Alfalfa silage inoculated with *Lactiplantibacillus plantarum* enhances milk fat content in dairy goats via modulating rumen microbial metabolism

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Introduction

Global demand for goat's milk products has risen due to their unique nutritional profile, particularly the health-beneficial milk fat components like PUFAs and CLA. Milk fat synthesis in goats depends on rumen microbial activity, which is modulated by dietary compounds, such as flavonoids. Studies show that flavonoids enhance rumen fermentation by promoting beneficial bacteria (e.g., Butyrivibrio) while inhibiting methane-producing microbes, ultimately improving milk fat yield and quelity. Alfalfa, a flavonoid-rich forage, sees increased flavonoids content during ensiling, especially when inoculated with *L. plantarum*. However, the long-term effects of flavonoid-enriched alfalfa silage on rumen microbiota and milk quality in dairy goats remain unexplored.

This study investigates how silage processing enhances alfalfa's nutritional value and elucidates its role in optimizing rumen metabolism and milk fat production, offering a sustainable strategy to improve livestock productivity.

Methods

Animals, management and experimental design

The animal experiment was conducted on a farm of Dingxi Jupencao husbandry Co., LTD (Dingxi, Gansu Province, China) from July to September in 2024. A total of twenty-two healthy mid-lactation Guanzhong dairy goats were used for the experiment , who were 2–3 years old with a mean body weight (BW) of 37.91±0.77 kg and milk yield of 0.61±0.01 kg/d. They were randomly assigned to 2 experimental groups and were offered diets containing 40% concentrate and 60% alfalfa silage inoculated with nothing (CK group) or inoculated with *L. plantarum* (treatment group, LP), which at an application rate of 1×10^5 colony-forming units/g of fresh weight. The goats were fed and milked twice a day at 06:00, 18:00, and received free access to water. Milking and recording daily milk yield before feeding.

Sample collection

Silage samples were collected after opening and the concentrate was collected weekly, both of which were immediately frozen at −20 °C. All silages and concentrates were manually mixed evenly for further determinations of nutritional composition, microbial community, and total flavonoid content. At the end of the experiment, milk samples were collected for the analysis of milk quelity, and rumen fluid were collected for the analysis of rumen volatile fatty acids (VFAs), microbiome and metabolome.

Statistical analysis

For phenotypic parameters of alfalfa silage and dairy goats, differences between groups were analysed using Student's t-tests, with statistical significance set at $P < 0.05$. Metagenomic comparisons between control (CK) and *L. plantarum*-treated (LP) groups were performed using the non-parametric Wilcoxon rank-sum test with Benjamini-Hochberg false discovery rate (FDR) correction ($q < 0.05$). For metabolomic profiling, metabolites with $P<0.05$ in Students' t-test and VIP (Variable Importance in theProjection) >1 in the first principal component of the OPLS-DA model were identified as differential metabolites.

Results

Butyrate was not detected in either group of silage and, LP silage exhibited significantly higher lactic acid and acetic acid content ($P<0.001$). The dry matter (DM) of LP silage was not adversely affected ($P>0.05$), while the neutral detergent fibers (NDF) and acidic detergent fibers (ADF) of LP silage was significantly reduced ($P<0.05$) compared to CK silage. This resulted in the production of more water-soluble carbohydrates (WSC) in LP silage ($P<0.05$). Moreover, compared to the silage in the CK group, the crude protein (CP) content was significantly higher in the LP group ($P<0.05$, Table 1).

The ensiling process promotes the release of flavonoid compounds from plant cell walls. Compared with the raw forage materials, the flavonoid content in silage shows a significant increase ($P<0.05$). Specifically in alfalfa silage, the LP group demonstrated significantly higher flavonoid content than the CK group ($P<0.05$, Figure 1).

Table 1 Fermentation characteristics and chemical composition of ensiled alfalfa

	CK	LP	*P*-value
Organic acids			
pH	4.90±0.01	4.81±0.05	>0.05
Lactate (g/kg, DM)	52.49±0.04	57.21±0.07	<0.001
Acetate (g/kg, DM)	50.80±0.01	65.61±0.12	<0.001
Propionate (g/kg, DM)	11.56±0.45	10.11±0.45	0.086
Butyrate (g/kg, DM)	NA	NA	-
Chemical components			
DM (g/kg, FW)	345.80±14.99	339.62±5.42	>0.05
NDF (g/kg, DM)	383.14±4.18	364.28±1.37	0.012
ADF (g/kg, DM)	280.88±2.63	265.05±0.24	0.004
CP (g/kg, DM)	177.24±0.25	181.61±1.39	0.021
NH_3-N (g/kg, DM)	16.13±0.56	14.89±0.39	>0.05
WSC (g/kg, DM)	8.93±0.62	12.49±0.91	0.032

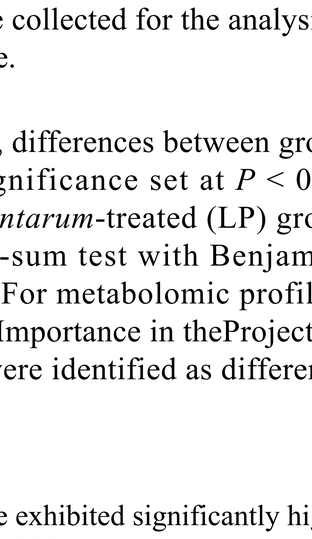

Figure 1. Determination of total flavonoid content in alfalfa samples

Results

Compared to the CK group, the total VFAs production in the rumen of dairy goats in the LP group increased significantly ($P<0.05$, Table 2). Specifically, the production of acetate, propionate, isobutyrate, and isovalerate of the LP dairy goats was significantly higher ($P<0.05$).

Feeding LP silage had no significant effect on milk yield in dairy goats ($P>0.05$). Compared to the CK group, the milk fat content in the LP group dariy goats increased significantly ($P<0.05$).

Table 2 Rumen fermentation parameters of dairy goats

	CK	LP	*P*-value
Total VFAs (mmol/L)	53.23±3.14	68.15±3.22	0.004
Acetate (mmol/L)	33.66±2.80	44.61±2.47	0.009
Propionate (mmol/L)	7.54±0.37	10.43±0.44	<0.001
A:P	4.39±0.22	4.28±0.15	0.69
Butyrate (mmol/L)	5.31±0.37	5.87±0.38	0.304
Isobutyrate (mmol/L)	1.06±0.03	1.30±0.05	0.001
Isovalerate (mmol/L)	1.05±0.03	1.59±0.08	<0.001
MCP (μg/mL)	80.25±6.58	77.44±6.32	0.76

Table 3 Analysis of milk production performance of dairy goats

Items	CK	LP	*P*-value
Milk yields			
Yields (g/d)	261.07±12.05	289.16±13.26	>0.05
Milk composition (%)			
Fat	2.88±0.15	3.45±0.20	0.046
Protein	3.92±0.23	4.18±0.23	>0.05
Lactose	4.33±0.05	4.48±0.09	>0.05
Total solids	11.53±0.37	11.88±0.70	>0.05

No significant differences were observed in α-diversity (Shannon index) of the rumen microbial communities of the goats between the CK and LP groups (Figure 2a), while β-diversity analysis revealed a significant microbial separation (Figure 2b). The most of differentially abundant bacteria genera were significantly upregulated in the LP group ($P<0.05$; Figure 2c), and all differentially abundant eukaryotic microbes among the Top30 taxa were significantly downregulated in the LP group ($P<0.05$; Figure 4d). Furthermore, dairy goats fed with flavonoid-enriched LP-group alfalfa silage exhibited significant upregulation of flavonoid degradation pathways (ko00946) in their rumen microbiota, indicating a microbial metabolic response to high flavonoid intake ($P<0.05$; Figure 4e).

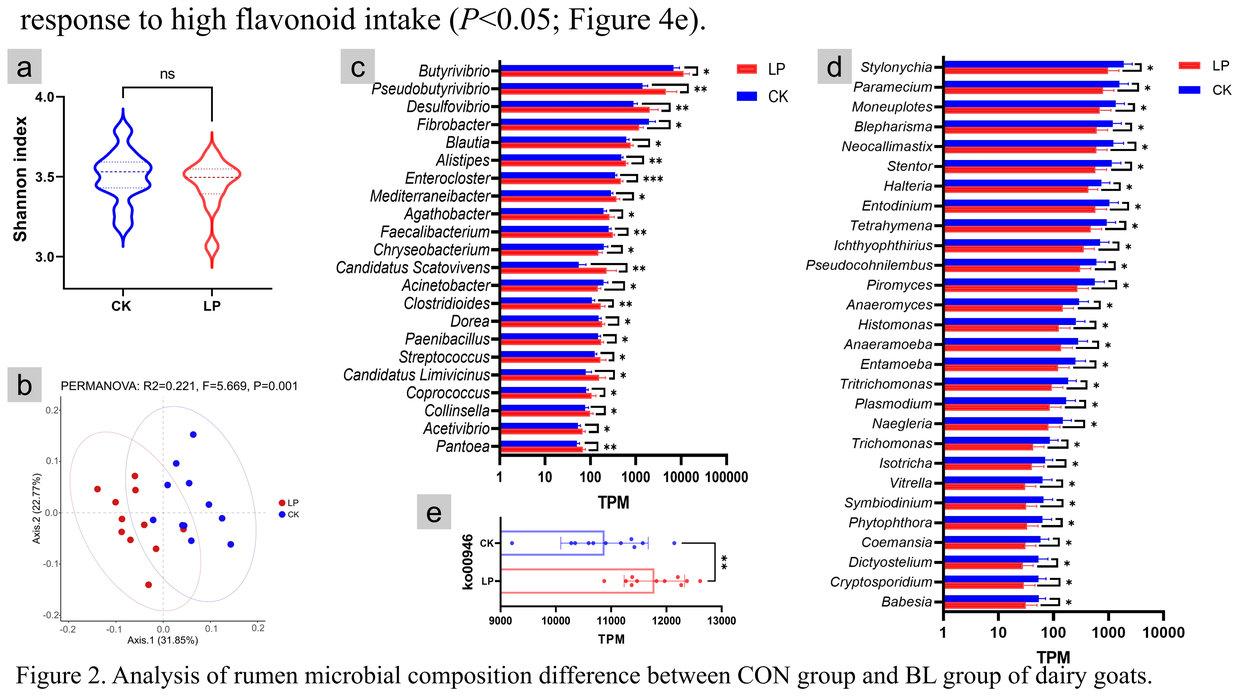

Figure 2. Analysis of rumen microbial composition difference between CON group and BL group of dairy goats.

Metagenomic analysis revealed systemic upregulation of KEGG metabolic modules (e.g., carbon/amino acid metabolism) in LP-group rumen microbiomes (Figure 3a). CAZyme profiling identified 26/30 differentially abundant enzymes (16GHs/6GTs/3CEs/1PL) as ATCC-enriched (Figure 3b). Integrated modeling (Figure 3c) demonstrated that rumen microbes utilize CAZymes to degrade dietary fiber into monosaccharides, which are metabolized into volatile fatty acids (VFAs). These VFAs are absorbed into circulation and subsequently contribute to milk fat synthesis in mammary epithelial cells.

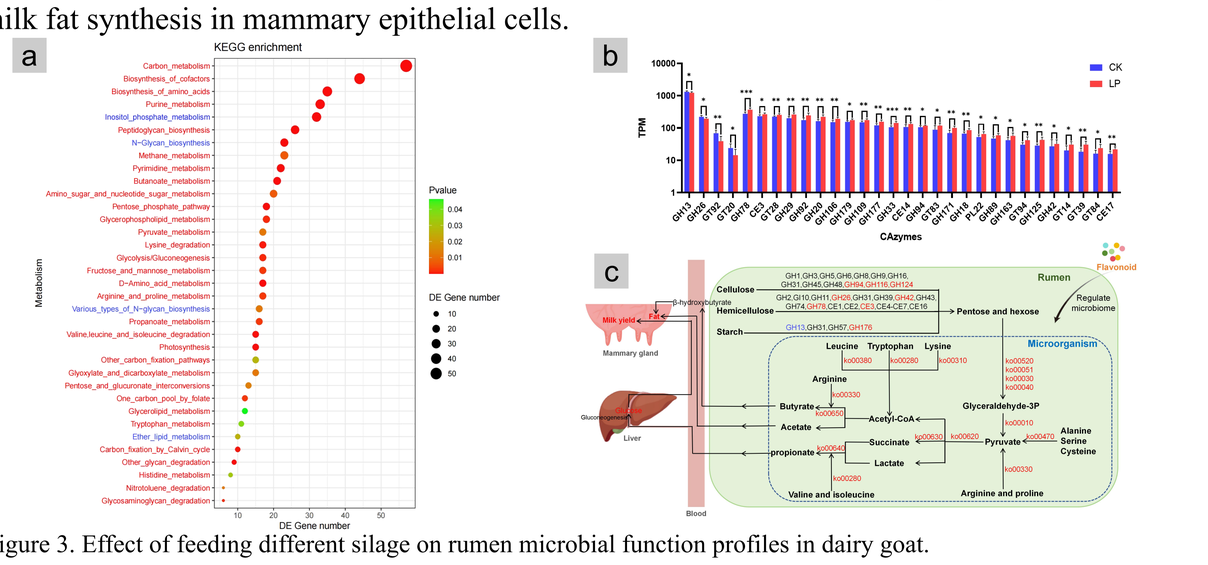

Figure 3. Effect of feeding different silage on rumen microbial function profiles in dairy goat.

Conclusions

Inoculation with *L. plantarum* significantly improved alfalfa silage quality and boosted total flavonoid content. Feeding this flavonoid-enriched silage to dairy goats induced marked changes in their rumen microbiota. Compared with the CK group, the LP group exhibited increased bacterial abundance alongside decreased eukaryotic microorganism populations. These microbial community shifts were accompanied by enhanced energy metabolism pathways, which elevated the production of milk fat precursors and ultimately led to higher milk fat content in the LP-group goats.