Effects of Cellulase, Lactobacillus plantarum, and Sucrose on Fermentation Parameters, Chemical Composition, and Bacterial Community of Hybrid Pennisetum Silage

Haoming Xiong, Yanchen Zhu, Zhiying Wen, Guangbin Liu, Yongqing Guo * and Baoli Sun *

Abstract: Hybrid Pennisetum (HP) is a perennial herb with a high yield and high quality, which makes it valuable for research as feed for herbivores. In order to make better use of hybrid Pennisetum as feed, this study studied the effects of cellulase (CE), Lactobacillus plantarum (LP), sucrose (SU), and their mixtures on fermentation parameters, chemical composition, and the bacterial community of hybrid Pennisetum silage. The experiment was divided into 7 treatments, silage treatment, and its abbreviations: CON (control group), CE (100 U/g FM cellulase), LP (1 × 10^6 cfu/g FM Lactobacillus plantarum), SU (1% FM sucrose), CE+LP (100 U/g FM cellulase + 1 × 10^6 cfu/g FM Lactobacillus plantarum), CE+SU (100 U/g FM cellulase + 1% FM sucrose), and LP+SU (1 × 10^6 cfu/g FM Lactobacillus plantarum + 1% FM sucrose). The silage bag was opened on the 60th day of ensilage for subsequent determination. The addition of CE and LP increased lactic acid content (p > 0.05). The pH and acetic acid of CE and LP were lower than CON (p < 0.05), and the crude protein content of CE was higher than CON. Cellulase and Lactobacillus plantarum can improve the quality of hybrid Pennisetum silage. Compared with Lactobacillus plantarum and sucrose, cellulase has better nutrition preservation and the ability to inhibit protein hydrolysis. 16S rRNA analysis showed that the dominant phyla were Firmicutes and Proteobacteria, and the dominant genera were Lactobacillus and Weissella. The changes in fermentation parameters and chemical components of hybrid Pennisetum silage caused by cellulase, Lactobacillus plantarum, sucrose, and their mixture may be the result of bacterial community changes.

1. Introduction

Hybrid Pennisetum (Pennisetum americanum × Pennisetum purpureum, HP) is a perennial herb, which is widely planted in tropical areas because of its large size, rapid growth, good adaptability to the environment and high yield [1]. HP can produce a large amount of biomass of about 40 tons per hectare per year [2]. Because of its high utilization efficiency of water and nitrogen, huge biomass, and high quality, it can be used as feed for herbivores and has research value [3]. In addition, it has been found that the silage HP can improve the daily gain and meat quality of meat rabbits [4]. However, the yield of grasses such as Pennisetum is greatly affected by the seasons, and it grows fast when it is rainy in summer and slow when it is dry in winter [5]. If the water content is high, the plants are easy to mildew and rot, so the storage mode of forage after harvest is very important. Ensiling is a method of preserving green plants by anaerobic fermentation, which allows the quality of the grass to be maintained for an extended period of time [6]. When the yield of the HP is high, silage can be processed to store the forage, and the nutritional ingredients of the forage can be retained to the greatest extent, the shelf life can be improved, and the palatability can be improved. However, tropical Gramineae plants have a rough stem and leaf structure and high cellulose content [5]. Moreover, HP
has the characteristics of high buffering capacity and low water-soluble carbohydrate (WSC) content [7]. Therefore, it is difficult to produce excellent silage.

Either directly adding sucrose [5] or adding cellulase to hydrolyze cellulose to produce glucose [8,9] can provide substrate for ensilage of hybrids *Pennisetum* and promote the proliferation of lactic acid bacteria. *Lactobacillus plantarum* has been widely used in silage experiments and production [10–13]. By consuming WSC, lactic acid (LA) is produced to reduce the pH of silage, so as to control the growth of other miscellaneous bacteria and improve the silage quality.

Therefore, the main purpose of this study is to evaluate the effects of different additives and mixed additives on the silage quality of HP. Pay special attention to the fermentation quality and bacterial community among different treatments.

2. Materials and Methods

2.1. Raw Materials and Silage Preparation

The hybrid *Pennisetum* was harvested in Meizhou City, Guangdong Province, China (longitude: 115.82 E, latitude: 24.52 N) in November 2020. No herbicides and fertilizers were used, and mowers were used for harvesting, leaving 2–3 cm of stubble. After harvesting, the stubble was cut into 1–2 cm pieces by hand with a paper cutter. The DM, CP, NDF, and ADF content of HP was 32.80%, 4.41%, 69.60%, and 43.16%. The experiment was divided into seven treatments, silage treatment, and its abbreviation: (1) no additive (CON); (2) 100 U/g cellulase (VTR Bio-Tech Co., Ltd., Zhuhai, China) of fresh matter (CE); (3) 1 × 10^6 cfu/g FM *Lactobacillus plantarum* (LP); (4) 1% sucrose (purity ≥ 99.5%; Shanghai Macklin Biochemical, Shanghai, China) of fresh matter (SU); (5) 100 U/g cellulase + 1 × 10^6 cfu/g FM *Lactobacillus plantarum* (CE+LP); (6) 100 U/g cellulose + 1% sucrose (CE+SU); (7) 1 × 10^6 cfu/g FM *Lactobacillus plantarum* + 1% sucrose (LP+SU), three repetitions per treatment. After that, the materials (not wilting, about 200 g) were put into plastic silo bags and sealed with a vacuum sealer. A total of 21 bags (7 treatments × 3 repetitions) were made and stored at room temperature (20–25 °C). After 60 days of silage, the silage bag was opened to determine the fermentation quality, chemical composition, and analysis of the bacterial community and metabolic spectrum.

2.2. Chemical Analysis

The bag was opened on a clean bench, a small amount of silage was removed from the seal, 5 g of each silage sample was mixed with 45 mL of deionized water, stored at 4 °C for 24 h, then filtered, and the pH value of the filtrate was measured with a glass electrode pH meter (PHS-3C, Inesa Scientific Instrument Co., Ltd., Shanghai, China). A Shodex RSpak KC-811s-dvb gel c column (8.0 mm × 30 cm; Shimadzu, Tokyo, Japan) analyzed the contents of acetic acid (AA), propionic acid (PA), and butyric acid (BA) [14]. LA was determined by p-hydroxyphenyl colorimetry [15]. The content of ammonia nitrogen (AN) was determined by phenol-sodium hypochlorite colorimetry [16].

The remaining samples were dried in an oven at 65 °C for 48 h, and the DM content of silage was analyzed. Then, they were ground by a knife grinder in the laboratory (FW100, Taisite Instrument Co., Ltd., Tianjin, China) until they passed through a 1 mm sieve. According to the method of the Official Analytical Chemists Association, the crude protein was analyzed by a Kjeltec 2300 auto-analyzer (FOSS Analytical AB, Hilleroed, Denmark) [17]. The contents of neutral washing fiber (NDF) and acid washing fiber (ADF) were determined without using thermostable amylase and sodium sulfite, determined by the A220 fiber analyzer (Ankom Technology Corp., Macedon, NY, USA) according to the method of Van Soest et al. [18].

2.3. Bacterial Community Analysis

A DNeasy power soil kit (QIAGEN, Inc., Venlo, TheNetherlands) was used to extract DNA samples and determine their purity, concentration, and integrity. Then, Pyrobest DNA polymerase (DR500A, TaKaRa, Kusatsu, Japan) and the primer pairs of 338F (5′-
ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GACTACHVGGGTATCTAATCC-3′) amplified the 16S rRNA V3–V4 regions of genomic DNA. After the amplification, Agencourt AMPure beads (Beckman Coulter, Indianapolis, IN) were adopted for the purification of PCR products, and the Picogreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) was used for quantification. Equimolar and paired-end sequencing (PE250) was performed via the Illumina Novaseq 6000 platform (Personal Biotechnology Co., Ltd., Shanghai, China). After high-throughput sequencing, QIIME (V 1.8.0) was adopted for the processing of sequenced data. The effective sequences were collected and grouped into operational taxonomic units (OTUs) using UCLUST with a 97 percent similarity criterion after being filtered to remove chimeric and low-quality sequences as indicated. An OTU table was created after a sample sequence from each OTU was chosen for further taxonomic categorization using the Basic Local Alignment Search Tool (BLAST). Sequence data analysis was carried out by QIIME and R software package (V 4.0.0). The α-diversity was analyzed by the α-diversity.py script in QIIME, using UniFrac distance measurement and visualization by principal coordinate analysis (PCoA), and the β-diversity analysis was carried out. The PICRUSt database was used to predict the function of microbiome [19].

2.4. Statistical Analysis

One-way ANOVA in SPSS 25.0 software was used to analyze silage fermentation parameters, chemical composition, and microbial α-diversity, among which the LSD method was used for multiple comparisons, and \( p < 0.05 \) was statistically significant.

3. Results

3.1. Fermentation Parameters of Hybrid Pennisetum Silage

The effects of Lactobacillus plantarum, sucrose, cellulose, and their combinations on fermentation parameters are shown in Table 1. Compared with CON, the pH of the two treatment groups of CE and LP decreased significantly \( (p < 0.05) \), while the other groups had no significant difference from CON \( (p > 0.05) \). The LA contents of CE, LP, and SU increased to some extent compared with CON, while CE+LP, CE+SU, and LP+SU decreased to some extent compared with CON, but none of them reached a significant level \( (p > 0.05) \). Compared with CON, the content of AA in CE+LP, CE+SU, and LP+SU increased significantly \( (p < 0.05) \), but the content of AA in CE, LP, and SU did not change significantly \( (p > 0.05) \). As for WSC, all experimental groups show different degrees of improvement compared with CON, especially CE, SU, and LP+SU \( (p < 0.05) \). However, AN, compared with CON, decreased to varying degrees in each experimental group \( (p < 0.05) \). BA was also detected in the SU group but not in other groups.

Table 1. Fermentation parameters of hybrid Pennisetum silage.

<table>
<thead>
<tr>
<th>Items</th>
<th>CON</th>
<th>CE</th>
<th>LP</th>
<th>Treatment</th>
<th>SU</th>
<th>CE+LP</th>
<th>CE+SU</th>
<th>LP+SU</th>
<th>SEM</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.70</td>
<td>3.61</td>
<td>3.60</td>
<td></td>
<td>3.68</td>
<td>3.71</td>
<td>3.72</td>
<td>3.72</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA (g/kg DM)</td>
<td>20.62</td>
<td>22.70</td>
<td>21.34</td>
<td></td>
<td>22.13</td>
<td>16.51</td>
<td>19.12</td>
<td>17.85</td>
<td>2.51</td>
<td>0.199</td>
</tr>
<tr>
<td>AA (g/kg DM)</td>
<td>9.58</td>
<td>9.78</td>
<td>9.62</td>
<td></td>
<td>10.36</td>
<td>14.58</td>
<td>13.99</td>
<td>11.37</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WSC (g/kg DM)</td>
<td>8.50</td>
<td>11.34</td>
<td>9.64</td>
<td></td>
<td>11.02</td>
<td>9.90</td>
<td>9.88</td>
<td>12.33</td>
<td>0.94</td>
<td>0.021</td>
</tr>
<tr>
<td>AN (g/kg DM)</td>
<td>0.87</td>
<td>0.69</td>
<td>0.62</td>
<td></td>
<td>0.62</td>
<td>0.66</td>
<td>0.66</td>
<td>0.72</td>
<td>0.07</td>
<td>0.031</td>
</tr>
<tr>
<td>BA (g/kg DM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>0.47</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CON, control group; CE, 100 U/g FM cellulase addition; LP, \( 1 \times 10^{6} \) cfu/g FM Lactobacillus plantarum addition; SU, 1% FM sucrose addition. DM, dry matter; FM, fresh matter; LA, lactic acid; AA, acetic acid; WSC, water soluble carbohydrates; AN, ammonia nitrogen; BA, butyric acid. SEM, standard error of means. Different lowercase indicates significant differences in the same column \( (p < 0.05) \).

3.2. Chemical Composition of Hybrid Pennisetum Silage

The chemical composition results are shown in Table 2. Relative to CON, DM was significantly reduced in all groups except for SU \( (p < 0.05) \), and the content of CE was

<table>
<thead>
<tr>
<th>Items</th>
<th>CON</th>
<th>CE</th>
<th>LP</th>
<th>Treatment</th>
<th>SU</th>
<th>CE+LP</th>
<th>CE+SU</th>
<th>LP+SU</th>
<th>SEM</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
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<td>3.60</td>
<td></td>
<td>3.68</td>
<td>3.71</td>
<td>3.72</td>
<td>3.72</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA (g/kg DM)</td>
<td>20.62</td>
<td>22.70</td>
<td>21.34</td>
<td></td>
<td>22.13</td>
<td>16.51</td>
<td>19.12</td>
<td>17.85</td>
<td>2.51</td>
<td>0.199</td>
</tr>
<tr>
<td>AA (g/kg DM)</td>
<td>9.58</td>
<td>9.78</td>
<td>9.62</td>
<td></td>
<td>10.36</td>
<td>14.58</td>
<td>13.99</td>
<td>11.37</td>
<td>0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WSC (g/kg DM)</td>
<td>8.50</td>
<td>11.34</td>
<td>9.64</td>
<td></td>
<td>11.02</td>
<td>9.90</td>
<td>9.88</td>
<td>12.33</td>
<td>0.94</td>
<td>0.021</td>
</tr>
<tr>
<td>AN (g/kg DM)</td>
<td>0.87</td>
<td>0.69</td>
<td>0.62</td>
<td></td>
<td>0.62</td>
<td>0.66</td>
<td>0.66</td>
<td>0.72</td>
<td>0.07</td>
<td>0.031</td>
</tr>
<tr>
<td>BA (g/kg DM)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>0.47</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

CON, control group; CE, 100 U/g FM cellulase addition; LP, \( 1 \times 10^{6} \) cfu/g FM Lactobacillus plantarum addition; SU, 1% FM sucrose addition. DM, dry matter; FM, fresh matter; LA, lactic acid; AA, acetic acid; WSC, water soluble carbohydrates; AN, ammonia nitrogen; BA, butyric acid. SEM, standard error of means. Different lowercase indicates significant differences in the same column \( (p < 0.05) \).
the lowest. Compared with CON, NDF, and ADF in each experimental group decreased to different degrees, and the contents of NDF and ADF in CE, LP, CE+LP, and CE+SU were significantly lower than those in CON ($p < 0.05$). In CP, CE, CE+LP, and CE+SU increased significantly compared with CON ($p < 0.05$), among which CE+SU performed the best, while LP and SU were not significant compared with CON ($p > 0.05$), while LP+SU decreased significantly ($p < 0.05$).

Table 2. Nutrient composition of hybrid *Pennisetum* silage.

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>CON</th>
<th>CE</th>
<th>LP</th>
<th>CE+LP</th>
<th>CE+SU</th>
<th>LP+SU</th>
<th>SEM</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (g/kg FM)</td>
<td>SU</td>
<td>324.88</td>
<td>298.07</td>
<td>305.24</td>
<td>323.45</td>
<td>308.29</td>
<td>308.73</td>
<td>315.26</td>
<td>3.42</td>
</tr>
<tr>
<td>NDF (g/kg DM)</td>
<td>CE+LP</td>
<td>625.31</td>
<td>547.63</td>
<td>588.99</td>
<td>607.22</td>
<td>585.59</td>
<td>565.00</td>
<td>614.48</td>
<td>14.93</td>
</tr>
<tr>
<td>ADF (g/kg DM)</td>
<td>CE+SU</td>
<td>339.88</td>
<td>288.13</td>
<td>307.56</td>
<td>318.45</td>
<td>313.78</td>
<td>291.92</td>
<td>330.05</td>
<td>11.60</td>
</tr>
<tr>
<td>CP (g/kg DM)</td>
<td>LP+SU</td>
<td>55.66</td>
<td>82.32</td>
<td>57.64</td>
<td>56.29</td>
<td>78.01</td>
<td>87.54</td>
<td>48.30</td>
<td>2.08</td>
</tr>
</tbody>
</table>

CON, control group; CE, 100 U/g FM cellulase addition; LP, $1 \times 10^6$ cfu/g FM *Lactobacillus plantarum* addition; SU, 1% FM sucrose addition; DM, dry matter; FM, fresh matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein. SEM, standard error of means. Different lowercase indicates significant differences in the same column ($p < 0.05$).

3.3. Bacterial Community of Hybrid *Pennisetum* Silage

3.3.1. Bacterial Diversity

In this study, the species diversity of $\alpha$-diversity was studied, including Good’s coverage, observed species index, chao1 index, Shannon index, Simpson index, and Pielou evenness. It can be seen from Table 3 that the Good’s coverage of all treatments is above 0.99, indicating that the sequencing results can reflect the information of most bacterial species in the sample, with Good’s coverage and reliable sequencing results; Chao 1 index and observed species index in LP+SU were significantly higher than those in CON ($p < 0.05$). The uniformity of Shannon, Simpson, and Pielou in CE+LP is significantly lower than that of CON ($p < 0.05$). The principal coordinate analysis of $\beta$-diversity in this study was carried out as shown in Figure 1. The significant segregation between CE/LP, LP+SU, and CON indicated that the bacterial compositions between CE/LP, LP+SU, and CON were different.

Table 3. The $\alpha$-diversity of bacterial community of hybrid *Pennisetum* silage.

<table>
<thead>
<tr>
<th>Items</th>
<th>Treatments</th>
<th>CON</th>
<th>CE</th>
<th>LP</th>
<th>CE+LP</th>
<th>CE+SU</th>
<th>LP+SU</th>
<th>SEM</th>
<th>$p$-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good’s coverage</td>
<td>SU</td>
<td>0.996</td>
<td>0.995</td>
<td>0.995</td>
<td>0.995</td>
<td>0.995</td>
<td>0.995</td>
<td>0.992</td>
<td>0.001</td>
</tr>
<tr>
<td>Observed species</td>
<td>CE+LP</td>
<td>386.53</td>
<td>370.60</td>
<td>412.53</td>
<td>354.37</td>
<td>400.40</td>
<td>402.13</td>
<td>606.10</td>
<td>61.91</td>
</tr>
<tr>
<td>Shannon index</td>
<td>CE+SU</td>
<td>4.19</td>
<td>4.34</td>
<td>3.83</td>
<td>3.88</td>
<td>3.48</td>
<td>4.07</td>
<td>4.63</td>
<td>0.25</td>
</tr>
<tr>
<td>Simpson index</td>
<td>LP+SU</td>
<td>0.81</td>
<td>0.85</td>
<td>0.79</td>
<td>0.78</td>
<td>0.71</td>
<td>0.81</td>
<td>0.82</td>
<td>0.03</td>
</tr>
<tr>
<td>Chao1 index</td>
<td>SU</td>
<td>462.06</td>
<td>478.40</td>
<td>517.75</td>
<td>457.50</td>
<td>503.57</td>
<td>515.10</td>
<td>765.93</td>
<td>97.49</td>
</tr>
<tr>
<td>Pielou evenness</td>
<td>CE+LP</td>
<td>0.49</td>
<td>0.51</td>
<td>0.44</td>
<td>0.46</td>
<td>0.40</td>
<td>0.47</td>
<td>0.50</td>
<td>0.02</td>
</tr>
</tbody>
</table>

CON, control group; CE, 100 U/g FM cellulase addition; LP, $1 \times 10^6$ cfu/g FM *Lactobacillus plantarum* addition; SU, 1% FM sucrose addition. Different lowercase indicates significant differences in the same column ($p < 0.05$).

3.3.2. Composition of Bacteria

Phylum analysis indicated that *Firmicutes* was the most important phylum in all treatments. Except for LP+SU, *Firmicutes* in other treatments accounted for more than 90% of the bacterial community (Figure 2A), and the relative abundance of *Proteobacteria* in LP+SU was significantly higher than that in other groups. At the genus level (Figure 2B), the most dominant bacteria in each group was *Lactobacillus*, followed by *Weissella*. Notably, compared with CON, the relative abundance of *Lactobacillus* in CE decreased, while that of LP, SU, CE+LP, and CE+SU increased. Compared with other groups, the relative abundance of *Weissella* in LP+SU decreased obviously, while the relative abundance of some other bacteria, such as *Leuconostoc* and *Bacillus*, increased.
Figure 1. Principal co-ordinate analysis (PCoA) of bacterial communities for hybrid *Pennisetum* silage (CON, control group; CE, 100 U/g FM cellulase addition; LP, $1 \times 10^6$ cfu/g FM *Lactobacillus plantarum* addition; SU, 1% FM sucrose addition).

Figure 2. Accumulation map of bacterial communities at the phylum (A) and genus (B) levels for hybrid *Pennisetum* silage (CON, control group; CE, 100 U/g FM cellulase addition; LP, $1 \times 10^6$ cfu/g FM *Lactobacillus plantarum* addition; SU, 1% FM sucrose addition).

3.3.3. Functions and Ways of Prediction

Based on ASV tree and ASV gene information in the Greengenes database, PICRUSt2 was used to predict the function of the hybrid *Pennisetum* silage microbial community. According to the functional annotation, action path, and abundance information of samples in the database, 20 functions with the highest abundance were selected, and their abundance information in each sample was drawn into the heat map. As can be seen from Figure 3, the first five prediction functions are: SPP; sucrose-6-phosphatase [EC: 3.1.3.24], ABC-2.A; ABC-2 type transport system ATP-binding protein, ABC-2.P; ABC-2 type transport system permease protein, gpmB; probable phosphoglycerate mutase [EC: 5.4.2.12], lacI, galR; LacI family transcriptional regulator. The first five ways of prediction are: biosynthesis of ansamycins, secondary bile acid biosynthesis, fatty acid biosynthesis, D-glutamine and D-glutamate metabolism, and lysine biosynthesis. Heat maps (Figure 3A,B) of predicted functions and pathways show that the addition of CE+LP, LP+SU, LP, and CE may affect the dominant functions and pathways.
Figure 3. Heatmap of the top 20 predicted functions (A) and pathways (B) of the bacterial communities analyzed via PICRUSt2 for hybrid Pennisetum silage (CON, control group; CE, 100 U/g FM cellulase addition; LP, $1 \times 10^6$ cfu/g FM Lactobacillus plantarum addition; SU, 1% FM sucrose addition).

4. Discussion

In this study, the soluble carbohydrate content of HP is low, and the soluble WSC is the key factor for ensilage success. Therefore, it is necessary to make the fermentation substrate have more WSC and establish beneficial bacteria [7]. The pH value is an important index to evaluate the quality of silage. Generally, silage with good fermentation is considered when the pH value is lower than 4.2 [20]. The pH of all treatments in this experiment was lower than 4.2, which met the standard of high-quality silage. The production of LA can rapidly reduce the pH of silage [21]. In this study, the pH value of CE and LP is lower than that of CON and other groups, and the LA content is higher than that of CON and other groups, which indicates that CE and LP have good fermentation effects, and adding LP and CE can promote silage fermentation. Li et al. (2019) added LP and CE to Pennisetum silage and reached a similar conclusion on pH and LA [22]. It can be considered that cellulase can degrade plant fiber into WSC at the early stage of ensilage, which can promote the reproduction of lactic acid bacteria, resulting in a rapid increase in LA and a decrease in pH [10]. Adding homologous fermentation strains such as LP can ferment silage to produce lactic acid, and the pH value can be reduced compared with untreated silage [23]. Sucrose also solved the problem of insufficient fermentation substrate in silage and promoted the production of LA. However, it is worth noting that in this experiment, when LP and CE were added at the same time, the content of LA decreased and the content of AA increased. The reason may be that cellulolytic enzymes can decompose structural carbohydrates and release pentose. Sugar can be further converted into D-xylose-5-phosphate, and then fermented into a mixture of LA and AA [24]. It may be that more WSC is decomposed by cellulase in the early stages, which leads to the proliferation of Lactobacillus plantarum. However, in the later stage, cellulose loses its activity in the low pH environment, and a large number of LPs consume WSC. When sugar is deficient, LP will metabolize LA into AA [25]. The content of AN can reflect the degree of hydrolysis of a polypeptide and the deamination of an amino acid or polypeptide [26]. Compared with CON, the AN content of each treatment group decreased, indicating that the ability of other groups to preserve protein was improved. The presence of butyric acid represents the fermentation of Clostridium, which is undesirable in silage [27]. Eating silage with high butyric acid will increase the probability of ketosis in lactating cows [28]. Butyric acid was detected in the
SU group, which indicated that the treatment was contaminated by *Clostridium*, and silage quality decreased.

In this experiment, it was found that the DM, NDF, and ADF of other groups all showed different degrees of decline compared with CON. The results showed that silage microbiota decomposed HP to different degrees. The decrease in DM may be due to microbial decomposition of silage nutrients into liquid and gas. Desta et al. (2016) proposed that when heterogeneous fermentation occurs in silage, the consumption of DM will increase, and a large amount of carbon dioxide will be produced [29]. In this study, the CP content of each group added with CE was higher than that of the other groups. This phenomenon may be due to the concentration effect caused by the loss of organic carbon in the fermentation process [30], the protein of the added enzyme, and the effect of cellulase on inhibiting protein degradation and promoting fiber degradation [31].

In the process of ensilage, microbial reactions are violent, and adding different additives will have different effects on the microbial community structure [32]. By using the 16S rRNA high-throughput sequencing technique, we can know the differences in bacterial communities among different additive treatment groups. The α-diversity can explain the richness, diversity, and evenness of species in bacterial communities. Good’s coverage is an index reflecting the depth of bacterial sequencing. Chao1 index and observed species index are used to indicate the richness of community species; the Shannon index and Simpson index are used to indicate the diversity of community species; and Pielou evenness reflects the evenness of the bacterial community. The results showed that the addition of CE and LP significantly reduced Pielou uniformity, Simpson index, and Shannon index. Similar to our research, Mu et al. (2020) found that the OTUs, Shannon index, and Chao1 index of amaranth silage added with CE and LP were lower than those of the control group. It may be that the addition of CE and LP promoted the proliferation of lactic acid bacteria and inhibited the growth of other harmful bacteria [33]. The addition of LP+SU significantly increased the observed species and Chao1 index. The β-diversity can reflect the difference in the bacterial community in each individual or treatment group. It can be seen that there is obvious separation between CE+LP, LP+SU, and CON, indicating that there are differences in bacterial composition among CE+LP, LP+SU, and CON. To sum up, the results of bacterial diversity are consistent with those of fermentation parameters and chemical components. After adding CE and LP on the surface, the structure of silage flora has changed greatly.

The bacterial community was closely related to the quality of silage. At the gate level, the dominant bacteria in all treatment groups were *Firmicutes* and Proteobacteria, which was consistent with the result of Chi et al. (2022) [34]. *Firmicutes* is an important microorganism in silage fermentation, and most of the bacteria involved in LA fermentation belong to *Firmicutes* [35], which can secrete a variety of cellulases, lipases, and proteases [36]. The average contents of *Firmicutes* and Proteobacteria in LP+SU were significantly different from those of other groups, and the fermentation direction was not consistent with that of other groups. Other gates are less abundant but also play an important role in silage.

At the genus level, the dominant bacteria are *Lactobacillus*, *Weissella*, and *Pediococcus*, which are different from the results of Chi et al. (2022) [34]; the mixed silage of hybrid *Pennisetum* and mulberry leaves is mainly attached to *Lactobacillus*, *Weissella*, *Pantoea*, and *Leuconostoc* bacteria during the entire ensiling period. The slightly different dominant bacteria may be due to the different climatic conditions and raw material growth conditions. *Lactobacillus* is a homofermentative lactic acid bacteria that can consume WSC to produce LA. *Weissella* is a heterologous fermentor that can consume WSC to produce LA, AA, and CO$_2$ [37]. It is worth noting that the proportion of *Lactobacillus* in the CE+LP group is higher than in other groups, but the proportion of LA in this group is the lowest, and the proportion of AA is the highest among all groups. *Lactobacillus*, as a homofermentative genus, will generally consume WSC and produce LA but will convert LA to AA in the absence of sugar [25]. Therefore, the reason may be that cellulase in the early stage of the CE+LP group decomposes more WSC, and the addition of exogenous LP makes LP more
advantageous than other groups. However, in the later stage, cellulose loses its activity in a low pH environment [38], making it difficult to produce new WSC. In the later stage, a large amount of LP will consume WSC, and LP metabolizes LA into AA when sugar is deficient. Bacillus often appears in large numbers in spoiled silage, and its spores are widely distributed in soil, water, and air [39]. Bacillus showed a variety of enzyme activities in silage, including protease, amylase, cellulase, and pectinase [40]. Bacillus is negatively correlated with CP content, and it may be that the proliferation of Bacillus produces more protease, which makes protein easier to decompose. Pseudomonas can inhibit pathogenic microorganisms during plant growth, which is helpful to promote plant growth, while the anaerobic environment after silage can inhibit the proliferation of Pseudomonas [41].

In Hao et al. (2020), Curbacillus was the most important proteolytic Curtobacterium, and its proteases are mostly metalloproteinases [42]. In this experiment, Pseudomonas was negatively correlated with pH and AA. The reason may be that with the decrease in pH, the activity of Pseudomonas was gradually inhibited, and Sun et al. (2021) reached a similar conclusion [43]. Curtobacterium is also negatively correlated with AA, which may be related to the inhibition of AA on microbiota [44].

PICRUSt2 was used to predict the metabolic functions and metabolic pathways of silage bacteria of silage HP. The results showed that the addition of CE+LP and LP increased ABC-2.P; ABC-2 type transport system permease protein, gpmB; probable phosphoglycerate mutase [EC: 5.4.2.12], dapE; succinyl-diaminopimelate desuccinylase [EC: 3.5.1.18], tagE; poly (glycerol-phosphate) alpha-glucosyltransferase [EC: 2.4.1.52]; and others. The addition of CE+LP and LP reduced the relative abundance of ABC.PA.S; polar amino acid transport system substrate-binding protein at the same time, but the addition of CE was the opposite. The performances of different additives in the first five ways are also quite different. The use of CE+LP increases the relative abundance of D-alanine metabolism and peptidoglycan biosynthesis pathways and decreases the relative abundance of biosynthesis of ansamycins and lysine biosynthesis pathways. The use of LP+SU increases the relative abundance of biosynthesis of ansamycins, lysine biosynthesis, and others. However, using LP will reduce the relative abundance of the first 20 pathways. The results showed that LP, SU, and CE, used alone or in combination, had a great influence on the function and pathway of the bacterial community in silage, but their action modes were quite different, which might be related to their action mechanisms in silage, and further research was needed.

5. Conclusions

This study shows that CE and LP can improve the fermentation quality of hybrid Pennisetum silage, and the fermentation type can be adjusted by changing the abundance of Lactobacillus and Weisella. The exogenous addition of Lactobacillus plantarum makes it occupy the dominant position quickly, produces LA faster, improves silage quality, and inhibits protein hydrolysis; cellulase and sucrose can provide substrate for silage fermentation, which is beneficial to the proliferation of Lactobacillus, while cellulase is more suitable for silage hybrid Pennisetum than sucrose. The addition of sucrose is beneficial to the proliferation of miscellaneous bacteria to a certain extent, so it is not suitable for silage hybrid Pennisetum. The mixed addition of LP and CE will not only increase the abundance of Lactobacillus in the silage HP but will also promote LA to AA after ensilage for a long time. Therefore, adding cellulase and Lactobacillus plantarum in hybrid Pennisetum silage alone has the best effect.

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