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Effects of Mulberry Branch and Leaves Silage on Microbial Community, Rumen Fermentation Characteristics, and Milk Yield in Lactating Dairy Cows

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Abstract: This study investigated the effects of mulberry branch and leaves (MBL) silage on milk yield, ruminal fermentation, and bacteria composition in dairy cows. Thirty-six mid-lactation cows were selected and randomly allocated into three groups. The control group (C) was fed on a total mixed ratio (TMR) diet, while the experimental groups were fed on TMR supplemented with 5% (L) and 10% (H) MBL silage. The experiment lasted for eight weeks, including two weeks of adaption. The results showed that Group H had an increased milk yield, milk fat content ($p < 0.05$), and 4% feed conversion ratio ($p = 0.10$). In addition, rumen propionic acid was significantly increased ($p < 0.05$), while acetate/propionate was significantly decreased ($p < 0.05$) in the high MBL silage group. The microbiome analysis showed that Bacteroides, Firmicutes, and Proteobacteria were the predominant phyla. Compared with Group C, the abundance of Bacteroides was significantly decreased ($p < 0.01$), while the Firmicutes and Proteobacteria were increased but not significantly different in Groups L and H. *Prevotella* was significantly decreased ($p < 0.05$) in the MBL silage groups, and *Succinivibrionaceae_UCG-001* was increased in Group H. The correlation analysis showed that eight bacterial species belonging to Firmicutes were positively correlated with propionic acid. However, four bacterial species belonging to the Bacteroides group were negatively correlated with propionic acid. In conclusion, feed supplementation with about 5–10% of MBL silage could modulate the rumen microbiota and fermentation, and increase the abundance of fiber-digesting, propionic acid synthesis and milk fat-related microorganisms, thus improving milk yield in dairy cows.

Keywords: mulberry branches and leaves; milk fat; rumen fermentation; microbiome; dairy cows

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1. Introduction

There is an increased shortage of forage in China due to increased animal production. Therefore, there is an urgent need to look for inexpensive, local feed sources [1]. Mulberry trees are fast-growing deciduous plants of the Moraceae family that are widely distributed in China [2]. Mulberry trees are multipurpose, and their fruits are used as food and for making wine, while the leaves are used as fodder due to the high biomass, crude protein content, and digestibility [3]. A previous study has shown that the tract and rumen digestibility of mulberry trees were in some cases comparable to alfalfa hay [4]. Meanwhile, the addition of mulberry leaves to feeds of ruminant animals reduces the need for expensive protein supplements [5,6]. Therefore, research is needed to evaluate the effects of feeding animals with mulberry.

The composition of gastrointestinal bacteria is relevant to the production performance of host animals [7]. Previous studies also showed that providing ensiled mulberry silage to dairy cows improved the immune and antioxidant function [8,9]. On the other hand, the application of mulberry leaves silage has shown that it could be used in finishing steer

diets without affecting the production performance [10]. Dietary changes usually affect the rumen microbiome. The rumen microbiome in dairy cows plays an essential role in animal health and performance [11,12]. Hence, we hypothesize that the partial replacement of forage and concentrate with 5% or 10% mulberry branch and leaves silage (MBL) affects the ruminal microbiome. Therefore, this study evaluated the effects of diets supplemented with MBL on the milk yield, rumen fermentation characteristics, and microbial community in dairy cows.

2. Materials and Methods

2.1. Diet and Animal Management

Mulberry trees were cultivated in the fields of Yinxiang Weiye Group Co., Ltd. (Shandong, China). The mulberry leaves and branches (MBL, GuiSang 62) were harvested at 0.8–1.0 m height (late growing period) and ensiled using 10% maize flour after chopping them to 2~3 cm by CLAAS (JAGUAR 960, Germany). After that, they were sprayed with a fermenting agent (Lactobacillus $\geq 2.0 \times 10^{10}$ CFU/g, Inner Mongolia Heimei Kesheng Biotechnology Co., Ltd., Inner Mongolia, China). The MBL silage was fermented for 45 days before being used for the feeding experiment.

A total of 36 mid-lactation Holstein dairy cows within the initial days in milk (DIM) = 159.41 ± 34.5 d, milk yield = 38.26 ± 3.18 kg, body weight = 665.17 ± 65.39 kg were randomly allocated into three groups (n = 12). The control group (Group C) was fed on a total mixed ratio (TMR), while the other two treatment groups were fed on TMR supplemented with 5% (Group L) and 10% (Group H) MBL silage. The three different TMRs were adjusted as isonitrogenous and isoenergetic diets, as shown in Table 1. Feeding and milking were carried out three times per day (07:00, 14:00 and 22:00). The experiment lasted eight weeks, including the first two weeks of adaptation and six weeks of the formal period.

Table 1. The ingredients and chemical composition of the experimental diets.

Item	Treatment [1]		
	C	L	H
	Ingredient (% of DM)		
Alfalfa hay	15.96	13.69	11.81
Oat hay	3.73	5.20	7.05
Mulberry silage	0	5.31	10.47
Corn silage	22.68	18.32	13.30
Cotton seed	5.47	5.45	5.44
Beet pulp	4.82	4.81	4.80
Maize meal	10.64	10.61	10.59
Flaked corn	14.19	14.15	14.12
Soybean meal	13.28	13.24	13.21
Rapeseed meal	2.87	2.86	2.86
Extruded soybean	1.12	1.12	1.11
DDGS [2]	0.74	0.74	0.74
Concentrate [3]	4.5	4.5	4.5
	Chemical composition		
DM, % of feed	48.29	48.61	48.50
CP, % of DM	16.73	16.86	16.88
NDF, % of DM	29.55	28.60	28.29
ADF, % of DM	17.33	17.12	17.06
EE, % of DM	5.59	5.34	5.27
Ash, % of DM	7.52	7.30	7.45
NE_L [4], Mcal/kg DM	1.77	1.78	1.78

[1] Group C was fed on non-mulberry silage diets; feeds in Groups L and H were supplemented with 5% and 10% mulberry silage diets, respectively. [2] DDGS: distillers dried grains with solubles. [3] The concentrate contained 26.89% fatty powder, 11.78% $CaHPO_4$, 20.67% Na_2HCO_3, 9.11% stone powder, 10.89% salt, 1.33% rumen-protected methionine, 9.78% yeast culture, 5.33% MgO and 4.22% premix. The premix (per kg of DM) contained Vitamin A 73 KIU, Vitamin E 1200 IU, Vitamin D 17 KIU, Se 20 mg, Cu 255 mg, Co 60 mg, Fe 40 mg, Zn 1635 mg, I 40 mg, and Mn 708 mg. [4] NE_L: net energy for lactation.

2.2. Samples Collection and Chemical Analysis

The dry matter intake (DMI) was calculated daily by the TMR and refusals every day. The dietary samples of MBL silage, TMRs, and residual samples were collected every two weeks during the experimental period. The samples were dried in an oven at 65 °C for 48 h and kept at room temperature for 24 h. After that, the samples were ground to pass through a 40-mesh sieve. Composite samples of forages and concentrates were analyzed for dry matter (DM), crude protein (CP), and ether extract (EE) [13]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined using Ankom A200 apparatus (Ankom Technology, Macedon, NY, USA) with heat-stable amylase and sodium sulfite (Fisher Scientific, Waltham, MA, USA). They were expressed inclusive of residual ash [14].

The chemical composition and fermentation quality of MBL silage were determined before feeding and are shown in Table 2. The milk samples (50 mL) were collected from each cow every sixth day of each week. The milk samples were collected three times per day during the milking time at a ratio of 4:3:3, as previously described (Wang et al., 2014). Potassium dichromate 0.6 mg/mL was added to the milk samples for preservation. After that, the milk samples were stored at 4 °C while awaiting further analysis. The milk components, including protein, fat, lactose, somatic cell counts (SCC), and milk urea nitrogen (MUN), were determined using an automatic ultrasonic milk composition analyzer (Bentley Instruments, Chaska, MN, USA).

Table 2. Chemical composition and fermentation quality of the mulberry branch and leaves silage.

Items [1]	Proportion, %	Items	Proportion, %
pH	4.89	Lactic acid (g/kg DM)	15.04
DM	28.93	Acetate (g/kg DM)	10.58
CP, % of DM	14.95	Propionate (g/kg DM)	2.75
EE, % of DM	5.40	Butyrate (g/kg DM)	-
ADF, % of DM	25.70	NH_3-N (g/kg Total N)	25.01
NDF, % of DM	37.18		
Ash, % of DM	9.10		
Ca, % of DM	0.70		
P, % of DM	0.27		

[1] DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber.

An oral stomach tube was used to collect the rumen fluid 3 h after morning feeding on the last day of the experiment [15]. The initial 150 mL rumen fluid was discarded. The pH value was determined immediately. Samples were collected from all cows. Every sample was aliquoted into three tubes. Liquid nitrogen was immediately added into one tube and stored for DNA extraction. The other sample was kept for the determination of NH_3-N, while the other sample was used for the determination of VFA [16,17]. The DNA extraction and sequencing of rumen fluid were determined according to our previous study [12].

2.3. Statistical Analysis

The ruminal fermentation characteristics were analyzed using SAS software (version 9.2, mixed model). The least squares means were calculated and separated by the PDIFF option in SAS. p-value ≤ 0.05 was considered statistically significant. Further, trends were declared significant at $0.05 < p \leq 0.10$.

The partial least squares discriminant analysis (PLS-DA), principal component analysis (PCA), and metabolic pathways spreading and enrichment analysis were analyzed using MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/, accessed on 12 October 2020). Correlation analyses between ruminal fermentation parameters and the top 25 genera of microbiota were determined using Spearman's correlation, and connections with $p < 0.05$ and $r > 0.54$ were retained.

3. Results

3.1. Milk Yield and Composition

As shown in Table 3, the milk yield and fat content were significantly higher in Group H than in Groups C and L ($p < 0.05$). In addition, the lactose yield was significantly higher in Groups C and H than in Group L ($p < 0.05$). Further, Group C had a significantly lower level of MUN than Groups L and H ($p < 0.05$). There were no differences in the dry matter intake (DMI), protein, and SCC content between the groups. However, the 4% feed conversion ratio (FCR) was increased in Group H ($p = 0.10$).

Table 3. Effects of feeding mulberry silage on lactation performance of dairy cows.

Item [1]	Treatment [2]			SEM	*p* Value		
	C	L	H		Treat	Week	T × W
DMI, kg/d	22.90	22.82	22.68	0.08	0.52	-	-
			Milk yield, kg/d				
Raw	36.34 ^{b}	36.54 ^{b}	38.21 ^{a}	1.34	0.27	<0.01	0.51
4% FCM	36.86 ^{b}	37.31 ^{b}	39.10 ^{a}	0.72	0.36	0.31	0.79
ECM	41.57 ^{b}	42.05 ^{b}	44.19 ^{a}	0.74	0.15	<0.01	0.71
Protein	1.30	1.31	1.39	0.03	0.13	0.04	0.65
Fat	1.49 ^{b}	1.51 ^{b}	1.59 ^{a}	0.03	0.05	<0.01	0.15
Lactose	1.79 ^{a}	1.73 ^{b}	1.84 ^{a}	0.04	0.12	<0.01	0.03
			Milk composition, %				
Protein	3.55	3.65	3.63	0.04	0.14	<0.01	0.93
Fat	4.10 ^{b}	4.14 ^{a}	4.16 ^{a}	0.09	0.66	<0.01	0.03
Lactose	4.76	4.79	4.78	0.26	0.66	<0.01	0.92
SCC, 10^3/mL	152.47	146.86	129.22	17.96	0.68	0.75	0.61
MUN, mg/dL	12.44 ^{b}	12.81 ^{a}	12.85 ^{a}	0.21	0.32	<0.01	<0.01
4% FCR	1.61 ^{b}	1.63 ^{b}	1.72 ^{a}	0.03	0.10	<0.01	0.24

^{a,b} Mean values within the treatment groups with different superscripts are significantly different ($p < 0.05$). [1] FCM: fat-corrected milk; ECM: energy-corrected milk; FCR: feed conversion ratio. ECM (kg/day) = 0.3246 × milk yield (kg/d) + 13.86 × fat yield (kg/d) + 7.04 × protein yield (kg/d). 4% FCM (kg/day) = 0.4 × milk yield (kg/d) + 15 × fat yield (kg/d). 4% FCR = 4% FCM/DMI. [2] Group C was fed on non-mulberry silage diets; feeds for Groups L and H were supplemented with 5 and 10% mulberry silage diets, respectively.

3.2. Rumen Fermentation Characteristics

Table 4 shows the rumen fermentation characteristics. There were no differences in the pH, ammonia nitrogen, total volatile acid, acetic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid concentrations in the rumen fluid. The concentration of acetic acid showed a decreasing trend ($p = 0.08$) with an increase in the MBL silage concentration. However, the propionic acid concentration increased significantly ($p < 0.05$) with an increase in the MBL silage concentration. In addition, the acetate/propionate ratio was significantly lower in Groups L and H than in Group C ($p < 0.05$).

Table 4. Effects of feeding MBL silage on the rumen fermentation characteristics in dairy cows.

Item	Treatment			SEM	*p* Value
	C	L	H		
pH	6.31	6.19	6.26	0.03	0.29
NH_3-H, mg/dL	11.92	12.23	12.33	0.43	0.92
		VFA, mmol/L			
Total VFA	134.13	131.57	133.50	1.66	0.57
Acetate (A)	85.42 ^{a}	81.57 ^{b}	80.69 ^{b}	1.05	0.08
Propionate (P)	27.74 ^{b}	30.56 ^{a}	30.96 ^{a}	0.58	0.04
Butyrate	17.23	17.82	17.33	0.33	0.75
Isobutyrate	1.85	1.66	1.81	0.06	0.39
Valerate	1.99	1.88	1.76	0.05	0.15

Table 4. *Cont.*

Item	Treatment			SEM	*p* Value
	C	L	H		
Isovalerate	1.17	1.17	1.15	0.03	0.95
A/P	2.89 [a]	2.61 [b]	2.64 [b]	0.05	0.02

[a,b] Least squares means within a row with different superscripts differ significantly ($p < 0.05$).

3.3. Ruminal Bacterial Communities

A total of 2115 OTUs were identified in the three groups. Among them, 1548 OTUs were found in all groups, accounting for 73.19% of the total OTUs (Figure 1A). Based on the PCoA graph (Figure 1B), the clouds derived from the three groups were separated from each other. There were no significant differences in all groups based on Sobs, ACE, Chao, Simpson, Shannon, and Coverage (Table 5). Besides, six bacterial phyla (Bacteroidota, Firmicutes, Proteobacteria, Actinobacteria, Patescibacteria, and Spirochaetes) were identified in the rumen fluid with a relatively high abundance (>1%) (Figure 2A). Further, a total of 247 bacterial taxa were identified at the genus level. *Prevotella*, *Succinivibrionaceae_UCG-001*, *norank_f_Muribaculaceae*, *NK4A214_group*, *Succiniclasticum*, *Lachnospiraceae_NK3A20_group*, *Ruminococcus*, *norank_f_norank_o_Clostridia_UCG-014*, *Rikenellaceae_RC9_gut_group*, and *Christensenellaceae_R-7_group* were the top ten most abundant bacteria at the genus level (Figure 2B).

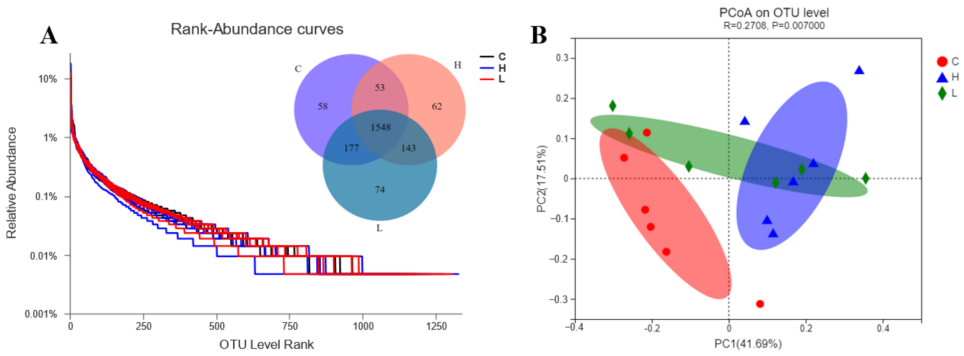

Figure 1. The rank-abundance curves are derived from the microbial OTU level. (**A**) The Venn graph illustrates the overlap of microbial OTUs among the treatment groups at a 3% dissimilarity level. (**B**) PCoA analysis of taxonomical classifications in the control (C) and mulberry silage (L, H) groups.

Table 5. The alpha diversity of the ruminal bacteria community in dairy cows.

Item	Treatments [1]			SEM	*p* Value
	C	L	H		
Sob	1189.67	1193.83	1146.17	23.83	0.69
Ace	1466.43	1481.10	1440.86	24.00	0.81
Chao	1461.27	1486.94	1454.22	26.60	0.88
Shannon	5.65	5.64	5.45	0.07	0.46
Simpson	0.02	0.02	0.02	<0.01	0.45
Coverage	0.99	0.98	0.99	<0.01	0.24

[1] Group C was fed on non-mulberry silage diets; Groups L and H were fed on feeds supplemented with 5 and 10% mulberry silage diets, respectively.

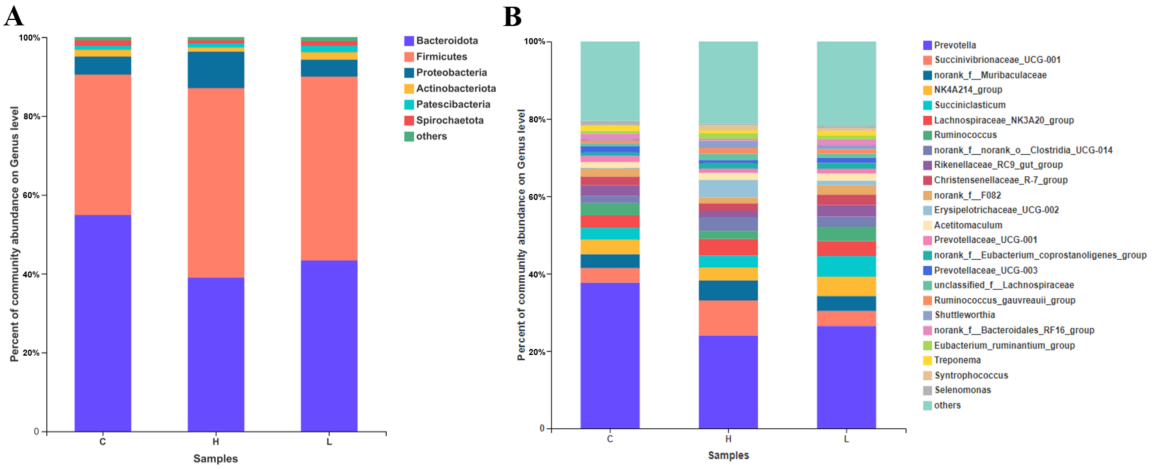

Figure 2. The distribution of rumen fluid bacterial taxa under (**A**) phylum and (**B**) genus levels between the treatments.

A total of 30 clades were identified as biomarkers among the three groups, of which nine were in Group C, 17 were in Group H, and four were in Group L (Figure 3). The nine clades identified in Group C included four genera belonging to Bacteroidota (*Prevotellaceae_UCG_003*, *Prevotellaceae_UCG_004*, *norank_f_251_o5*, and *Rikenellaceae_U29_B03*), two genera belonging to Proteobacteria (*Succinimonas* and *Anaerobiospirillum*), and one genus belonging to Elusimicrobiota (*Endomicrobium*), Actinobacteriota (*Corynebacterium*), and Desulfobacterota (*Mailhella*). The 17 clades identified in Group H included two genera belonging to Bacteroidota (*Rikenellaceae_SP3_e08* and *unclassified_o_Bacteroidales*), 14 genera belonging to Firmicutes (*Lachnoclostridium*, *Shuttleworthia*, *Syntrophococcus*, *unclassified_f_Lachnospiraceae*, *Ruminococcus_gauvreauii_group*, *Eubacterium_ruminantium_group*, *norank_f _Selenomonadaceae*, *Dialister*, *Eubacterium_eligens_group*, *Coprococcus*, *Monoglobus*, *Lactobacillus*, *Catonella*, and *Lachnospiraceae_XPB1014_group*) and one genus belonging to the Desulfobacterota (*Desulfovibrio*). The four clades identified in Group L included three genera belonging to Firmicutes (*Saccharofermentans*, *unclassified_p_Firmicutes*, and *Lachnospiraceae_FE2018_group*) and one genus belonging to Actinobacteriota (*unclassified_f_Atopobiaceae*).

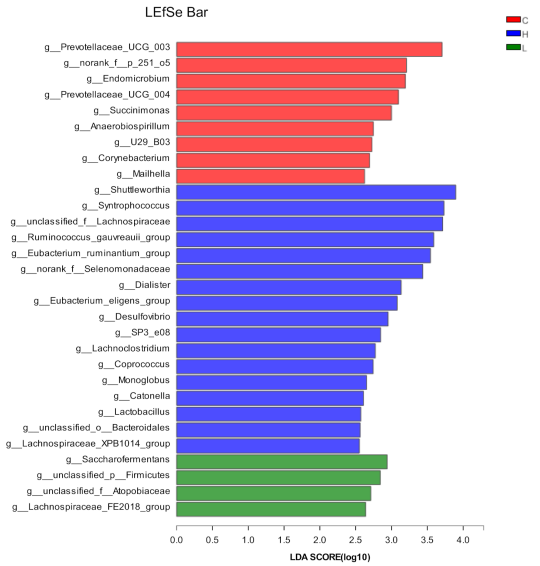

Figure 3. The abundance of rumen fluid bacteria in the control (C) and mulberry silage (L, H) groups. Red, blue and green bars represent the bacterial community of the control group and Groups L and H with a significantly higher abundance compared with that in other groups, respectively.

3.4. Correlation Analysis between the Ruminal Microbiome and Fermentation Parameters

The bacterial communities were mainly correlated with the rumen propionate concentration and the acetate/propionate ratio (Figure 4). In detail, *Ruminococcus*, *norank_f_Bacteroidales_RF16_group*, *NK4A214_group*, *Prevotellaceae_UCG-003*, and *Rikenellaceae_RC9_gut_group* were positively correlated with the acetate/propionate ratio ($p < 0.05$). However, *Shuttleworthia*, *Erysipelotrichaceae_UCG-002*, *Syntrophococcus*, *norank_f_norank_o_Clostridia_UCG-014*, *Eubacterium_ruminantium_group*, *unclassified_f_Lachnospiraceae*, *Ruminococcus_gauvreauii_group*, and *Succinivibrionaceae_UCG-001* were negatively correlated with the acetate/propionate ratio ($p < 0.05$). In addition, *Succinivibrionaceae_UCG-001*, *norank_f_norank_o_Clostridia_UCG-014*, *Erysipelotrichaceae_UCG-002*, *Shuttleworthia*, *unclassified_f_Lachnospiraceae*, *Ruminococcus_gauvreauii_group*, *Eubacterium_ruminantium_group*, and *Syntrophococcus* were positively correlated with propionate ($p < 0.05$). However, *Rikenellaceae_RC9_gut_group*, *norank_f_Bacteroidales_RF16_group* and *Prevotellaceae_UCG-003* were negatively correlated with propionate ($p < 0.05$).

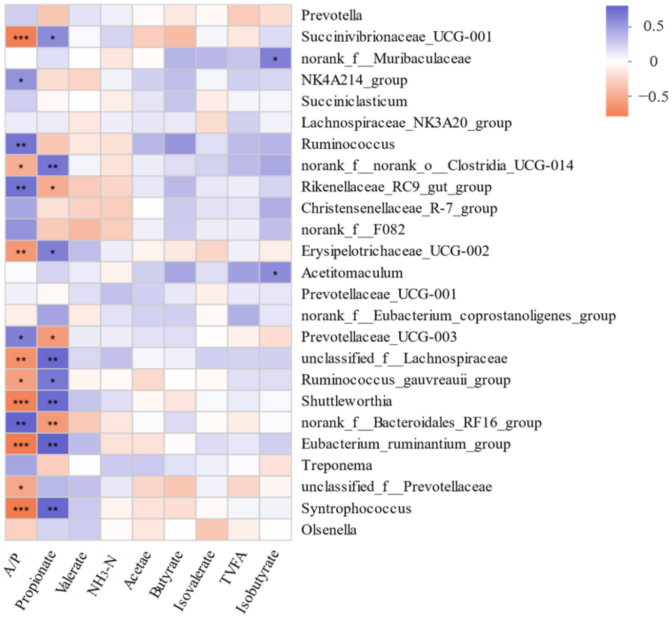

Figure 4. Correlation analyses between the fermentation characteristics and top 25 rumen bacterial genera. The blue squares show the positive correlations, while orange squares show the negative correlations. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. NH_3-N = ammonia nitrogen, A/P = the ratio of acetate to propionate, TVFA = total volatile fatty acid.

4. Discussion

Milk production in dairy cows mainly depends on the feed intake and quality, with the quality of roughage, green feed and dietary protein being the main contributing factors. In this study, although the CP and lactic acid of MBL silage was relatively lower than that of mulberry leaves silage, the quality was comparable to corn silage [9]. This means that it could be an available resource for dairy cows. There were no significant differences in the dry matter intake between the groups. However, Group H showed an increased milk production. A previous study showed that fermented mulberry leaves had significantly increased levels of bioactive components, CP and EE, and that the crude fiber content and anti-nutritional factors were significantly decreased [18]. Ensiling MBL could reduce the levels of lignin in the branches and increase the levels of soluble reducing sugars, leading to a higher rate of degradation in the rumen. Therefore, we hypothesized that the improved milk production was due to the enhanced digestibility of MBL in the rumen.

Milk fat can be synthesized de novo from acetic acid and β-hydroxybutyric acid produced during rumen fermentation (40–45%) or could be directly obtained from feeds (55–60%) [19,20]. Previous studies showed that plant flavonoids could promote the devel-

opment of mammary glands, improve milk quality, and modulate the ruminal microbiome and fermentation [21,22]. In addition, fatty acids such as palmitic acid and octadecenoic acid could promote the synthesis of milk fat [23]. The present study showed that increasing the concentration of MBL silage resulted in a significant increase in the milk fat yield, attributed to the effect of flavonoids in MBL silage on rumen microorganisms [22].

Bacteroidota, Firmicutes and Proteobacteria were shown to be the dominant phyla in the rumen, consistent with a previous study [24]. Bacteria of the Bacteroidota phylum are mainly considered degraders of protein and nonstructural polysaccharides [25]. *Prevotella* belongs to the phylum Bacteroidetes, which can degrade and utilize structural polysaccharides in plants such as pectin, starch and xylan. However, they have to be co-cultivated with cellulolytic bacterium [26]. *Rumenococcus* and *Laurespirillum* are Firmicutes bacteria that degrade fiber. The decomposition of fiber can affect the function of carbohydrate enzymes [27]. In addition, Proteobacteria are involved in the formation of biofilms and the digestion of soluble carbohydrates [28]. In this study, there were no significant differences in the composition of the dominant bacteria phyla between the groups, and this was consistent with a previous study on finishing steers [10]. However, the proportions of the different bacteria phyla between the groups showed large differences. There was a significant decrease in the abundance of Bacteroides and a significant increase in the abundance of Proteobacteria in the group fed on high MBL silage.

A previous study showed that MBL alkaloids had an inhibitory effect on α-glucosidase (the key enzyme involved in carbohydrate digestion) [29]. It is speculated that the alkaloids in MBL reduce the body's absorption and utilization of carbohydrate. Thus, the available glycogen substrates for *Bacillus* and *Prevotella* are reduced, which in turn leads to a decrease of the abundance. In this study, the LDA graph showed that the abundance of Lachnospiraceae (*Lachnospiraceae_NK3A20_group*) and Ruminococcaceae (*Ruminococcaceae_NK4A214_group*) was significantly higher than in the control group, suggesting that the MBL silage led to an increase in the abundance of rumen fiber-degrading bacteria. The ratio of the Firmicutes and Bacteroides *Desulfovibrio* (Proteobacteria), *Lactobacillus*, *Laospirillaceae*, *Eubacteria* and *Microbacteria* (Firmicutes) were shown to be positively correlated with the milk fat yield. However, the abundance of *Prevotella* was shown to be negatively correlated with the milk fat yield [30]. In this study, the ratio of Firmicutes and Bacteroides was shown to be increased in the high MBL group. However, *Prevotella* was significantly decreased in the high MBL group. The LDA graph showed that the *Lacetospiraceae*, *Eubacteria*, *Microbacteria*, *Lactobacillus* and *Desulfovibrio* in Group H were significantly higher than in Group C. Therefore, the MBL silage significantly increased the abundance of rumen microorganisms related to milk fat synthesis, thereby increasing the milk fat yield in dairy cows.

In this study, *Succinivibrionaceae_UCG-001*, belonging to Proteobacteria, was the second most abundant bacteria out of all the samples. A previous study showed that the feed efficiency was positively correlated to the abundance of *Succinivibrio* and *Enterococcus faecalis* (Firmicutes). *Succinivibrio* can convert succinic acid into propionic acid, thus increasing the ruminal levels of propionic acid and consequently increasing the lactose and milk yield [31]. *Succinivibrio* and *Enterococcus faecalis* had a higher abundance in Group H than in Groups C and L. Further, the correlation analysis showed that *Succinivibrionaceae_UCG-001* was positively correlated with propionic acid levels. This explains the increase in the ruminal propionic acid levels and feed efficiency.

The correlation analysis between rumen fermentation and microorganisms showed that propionic acid was positively correlated with Firmicutes bacteria (*norank_f_norank_o_Clostridia_UCG-014*, *unclassified_f_Lachnospiraceae*, *Eubacterium_ruminantium_group*, *Shuttleworthia*, *Syntrophococcus*, *Succinivibrionaceae_UCG-001*, *Ruminococcus_gauvreauii_group* and *Erysipelotrichaceae_UCG-002*) in the high MBL silage group. However, propionic acid was negatively correlated with Bacteroides bacteria (*norank_f_Bacteroidales_RF16_group*, *Selenomonas*, *Rikenellaceae_RC9_gut_group* and *Prevotellaceae_UCG-003*) in Group C. These results suggest that MBL silage was associated with an increased abundance of propionic acid synthesizing microorganisms of the phylum Firmicutes in the rumen.

5. Conclusions

MBL silage can be used as an alternative feed source in lactating dairy cows. Further, feed supplementation with about 5% to 10% of MBL silage could modulate the rumen microbiota and fermentation, and increase the abundance of fiber-digesting, propionic acid synthesis and milk fat-related microorganisms, consequently improving milk yield in dairy cows.

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