyzed using one-way ANOVA procedure of SAS 9.1 statistical software. Duncan' s multiple comparison test was applied when significant differences were detected. Differences were considered significant at $P<0.05$.

Results

2.1 Effects of Different Biological Agents on Nutrient Apparent Digestibility in Dorper×Thin-Tailed Han Crossbred Lambs

As shown in Table 2 , the apparent digestibility of dry matter (DM) and acid detergent fiber (ADF) in all experimental groups was significantly higher than that in the control group ($P<0.05$). The enzyme-bacteria agent group exhibited the highest neutral detergent fiber (NDF) apparent digestibility, which was significantly higher than that in the monensin and *Bacillus licheniformis* groups ($P<0.05$). The enzyme-bacteria agent group also showed significantly higher apparent digestibility of organic matter (OM) and ADF compared to the control and monensin groups ($P<0.05$).

Table 2 Effects of different biological agents on nutrient intake and apparent digestibility in Dorper×thin-tailed Han crossbred lambs

Items	Control	Monensin	*Bacillus licheniformis*	*Saccharomyces cerevisiae*	Enzyme-bacteria agent	P-value
DM						
Intake (g)	623.45[a]	554.40	552.73	534.43	531.68	
Excretion (g)	58.68	61.90	61.73	63.70	64.98[a]	
Apparent digestibility (%)	58.53	61.63	61.85	63.78	65.18[a]	<0.001
OM						
Intake (g)						
Excretion (g)						
Apparent digestibility (%)	58.95	60.40	60.95	63.85[a]	65.18[a]	<0.001
NDF						
Intake (g)	209.58[a]	188.43	187.75	187.80	180.13	

Items	Control	Monensin	*Bacillus licheniformis*	*Saccharomyces cerevisiae*	Enzyme-bacteria agent	P-value
Excretion (g)						
Apparent digestibility (%)	42.48	46.88	47.70	47.80	50.65[a]	<0.001
ADF						
Intake (g)						
Excretion (g)						
Apparent digestibility (%)						

In the same row, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), while different lowercase letter superscripts indicate significant difference (P<0.05). The same as below.

2.2 Effects of Different Biological Agents on Energy Metabolism in Dorper×Thin-Tailed Han Crossbred Lambs

As shown in Table 3 , all experimental groups exhibited significantly higher apparent digestibility of GE and metabolizability of GE compared to the control group (P<0.05). Although differences were not significant (P>0.05), DE in the monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and enzyme-bacteria agent groups increased by 4.2%, 3.7%, 7.3%, and 11.4%, respectively, compared to the control group. Both ME and metabolizability of DE were higher in all experimental groups than in the control group, following the order: enzyme-bacteria agent > *Saccharomyces cerevisiae* > monensin > *Bacillus licheniformis* > control, though differences among groups were not significant (P>0.05).

Table 3 Effects of different biological agents on energy metabolism in Dorper×thin-tailed Han crossbred lambs

Items	Control	Monensin	*Bacillus licheniformis*	*Saccharomyces cerevisiae*	Enzyme-bacteria agent	P-value
GE intake (MJ/d)	11.25^{a}	9.95	9.70	9.73	9.70	
FE (MJ/d)						
UE (MJ/d)						
DE (MJ/d)						
ME (MJ/d)	58.00	62.03^{a}	62.38^{a}	63.20^{a}	64.08^{a}	
Apparent digestibility of GE (%)	46.63	50.20^{a}	50.45^{a}	51.43^{a}	52.38^{a}	
Metabolizability of GE (%)						
Metabolizability of DE (%)						

2.3 Effects of Different Biological Agents on Nitrogen Metabolism in Dorper×Thin-Tailed Han Crossbred Lambs

As shown in Table 4 , all experimental groups showed significantly higher nitrogen apparent digestibility than the control group (P<0.05). Total nitrogen excretion in all experimental groups tended to be lower than in the control group, though differences were not significant (P>0.05). Compared to the control group, retained nitrogen in the monensin, *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and enzyme-bacteria agent groups increased by 8.3%, 17.8%, 9.6%, and 15.4%, respectively. Nitrogen utilization efficiency increased by 13.3%, 22.5%, 12.9%, and 15.1%, respectively, and biological value of nitrogen increased by 7.5%, 15.3%, 3.4%, and 3.2%, respectively, though these differences were not significant (P>0.05). The enzyme-bacteria agent group exhibited significantly higher apparent digestible nitrogen and nitrogen apparent digestibility compared to the control, monensin, and *Bacillus licheniformis* groups (P<0.05).

Table 4 Effects of different biological agents on nitrogen metabolism in Dorper×thin-tailed Han crossbred lambs

Items	Control	Monensin	*Bacillus licheniformis*	*Saccharomyces cerevisiae*	Enzyme-bacteria agent	P-value
N intake (g/d)	14.35^{a}	12.43	12.20	11.60	11.28	
Fecal N (g/d)						
Urinary N (g/d)	27.43	27.70	28.03	29.03^{a}	30.63^{a}	
Total N excretion (g/d)	69.73	71.48^{a}	73.10^{a}			<0.001
Apparent digestible N (g/d)	65.65	69.05				
Retained N (g/d)						
Nitrogen apparent digestibility (%)						

Items	Control	Monensin	*Bacillus licheniformis*	*Saccharomyces cerevisiae*	Enzyme-bacteria agent	P-value
Utilization efficiency of N (%)						
Biological value of N (%)						

Discussion

Monensin is a polyether ionophore antibiotic that facilitates ion transport across cell membranes [20]. By altering cellular ion balance, it kills protozoa and inhibits the growth of Gram-positive bacteria in the rumen, thereby improving rumen fermentation and reducing methane production. Although monensin leaves minimal residue in animal tissues, its excretion into the environment may affect human health through ecological cycles, ultimately leading to its phase-out. Probiotics, featuring non-polluting and residue-free characteristics, can inhibit harmful bacteria, improve rumen microbial flora, enhance immunity and daily gain, and improve feed conversion efficiency. Research has shown that compound probiotic preparations outperform single-strain preparations [21], and enzyme-bacteria agents can promote weight gain in goats [22]. Another report from our research group demonstrated that all biological agents improved average daily gain and feed conversion efficiency in mutton sheep, with the enzyme-bacteria agent showing the best results [23].

3.1 Effects of Different Biological Agents on Nutrient Apparent Digestibility in Dorper×Thin-Tailed Han Crossbred Lambs

Apparent digestibility of DM and OM reflects the comprehensive digestive characteristics of animals. Ruminant DM intake typically ranges from 2% to 4% of body weight [24]. In this experiment, DM intake accounted for 4% of body weight across all groups, which falls within the normal range. The results indicated that *Bacillus licheniformis*, *Saccharomyces cerevisiae*, and enzyme-bacteria agents significantly improved DM and OM apparent digestibility, while monensin only significantly improved DM apparent digestibility. Monensin can kill protozoa [25] and inhibit Gram-positive bacteria growth, thereby improving rumen fermentation. Studies have shown that feeding monensin to lambs does not significantly affect DM intake but improves feed conversion efficiency [11], though other research found no effect on nutrient apparent digestibility [26]. *Bacillus licheniformis* can lower gastrointestinal pH, improve microbial environment, produce nutrients such as vitamins, amino acids, and organic acids, and supplement enzymes including protease, lipase, amylase, and cellulase, while also possessing amylase- and protease-like activities. Yeast can produce secondary metabolites such as β-glucan, mannan, and organic acids during metabolism, effectively reducing gastrointestinal pH, and exhibits strong amylase-, protease-, and lipase-like activities. Yeast culture contains various nutrients including organic acids, vitamins, calcium, and phosphorus [27]. These findings suggest that both *Bacillus licheniformis* and *Saccharomyces cerevisiae* can improve nutrient apparent digestibility. The small intestine is the primary site for nutrient digestion and absorption. Lin et al. [1] reported that antibiotics, probiotics, and enzyme-bacteria agents all promote the morphological development of duodenal, jejunal, and ileal villi, potentially enhancing nutrient digestion and absorption through improved intestinal structure. The higher DM and OM apparent digestibility in the enzyme-bacteria agent group may be attributed to the active protease providing not only exogenous enzymes but also essential nutrients for probiotic growth, creating a synergistic effect.

Ruminants can utilize structural carbohydrates such as cellulose and hemicellulose that are difficult for monogastric animals to digest due to their specialized rumen structure. NDF and ADF apparent digestibility reflect the degree of dietary utilization in ruminants. Effective fiber degradation in the rumen provides energy for both the animal and rumen microorganisms and ensures healthy saliva secretion, rumination, rumen fluid buffering, and rumen wall health [29]. Therefore, fiber digestibility is of great significance in ruminants. ADF includes silicates, cellulose, and lignin [30], while NDF is currently considered the best indicator for fiber as it effectively separates structural from non-structural carbohydrates [31]. NDF degradation is primarily completed by relatively stable fiber-degrading bacteria in the rumen [24]. In this experiment, all biological agents significantly improved ADF apparent digestibility compared to the control group, while only the enzyme-bacteria agent significantly improved NDF apparent digestibility, with other agents showing a trend toward improvement. Probiotic preparations contain live microorganisms that affect intestinal colonization and composition. Fu et al. [32] reported that probiotics can increase the populations of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Butyrivibrio fibrisolvens* in the rumen. The enzyme-bacteria agent may have improved fiber utilization by increasing fiber-degrading bacterial populations in the rumen.

3.2 Effects of Different Biological Agents on Energy Metabolism in Dorper×Thin-Tailed Han Crossbred Lambs

Animal energy metabolism refers to the energy generated during nutrient transformation or energy expenditure during physical activity, essentially representing a complex process of biological oxidation and reduction. Animals primarily obtain energy through nutrient oxidation, generally via three cycles: glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation [27]. Ruminants experience greater energy losses during digestion than non-ruminants, mainly due to rumen fermentation energy losses, with methane production being the primary pathway. In this experiment, all biological agents improved DE and ME and significantly increased apparent digestibility and metabolizability of GE. This may be because both monensin and probiotics can alter rumen fermentation, reduce acetate content, increase propionate content, and decrease the acetate/propionate ratio [33-34]. Acetate promotes methane production that is eructated and lost as unusable energy, while propionate serves as a substrate for glucose synthesis, providing available energy. Han [34] reported that monensin significantly reduced total volatile fatty acid content, butyrate content, and acetate/propionate ratio while increasing propionate content and reducing methane production, though the effect was time-dependent. Xiao et al. [27] found that feeding *Bacillus* and yeast to mutton sheep significantly reduced daily methane emissions and improved apparent digestibility and metabolizability of GE, consistent with our results. Additionally, probiotics may affect intestinal colonization, thereby influencing local and systemic immune function [32] and reducing energy losses due to stress. In this experiment, all experimental groups showed numerically higher DE, ME, apparent digestibility of GE, metabolizability of GE, and metabolizability of DE compared to the control group, indicating that all biological agents improved energy metabolism, with the enzyme-bacteria agent showing the best effect.

3.3 Effects of Different Biological Agents on Nitrogen Metabolism in Dorper×Thin-Tailed Han Crossbred Lambs

After dietary protein enters the rumen, a portion is degraded by rumen microorganisms to form rumen-degradable protein for microbial degradation and synthesis of microbial protein, which is ultimately digested and absorbed by the host. The remaining portion bypasses the rumen as rumen-undegradable protein entering the lower digestive tract, with undegraded nitrogen and endogenous nitrogen eventually excreted in feces and urine [35]. Therefore, protein digestion and metabolism in ruminants is primarily influenced by rumen microbial degradation, as well as nitrogen excretion in feces and urine. Some studies have reported fecal nitrogen exceeding urinary nitrogen, while others have shown the opposite. In this experiment, fecal nitrogen was higher than urinary nitrogen in all groups except the enzyme-bacteria agent group. All biological agents tended to reduce total nitrogen excretion and significantly improved nitrogen apparent digestibility. This may be because monensin can directly or indirectly reduce protein degradation and deaminase activity in the rumen, decrease dietary protein degradation, increase rumen-undegradable protein flow, and inhibit populations of highly ammonia-producing bacteria involved in protein metabolism, thereby reducing rumen ammonia production [36]. Both monensin and probiotics can improve rumen fermentation and increase propionate content, which can effectively prevent amino acid bioconversion to volatile fatty acids, carbon dioxide, and ammonia. Propionate also participates in gluconeogenesis, indirectly reducing hepatic degradation of gluconeogenic amino acids and improving nitrogen utilization efficiency [37].

Retained nitrogen directly indicates the degree of dietary protein utilization, whereas nitrogen apparent digestibility cannot accurately reflect the extent of digestion and absorption. Therefore, retained nitrogen is more meaningful than nitrogen apparent digestibility [24] and serves as a direct indicator of nitrogen utilization in mutton sheep. In this experiment, biological agents had minimal effects on nitrogen intake and fecal nitrogen, but urinary nitrogen varied considerably among treatment groups, with the lowest value observed in the *Bacillus licheniformis* group. Consequently, retained nitrogen and nitrogen apparent digestibility were higher in all experimental groups than in the control group, with the *Bacillus licheniformis* group showing the highest values. Since retained nitrogen directly affects nitrogen utilization efficiency, the *Bacillus licheniformis* group exhibited the highest nitrogen utilization efficiency. Nitrogen utilization efficiency was generally high across all groups in this experiment. Although the feeding standard employed was suitable for Dorper×thin-tailed Han crossbred lambs [38], the control group results were higher than those reported by Wan et al. [39] using the same standard, possibly because dietary protein content was higher than in the previous study or because Leymus chinensis supplementation promoted gastrointestinal development and improved nitrogen digestion and absorption. The results demonstrate that all biological agents improved nitrogen digestion and utilization, promoted nitrogen absorption, and could prevent water and soil eutrophication, benefiting environmental protection. Overall, the enzyme-bacteria agent showed the best comprehensive effect.

Conclusion

Under the conditions of this experiment:

1. Monensin improved nutrient apparent digestibility, reduced FE and fecal nitrogen, and increased apparent digestibility of GE, metabolizability of GE, and nitrogen apparent digestibility in mutton sheep.

2. The probiotic additives *Bacillus licheniformis* and *Saccharomyces cerevisiae* produced effects comparable to monensin.

3. The enzyme-bacteria agent significantly improved nutrient apparent digestibility, energy metabolism, and nitrogen metabolism, demonstrating

superior effects to single probiotic preparations.

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