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ARTICLE

Effects of a Yeast–Lactobacilli Probiotic on Rumen Volatile Fatty Acids and Serum Glucose Concentration in Lambs

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Abstract
This study evaluated the effects of a probiotic supplement containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* on rumen fermentation characteristics and glucose metabolism in lambs during the transition from milk to solid feed. Lambs were assigned to a control group and two probiotic treatment groups. Rumen volatile fatty acids (VFAs), blood glucose concentration, and rumen protozoal populations were measured during the weaning period.
At 60 days of age, lambs receiving the probiotic treatment in Experimental Group I exhibited significantly higher concentrations of acetic acid (28.72 ± 5.2 mmol L^{-1}), propionic acid (8.77 ± 0.5 mmol L^{-1}), and butyric acid (4.03 ± 0.56 mmol L^{-1}) compared with the control group ($P < 0.05$). Although total VFA concentration was highest in Experimental Group I (44.4 ± 5.78 mmol L^{-1}), differences among groups were not statistically significant ($P > 0.05$). Blood glucose concentrations increased rapidly during the first 6 h after birth, rising from 0.79 ± 0.8 to 4.53 ± 0.2 mmol L^{-1} ($P < 0.001$). At 45 days of age, probiotic-treated lambs had significantly higher blood glucose concentrations than control lambs ($P < 0.001$); however, these differences were no longer evident at 60 days of age ($P > 0.05$).
The number of rumen ciliates did not differ significantly among treatments ($P > 0.05$), although all groups were dominated by the genera *Entodinium*, *Diplodinium*, and *Isotricha*. The results indicate that probiotic supplementation can enhance rumen fermentation and improve glucose metabolism during the weaning transition, particularly through increased production of volatile fatty acids.

Keywords: rumen fermentation, weaning transition, protozoal population, blood metabolites, microbial supplementation, small ruminants

Introduction

Probiotics are widely used as feed additives in livestock production because of their beneficial effects on animal health, nutrient utilization, growth performance, and immune function [1]. In ruminants, probiotic supplementation has attracted increasing attention as an alternative to antibiotic growth promoters due to its ability to improve rumen microbial activity and digestive efficiency [2]. Among the most commonly used probiotic microorganisms are yeast (*Saccharomyces cerevisiae*) and lactic acid bacteria, including *Lactobacillus plantarum* and *Lactobacillus acidophilus*, which have been reported to enhance rumen fermentation and stabilize the gastrointestinal microbial ecosystem.

Previous studies have shown that probiotic supplementation can alter rumen fermentation patterns by increasing the production of volatile fatty acids (VFAs) and reducing ruminal ammonia concentrations [3]. Volatile fatty acids, primarily acetate, propionate, and butyrate, are the major end-products of microbial fermentation in the rumen and represent the principal energy source for ruminant animals [4]. Among these metabolites, propionate is particularly important because it serves as the primary precursor for hepatic gluconeogenesis and contributes directly to the maintenance of blood glucose concentrations.

The transition from milk feeding to solid feed represents a critical developmental stage in lambs. During the neonatal period, glucose is mainly derived from the digestion and absorption of lactose in milk. As lambs mature and begin consuming forage and concentrate feeds, rumen fermentation becomes increasingly important, resulting in greater production of VFAs and a gradual shift in energy metabolism [4]. Consequently, changes in rumen fermentation during the weaning period may influence blood glucose concentrations and overall metabolic adaptation.

Blood glucose concentration is an important physiological indicator of energy metabolism in young lambs. Previous studies have demonstrated that glucose levels undergo rapid changes during the first hours and days after birth and gradually stabilize as rumen development progresses [5,6]. Because propionate produced during ruminal fermentation serves as a major substrate for glucose synthesis, factors that modify rumen microbial activity may indirectly affect glucose metabolism.

Rumen protozoa constitute an important component of the rumen microbial ecosystem. These microorganisms participate in the degradation of structural and non-structural carbohydrates and contribute to the production of volatile fatty acids through fermentation processes [7]. Changes in protozoal populations may therefore influence rumen fermentation characteristics and the availability of metabolic substrates for the host animal [8].

Although the effects of probiotics on rumen fermentation have been investigated in adult ruminants, information regarding their influence on volatile fatty acid production, blood glucose concentration, and rumen protozoal populations during the weaning transition in lambs remains limited. Therefore, the objective of this study was to evaluate the effects of a probiotic feed supplement containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* on rumen fermentation characteristics, blood glucose concentration, and rumen protozoal populations in lambs during the transition from milk to solid feed. It was hypothesized that probiotic supplementation would enhance rumen fermentation, increase volatile fatty acid production, and support glucose metabolism during this critical developmental period.

Materials and Methods

Probiotic Supplement

The probiotic supplement used in this study contained *Saccharomyces cerevisiae* (1×10^9 CFU g^{-1}) and the commercial preparation “Lactobacterin-STH,” which contained indigenous strains of *Lactobacillus plantarum* and *Lactobacillus acidophilus* (2×10^8 CFU g^{-1}). The product also contained 17 essential amino acids, including methionine, arginine, and lysine, and consisted of approximately 50–60% microbial protein.

Experimental Animals and Treatments

The study was conducted in the 3rd subdistrict of Bayantsagaan soum, Tuv Province, Mongolia, during the spring–summer production season of 2024. A total of 45 healthy male lambs born between 1 and 5 April 2024 were selected based on similar live body weight and randomly allocated to three treatment groups (n = 15 per group).

Lambs in the control group received no probiotic supplementation. Lambs in Experimental Group I were supplemented daily with 20 g of a probiotic mixture containing *Saccharomyces cerevisiae* and lactobacilli (*Lactobacillus plantarum* and *L. acidophilus*) at a ratio of 70:30. Lambs in Experimental Group II received 20 g day^{-1} of a probiotic mixture containing *S. cerevisiae* and lactobacilli at a ratio of 50:50. Probiotic supplementation was administered daily from 25 to 45 days of age.

Throughout the experimental period, all lambs remained with their dams and were managed under traditional pasture-based grazing conditions. Animals had continuous access to natural pasture and water, and all husbandry practices were carried out according to local management procedures.

Rumen Fluid Collection and Analysis

Rumen fluid samples were collected using a stomach tube from lambs in all groups at 45 and 60 days of age. Samples were collected approximately 90 min after feeding at day 45 and 120 min after feeding at day 60. Each sample was divided into two subsamples. One subsample was preserved for protozoal analysis, while the second was used for volatile fatty acid (VFA) determination. Samples were transported to the laboratory under refrigerated conditions.

Rumen protozoa were identified microscopically, and total counts and species composition were determined using standard identification keys. For VFA analysis, rumen fluid was centrifuged, and the supernatant was preserved with orthophosphoric acid. Total VFA concentration and individual VFAs (acetate, propionate, and butyrate) were quantified by high-performance liquid chromatography (HPLC) using external standards.

Blood Collection and Glucose Analysis

Blood samples were collected from the jugular vein at birth (0 h), and at 1, 6, 24, and 48 h after birth, as well as at 45 and 60 days of age. Serum was separated by centrifugation and stored until analysis.

Serum glucose concentration was determined using a commercial Rotor-Gene diagnostic kit following the manufacturer's instructions. Absorbance was measured at 500 nm using a spectrophotometer, and glucose concentrations were calculated using calibration standards.

Statistical Analysis

Data were analyzed using SPSS and Microsoft Excel software. Results are presented as mean ± standard deviation (SD) or standard error (SE), as appropriate. Differences among treatments were evaluated using one-way analysis of variance (ANOVA), and statistical significance was declared at $P < 0.05$.

Results

Effect of Probiotic Supplementation on Volatile Fatty Acid Concentration and Composition

The total concentration and composition of volatile fatty acids (VFAs) in rumen fluid are presented in Table 1 and Figure 1.

The highest total VFA concentration was observed in Experimental Group I (44.43 ± 5.78 mmol L^{-1}), followed by Experimental Group II (32.74 ± 5.99 mmol L^{-1}) and the control group (32.08 ± 6.32 mmol L^{-1}). Despite the numerically greater concentration in Experimental Group I, differences among groups were not statistically significant ($P > 0.05$).

Acetic acid was the predominant VFA in all groups, accounting for 63.68–64.65% of total VFAs. Lambs in Experimental Group I exhibited higher concentrations of acetic acid (28.72 ± 5.20 mmol L^{-1}), propionic acid (8.77 ± 0.50 mmol L^{-1}), and butyric acid (4.03 ± 0.56 mmol L^{-1}) than those in Experimental Group II and the control group.

The relative proportion of propionic acid ranged from 18.11% to 19.73%, whereas butyric acid accounted for 9.07–9.56% of total VFAs. Minor VFAs, including isobutyric, valeric, and isovaleric acids, collectively represented less than 7% of total VFAs in all groups.

No significant differences were detected among treatments in the relative proportions of individual VFAs ($P > 0.05$).

Table 1.

Total concentration and composition of volatile fatty acids (VFAs) in rumen fluid of lambs receiving different probiotic treatments

No	**Volatile Fatty Acid, parameter**	**Differences between experimental groups**				**Control n=4**	
		Experimental group I n=4		Experimental group II n=4			
1	**Total VFA (mmol/L)**	**44.43 ± 5.78**	**Percent**	**32.74±5.99**	**Percent**	**32.08±6.32**	**Percent**
2	Acetic acid	28.72±5.2	64.65	21.02±6.5	64.20	20.43±4.5	63.68
3	Propionic acid	8.77±0.5	19.73	5.93±1.5	18.11	6.27±0.9	19.54
4	Butyric acid	4.03±0.56	9.07	3.13±1.2	9.56	2.98±0.5	9.28

5	Isobutyric acid	0.79±0.09	1.78	0.74±0.25	2.26	0.71±0.18	2.21
6	Valeric acid	0.78±0.02	1.76	0.60±0.17	1.83	0.63±0.12	1.96
7	Iso-valeric acid	1.34±0.02	3.01	1.34±0.41	4.04	1.07±0.25	3.33

Among the volatile fatty acids (VFAs) formed in the rumen of lambs, the proportion of acetic acid averaged 64.65% and 64.20% in experimental groups I and II, respectively, while it was around 63.68% in the control group lambs that continuously grazed with their dams. The proportion of propionic acid was 19.73% in experimental group Iand 19.54% in the control group, while it was approximately 18.11% in group II. Meanwhile, butyric acid and its isomers accounted for 11.82% in group II, and valeric acid with its isomers reached 5.87%, which were slightly higher than those in group and the control group.

Based on the above findings, it can be suggested that the probiotic supplement containing a combination of yeast and lactic acid bacteria may have a potentially positive effect on the production of volatile fatty acids (VFAs)—a key indicator of rumen development in lambs—as well as on the relative proportions of individual VFA components. For example, in experimental II, the proportion of butyric acid and valeric acid, including their respective isomers, within the volatile fatty acid (VFA) profile was slightly higher compared to experimental I and the control.

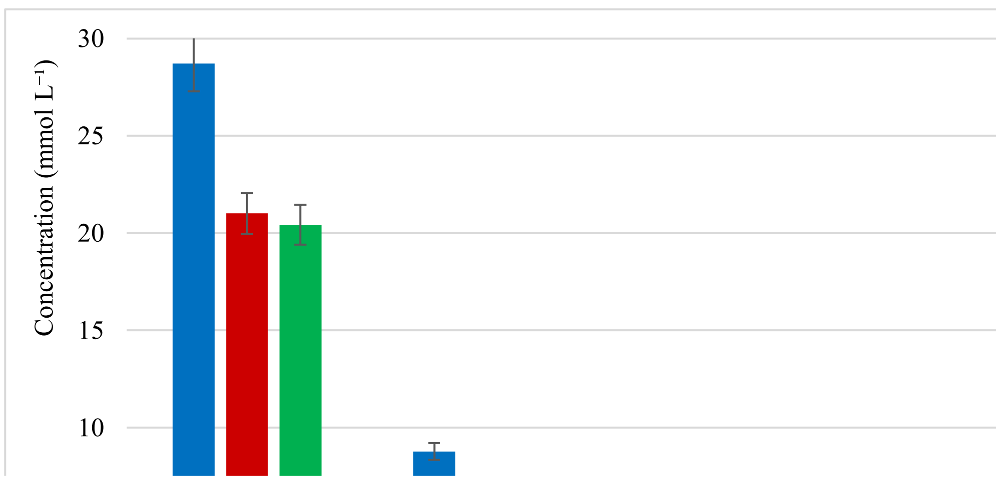

Figure 1. Concentrations of individual volatile fatty acids (VFAs) in rumen fluid of lambs receiving different probiotic treatments. Bars represent mean ± standard error (SE)

Effect of Probiotic Supplementation on Serum Glucose Concentration

Serum glucose concentrations in lambs at different ages are presented in Figure 2 and Table 2.

At birth, the mean serum glucose concentration was 0.79 ± 0.8 mmol L^{-1}. Glucose concentration increased significantly during the first hours after birth, reaching 2.32 ± 0.9 mmol L^{-1} at 1 h and peaking at 4.53 ± 0.2 mmol L^{-1} at 6 h ($P < 0.001$). Thereafter, glucose concentrations declined to 3.04 ± 0.9 mmol L^{-1} at 24 h and 3.01 ± 0.7 mmol L^{-1} at 48 h of age.

At 45 days of age, lambs in Experimental Group I and Experimental Group II exhibited significantly higher serum glucose concentrations (5.8 ± 0.8 and 5.3 ± 0.9 mmol L^{-1}, respectively) than lambs in the control group (3.9 ± 0.9 mmol L^{-1}; $P = 0.001$). However, by 60 days of age, serum glucose concentrations were similar among groups, averaging approximately 4.2 mmol L^{-1}, and no significant differences were detected ($P > 0.05$).

These results indicate that serum glucose concentration changed markedly during the early postnatal period and differed among treatments at 45 days of age but not at 60 days of age.

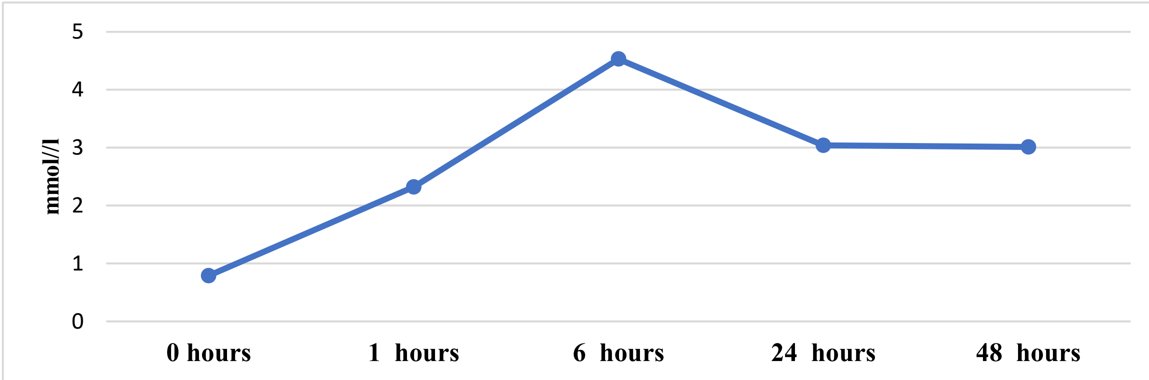

Figure 2. Serum glucose concentration in neonatal lambs during the first 48 h after birth.
Values represent mean ± standard error (SE)
Serum glucose concentration (mmol L^{-1})

In general, ruminant animals tend to have lower blood glucose levels compared to non-ruminants, a phenomenon attributed to the energy supply derived from volatile fatty acids (VFAs) produced in the rumen. Based on this principle, our study aimed to determine both the concentration of VFAs in the rumen and the corresponding serum glucose levels of lambs during the transition period. Glucose levels were measured at specific time points after birth (0, 1, 6, 24, and 48 hours), as well as at 45 and 60 days of age. The results are presented in Figure 2.

This parameter peaked at 6 hours postpartum, reaching the highest level of 4.53 ± 0.2 mmol/L, before gradually decreasing over time, as previously described. In contrast, this indicator showed that the blood serum glucose concentration of lambs in the experimental groups at 45 days of age was 5.8 ± 0.8 mmol/L in experimental I and 5.3 ± 0.9 mmol/L in experimental II, which were significantly higher than that of the control (3.9 ± 0.9 mmol/L) ($P < 0.001$).

By 60 days of age, serum glucose levels in both experimental and control groups stabilized at approximately 4.20 mmol/L, with no statistically significant difference observed ($P>0.05$).

These findings suggest that the dual-strain probiotic supplement may have a positive effect on blood glucose levels during the rumen development (transition) phase in lambs. In particular, the significantly elevated glucose concentration at 45 days of age in the experimental group provides strong evidence of the probiotic's potential influence during early postnatal growth.

Table 2.

Serum glucose concentration of lambs at 45 and 60 days of age (mmol L^{-1})

	Groups			
Age (days)	Experimental I (n = 7)	Experimental II (n = 7)	Control (n = 7)	P-value
45	5.8 ± 0.8	5.3 ± 0.9	3.9 ± 0.9	0.001
60	4.2 ± 0.4	4.2 ± 0.5	4.2 ± 0.6	>0.05

Effect of Probiotic Supplementation on Rumen Protozoal Population

The total number and species composition of rumen protozoa in lambs at 60 days of age are presented in Table 3. The mean protozoal population was 343.3 ± 16.4 × 10^3 mL^{-1} in Experimental Group I, 351.6 ± 15.4 × 10^3 mL^{-1} in Experimental Group II, and 340.0 ± 14.7 × 10^3 mL^{-1} in the control group. No significant differences were observed among treatments ($P > 0.05$).

Microscopic examination identified six protozoan genera commonly found in the rumen of grazing sheep, namely *Entodinium, Diplodinium, Epidinium, Isotricha, Ophryoscolex, and Dasytricha*. Among these, *Entodinium* was the dominant genus in all groups, accounting for approximately 57–61% of the total protozoal population.

The abundance of individual protozoan genera was similar among treatments. Counts of *Diplodinium, Epidinium, Isotricha, Ophryoscolex*, and *Dasytricha* showed only minor numerical variation and were not significantly affected by probiotic supplementation.

Table 3.

Total number and species composition of rumen protozoa in lambs at 60 days of age ($\times 10^3$ mL^{-1})

Differences between groups	Total number n=3	Protozoan types					
		Entodinium	*Diplodinium*	*Epidinium*	*Isotricha*	*Ophryoscolex*	*Dasytricha*
Group I	343.3±16.4	196.6±10.2	58.3±6.2	35.0 ±4.0	21.6±2.3	18.3±2.3	13.3±2.3
Group II	351.6±15.4	200.0±8.1	63.3±6.2	36.6±3.1	21.6±2.4	20.0±4.0	10.0±4.0
Control	340.0±14.7	206.6±20.5	53.3±6.2	33.3±4.7	21.6±6.2	16.6±2.3	8.3±2.3

Values are presented as mean ± standard deviation (SD). No significant differences among treatments were detected ($P > 0.05$).

Discussion

The present study evaluated the effects of a probiotic supplement containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* on rumen fermentation, blood glucose concentration, and rumen protozoal populations in lambs during the transition from milk to solid feed. The results demonstrated that probiotic supplementation increased the concentration of volatile fatty acids (VFAs), particularly acetate, propionate, and butyrate, and was associated with higher serum glucose concentrations during the weaning period. Volatile fatty acids are the primary products of microbial fermentation in the rumen and constitute the major energy source for ruminant animals. In the present study, lambs receiving the probiotic treatment in Experimental Group I exhibited the highest concentrations of total VFAs and the major fermentation products, including acetate, propionate, and butyrate. Although differences in total VFA concentration were not statistically significant, the numerical increase suggests that probiotic supplementation enhanced rumen fermentation activity. Similar effects have been reported in previous studies, where probiotic supplementation improved ruminal microbial activity and increased VFA production through stimulation of beneficial microbial populations [3,11].

Among the VFAs, propionate is particularly important because it serves as the primary precursor for hepatic gluconeogenesis in ruminants [1,10]. The higher propionate concentration observed in Experimental Group I was accompanied by significantly greater serum glucose concentrations at 45 days of age compared with the control group. This relationship suggests that enhanced ruminal fermentation may have increased the availability of gluconeogenic substrates, thereby supporting glucose synthesis during the critical transition period. Previous studies have similarly demonstrated that increased ruminal production of propionate contributes to improved glucose metabolism and energy status in young ruminants [1,10].

Blood glucose concentrations changed markedly during early postnatal development. The rapid increase observed during the first six hours after birth was followed by a decline during the subsequent 24–48 hours, reflecting the normal physiological adaptation of neonatal lambs to extrauterine life and milk consumption. At 45 days of age, however, probiotic-treated lambs maintained significantly higher serum glucose concentrations than the control group. This finding may indicate improved energy metabolism during the period when lambs increasingly depend on ruminal fermentation and gradually reduce their reliance on milk-derived nutrients. By 60 days of age, glucose concentrations were similar among treatments, suggesting that the metabolic advantage associated with probiotic supplementation was most pronounced during the active phase of rumen development.

Despite the observed increases in VFA production and serum glucose concentration, probiotic supplementation did not significantly affect the abundance or species composition of rumen protozoa. The dominant genera identified in all groups were *Entodinium*, *Diplodinium*, *Epidinium*, *Isotricha*, *Ophryoscolex*, and *Dasytricha*, which are characteristic of grazing sheep. The absence of significant differences among treatments suggests that the probiotic primarily influenced bacterial fermentation processes rather than protozoal population dynamics. This interpretation is consistent with previous reports indicating that probiotic supplementation can alter microbial activity and fermentation efficiency without necessarily changing protozoal abundance [11].

The improved fermentation characteristics observed in the probiotic-treated lambs may be associated with enhanced growth and activity of beneficial ruminal microorganisms, particularly cellulolytic and lactic acid-utilizing bacteria. Increased microbial activity can improve the degradation of plant carbohydrates and promote the production of fermentation end-products, especially propionate and butyrate. Consequently, greater availability of these metabolites may contribute to improved energy supply during the transition from milk to pasture-based feeding.

Overall, the findings of this study indicate that supplementation with a combined yeast–lactobacilli probiotic can support rumen development and carbohydrate metabolism in lambs during the weaning period. The positive responses observed in VFA production and serum glucose concentration suggest that probiotic supplementation may enhance metabolic adaptation during the transition from milk to solid feed, thereby supporting animal health and productivity under pasture-based production systems.

Conclusion

Supplementation with a probiotic containing *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* influenced rumen fermentation and glucose metabolism in lambs during the weaning period. Probiotic-treated lambs showed higher concentrations of major volatile fatty acids and significantly greater serum glucose concentrations at 45 days of age than control lambs. However, probiotic supplementation did not significantly affect rumen protozoal populations. These findings suggest that combined yeast–lactobacilli probiotics may support rumen development and metabolic adaptation during the transition from milk to solid feed.

Author's contribution

P.D and T.B conducted the experiments and prepared the manuscript. Ts.L. and B.S. performed the laboratory analyses and contributed to the statistical analysis of the data. U.D. and Z.M. contributed to the experimental design and the preparation of the literature review. D.l., J.S. and D.Sh. supervised the research, served as principal investigators of the project, and contributed to the overall study design, manuscript review, and revision. All authors reviewed and approved the final version of the manuscript.

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