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An Assessment on the Fermentation Quality and Bacterial Community of Corn Straw Silage with Pineapple Residue

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Simple Summary: Pineapple residue (PR) is a potential feed for ruminants, providing a rich source of fiber, protein, and water-soluble carbohydrates. However, PR has high water content, which may make storage difficult. For ruminants, corn straw (CS) is a common source of roughage, and it is often used for silage. Mixed ensiling of PR with CS may provide a solution to the problem of PR being difficult to preserve. The aim of this study was to evaluate the chemical composition, fermentation quality, and microbial community of CS silage mixed with PR. Mixed CS with appropriate PR silage showed a lower pH value and higher lactate acid and acetic acid content. Also, the addition of PR lead to an increase in the relative abundance of *Lactobacillus* in mixed silage.

Abstract: The effects of pineapple residue (PR) on fermentation quality, chemical composition, and bacterial community of corn straw (CS) silage were evaluated. CS was ensiled with 0% control group (CON), 15% (P1), 30% (P2), and 45% (P3) PR on a fresh matter (FM) basis for 45 days. P3 had lower dry matter (DM) and crude protein (CP) contents but higher ammonia-N (NH_3-N) content than the other three groups ($p < 0.05$). Compared with the other groups, P1 had lower a pH and higher lactic acid and acetic acid contents ($p < 0.05$). The lactic acid bacteria count in P1 was higher than in P2 and P3 ($p < 0.05$); the number of yeast in P2 was higher than in the other groups ($p < 0.05$). With the increasing proportion of PR addition, the relative abundance of *Lactobacillus* gradually increased, and the dominant genus in P3 was *Acetobacter*. In summary, the addition of PR can improve the quality of CS silage, and the optimum addition ratio for PR was 15% on a FM basis.

Keywords: pineapple residue; corn straw; silage quality; microbial diversity

Citation: Li, D.; Xie, H.; Zeng, F.; Luo, X.; Peng, L.; Sun, X.; Wang, X.; Yang, C. An Assessment on the Fermentation Quality and Bacterial Community of Corn Straw Silage with Pineapple Residue. *Fermentation* **2024**, *10*, 242. https://doi.org/10.3390/fermentation10050242

Academic Editors: Wansup Kwak and Siran Wang

Received: 9 April 2024
Revised: 28 April 2024
Accepted: 29 April 2024
Published: 30 April 2024

1. Introduction

Corn straw (CS) is a major field crop residue produced in large amounts that is rich in carbohydrates (cellulose and hemicellulose) and provides good roughage sources for ruminants [1,2]. However, large amounts of CS are discarded during the harvest season, leading to low CS utilization and a loss of a valuable feed resource. In order to preserve the quality of roughage, silage is commonly used in dairy farming. CS is one of primary crops for ensiling compared with other silage [3]. Silage of CS may prolong its storage time, increase its utilization efficiency, improve feed palatability, and enhance the production performance of animals [4]. Therefore, by incorporating corn stover into the feed system for ruminants, not only can it effectively utilize agricultural waste resources and reduce feed costs, but it is also expected to improve the production performance and quality of livestock and poultry products. In addition, this practice also helps to solve the environmental

problems caused by stover burning, providing an environmentally friendly solution for a sustainable development path. The scientific utilization of corn stover is not only related to the development of agriculture and animal husbandry, but it is also an important contribution to sustainable agriculture and resource recycling.

Pineapple belongs to the *Bromeliaceae* family and is a high-quality and high-yield tropical fruit in tropical and subtropical regions, known for its aromatic, sweet, and crisp flavor, as well as its rich content of sugars, minerals, and vitamins [5–7]. In recent years, China has seen a steady increase in pineapple production, with a total output reaching 1.733 million tons in 2019 [8]. Pineapple residue (PR), which refers to the leftover pineapple peels and residual pulp after pressing juice, canning, or wine making, accounts for approximately 50–70% of the entire fresh fruit weight [9]. PR has similar nutritional content to pineapple pulp, rich in crude protein (CP), ether extract (EE), crude fiber (CF), and vitamin C, making it a good feed resource [10,11]. However, the majority of PR is discarded as waste, resulting in a severe waste of resources and serious environmental pollution [12]. If PR is scientifically and reasonably developed and utilized as animal feed, it can turn waste into treasure and reduce feed costs. Liu et al. found that PR is a high-quality feed resource for cattle with good feed intake and digestion properties [13]. Yang et al. found that the partial substitution of fermented PR for whole corn silage in the diet did not impair or affect the productive performance of goats [14]. Gowda et al. reported that fed dairy cattle with silage pineapple waste could improve lactation productivity [15]. Suksathit et al. reported that pineapple waste silage can improve nutrient digestibility in livestock compared to hay silage [16].

Several studies reported that mixed ensiling improves silage quality and promotes stability of the fermentation process compared to fermentation alone [17,18]. Denek et al. found that tomato pomace silage fermented and preserved well with the addition of wheat straw and wheat grain [19]. Ni et al. found that mixed ensiling of forage soybean with crop corn or sorghum could reduce the pH value, enhance *Lactobacillus* abundance, and improve the forage soybean silage quality [20]. Li et al. found that mixed silage of the banana pseudostem and fresh maize stover decreased the pH value, *Enterobacteriaceae* count, yeast, and mold count [21]. Thus, we hypothesized that ensiling CS and PR together could inhibit undesirable fermentation and improve silage fermentation quality. However, to our knowledge, relatively little information is available on the fermentation characteristics and microbial diversity of CS ensiled alone or in combination with PR, and they are poorly understood. These studies showed positive effects of feeding animals with fermented PR, providing strong support for the widespread use of pineapple pomace in ruminant feeding and demonstrating that PR is an ideal feed for ruminants.

Therefore, this experiment aims to assess the effects of adding different ratios of PR on CS silage quality and microbial diversity and ultimately provide a scientific basis for the rational utilization of PR as ruminant feed.

2. Materials and Methods

2.1. Ensiling Materials and Silage Preparation

CS and PR were obtained from the Pasture Research Base of Guangxi Buffalo Research Institute in Nanning, China in May 2023. PR includes pineapple epidermis, leaf crown, and part of fruit pulp. Moreover, the CS refers to the residues remaining after the corn cobs are harvested. This material predominantly includes the green stem and leaves, which are typically at a late vegetative stage. The chemical compositions of CS and PR are listed in Table 1. Fresh CS and PR were cut into 1 to 2 cm pieces. Then, they were spread out flat on the ground to dry out moisture to about 70%. For silage preparation, CS was mixed with varying percentages of PR and ensiled under anaerobic conditions. Silages were prepared using a small-scale system of silage fermentation. Specifically, mixes were prepared with 0% PR (control), 15% PR (P1), 30% PR (P2), and 45% PR (P3), based on fresh matter. Samples were weighed and mixed uniformly according to the specified addition ratios, packed into polyethylene film bags (1000 g per bag), vacuum-sealed using a vacuum packaging

machine (DZ500; Gzrifu Co. Ltd., Guangzhou, China), and then stored in the dark at room temperature (20–30 °C). After 45 days, the polyethylene film bag was opened for the test.

Table 1. Chemical composition of corn straw and pineapple residue.

Items [1]	DM	CP	NDF	ADF	Ash	Ca	P
	(%)	(% DM)					
corn straw	26.92	13.10	68.39	38.39	9.36	0.73	0.23
pineapple residue	18.47	8.02	54.94	28.06	9.95	0.39	0.16

[1] D[1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

2.2. Chemical Analysis

The raw material and silage samples (500 g) were subjected to a consistent drying process at 65°C in an oven (LBAO-250; STIK Instrument Equipment shanghai Co., Ltd. Shanghai, China) until they reached a stable weight. Subsequently, they underwent pulverization using a pulverizer (FS200; Guangzhou Bomin Electrical and mechanical equipment Co. Ltd., Guangzhou, China), while ensuring the particles passed through a 1 mm screen for uniformity. To assess the chemical compositions, several analyses were conducted following standard protocols outlined by the AOAC. Specifically, the dry matter (DM), CP, and Ash contents were determined using methods 934.01, 976.05, and 942.05, respectively [22]. Additionally, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to procedures in Van Soest [23].

2.3. Fermentation Characteristics Analysis

Silage samples (20 g) were mixed with 180 mL of distilled water and stored at 4 °C for 24 h [24]. Then, filtration was carried out using two layers of gauze, and the filtrate was used for determining pH, NH_3-N, microbial crude protein (MCP), and organic acid contents. The pH value was assessed using a portable pH meter (pH8180-0-00; Smart sensor Co., Ltd., Dongguan, China). Ammonia-nitrogen (NH_3-N) was determined with the phenol-hypochlorite procedure [25]. MCP was determined using procedures described by Bradford [26]. The organic acid contents, including those of lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA), were measured via high-performance liquid chromatography (1260 Infinity II; Agilent Technologies, Inc., Waldbronn, Germany) according to Xie et al. [27]. The specific methods were as follows: Shodex RSpak KC–811 (8.0 mm × 300 mm; Showa Denko K.K., Tokyo, Japan); DAD detector set at 210 nm; elution process was carried out using 3 mmol/L $HClO_4$ as the eluent at a flow rate of 1.0 mL/min; temperature was 50 °C; and sample size was 5.0 μL.

Silage samples (10 g) were blended with 90 mL of sterilized water and serially diluted from 10^{-1} to 10^{-5} in sterilized water. Lactic acid bacteria (LAB) were measured by means of a plate count on De Man–Rogosa–Sharpe (MRS) agar (Qingdao Haibo Biotechnology Co., Ltd., Qingdao, China) and incubated at 37 °C for 48 h. Yeast were counted on potato dextrose agar (Qingdao Haibo Biotechnology Co., Ltd.) after incubation for 48 h at 37 °C. Numbers of colonies were considered to indicate the numbers of viable microorganisms (cfu g^{-1} of fresh matter [FM]) [28].

2.4. Microbial Analysis

Silage samples (15 g) were aseptically combined with 180 mL of sterile phosphate-buffered saline (PBS). This mixture was subsequently incubated at a constant temperature of 37 °C for 2 h under agitation at 200 r/min to ensure that the microbial population mixed into the buffer. Then, the samples were filtered through two layers of a sterile gauze to remove larger particulate matter. A volume of 70 mL of the resulting filtrate was then subjected to centrifugation at 12,000 r/min for 5 min at a temperature of 4 °C. This high-speed centrifugation facilitated the sedimentation of the microbial population, and the supernatant was discarded. Subsequently, it was washed 2 times with sterile PBS to remove any residual im-

purities. The precipitate was resuspended in 1–2 mL of the same buffer. Genomic DNA was extracted using the cetyltrimethy-lammonium bromide (CTAB) method [29]. DNA samples were sent to Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) for sequencing and analysis. The V3–V4 regions of the 16S rRNA gene were processed for amplification with the primers. The following primers were used: 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). For the polymerase chain reactions, we used 4 μL of 5× FastPfu Buffer (TransGen Biotech Co., Ltd., Beijing, China), 2 μL of dNTPs (2.5 mmol·L^{-1}), 0.8 μL of Forward Primer (5 μmol·L^{-1}) (TransGen Biotech Co., Ltd., Beijing, China), 0.8 μL of Reverse Primer (5 μmol·L^{-1})(TransGen Biotech Co., Ltd., Beijing, China), 0.4 μL of FastPfu Polymerase (TransGen Biotech Co., Ltd., Beijing, China), 0.2 μL of BSA (TransGen Biotech Co., Ltd., Beijing, China), 10 ng of Template DNA (TransGen Biotech Co., Ltd., Beijing, China), and added ddH_2O to obtain 20 μL of total volume. The steps included initialization at 95 °C for 3 min, 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 45 s, and a final elongation at 72 °C for 10 min. The PCR amplification products were detected via 2% agarose gel electrophoresis [30]. Purified DNA was sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) at Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). The sequences obtained from the MiSeq platform were processed using QIIME software (version 1.9.1). The sequence data reported in this study were archived in the Sequence Read Archive (SRA) under the accession number PRJNA1060478.

2.5. Statistical Analysis

Data on the fermentative characteristics were analyzed with one-way ANOVA using SPSS (SPSS 26.0 program, SPSS Inc., Chicago, IL, USA). The Alpha diversity index was calculated using Mothur software (version v.1.30.2). The histogram and heatmap were analyzed using R software (version v.3.3.1). There were significant differences only when the probability level was lower than 0.05 ($p < 0.05$).

3. Results

3.1. Chemical Compositions

The lowest DM content was found in P3, flowed by P2, P1, and CON ($p < 0.05$) (Table 2). The CP content in P3 was lower than those in CON and P1 ($p < 0.05$). The differences in NDF and ADF content among all silages were not significant ($p > 0.05$). The Ash content in P1 was lower than that in P2 and P3 ($p < 0.05$). The Ca content in P1 was lower than that in CON and P2 ($p < 0.05$).

Table 2. The effects of adding pineapple residue on the chemical compositions of corn straw silage.

Items [1]	CON	P1	P2	P3	*p*-Value
DM (%)	27.55 ± 0.31 [a]	26.40 ± 0.56 [b]	25.35 ± 0.63 [b]	23.61 ± 1.00 [c]	<0.001
CP (% DM)	13.21 ± 0.27 [a]	12.93 ± 0.14 [a]	12.55 ± 0.30 [ab]	11.71 ± 1.13 [b]	0.007
NDF (% DM)	61.69 ± 1.15	60.25 ± 1.65	60.76 ± 0.50	62.35 ± 2.88	0.277
ADF (% DM)	36.56 ± 0.80	35.97 ± 1.00	36.48 ± 0.40	38.16 ± 3.26	0.267
Ash (% DM)	9.28 ± 0.21 [ab]	9.16 ± 0.17 [b]	9.52 ± 0.05 [a]	9.53 ± 0.22 [a]	0.008
Ca (% DM)	0.67 ± 0.03 [a]	0.59 ± 0.01 [c]	0.64 ± 0.03 [ab]	0.60 ± 0.01 [bc]	0.001
P (% DM)	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	<0.001

[1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage. [a–c] Different letters in the same row indicate significant differences $p < 0.05$.

3.2. Fermentation Quality

P1 had a lower pH value but higher LA and AA content compare with the other group ($p < 0.05$) (Table 3). PA was not detected in any of the other three groups, except for P3. BA was not detected in any silages. NH_3-N content in P3 was higher than in the other groups ($p < 0.05$). NH_3-N content of all silage mixtures tended to increase after the addition of PR,

resulting in CON silage having lower NH_3-N content than the other silages. The number of LAB in P1 was higher than in P2 and P3 ($p < 0.05$). The number of yeast in P2 was higher than the other groups ($p < 0.05$), followed by that in the P3, CON, and P1 silages.

Table 3. The effects of adding pineapple residue on the fermentation quality of corn straw silage.

	Groups				
Items [1]	**CON**	**P1**	**P2**	**P3**	***p*-Value**
pH	4.28 ± 0.07 [b]	4.18 ± 0.04 [c]	4.35 ± 0.05 [ab]	4.43 ± 0.06 [a]	<0.001
Lactate acid (g/kg DM)	15.81 ± 0.01 [b]	17.15 ± 0.01 [a]	14.78 ± 0.02 [c]	13.88 ± 0.01 [d]	<0.001
Acetic acid (g/kg DM)	10.33 ± 0.01 [b]	12.54 ± 0.03 [a]	9.69 ± 0.02 [c]	9.56 ± 0.02 [d]	<0.001
Propionic acid (g/kg DM)	ND	ND	ND	0.91 ± 0.04	-
Butyric acid (g/kg DM)	ND	ND	ND	ND	-
NH_3-N (g/kg DM)	1.19 ± 0.02 [c]	1.22 ± 0.02 [c]	1.29 ± 0.04 [b]	1.47 ± 0.05 [a]	<0.001
MCP (mg/mL)	1.34 ± 0.38	1.01 ± 0.12	1.08 ± 0.25	1.38 ± 0.20	0.090
Microbial population (lg CFU/g of FM)					
Lactic acid bacteria	6.69 ± 0.02 [a]	6.73 ± 0.02 [a]	6.58 ± 0.06 [b]	6.59 ± 0.03 [b]	<0.001
Yeast	2.22 ± 0.04 [c]	2.21 ± 0.09 [c]	2.66 ± 0.06 [a]	2.48 ± 0.07 [b]	<0.001

[1] NH_3-N, ammonia-N; MCP, microbial crude protein; ND, not detected; CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage. [a–d] Different letters in the same row indicate significant differences $p < 0.05$.

3.3. Microbial Analysis

The Shannon index and Simpson index were not significant among the four groups ($p > 0.05$) (Table 4). The Chao1 index in P3 was higher than in P2 ($p < 0.05$).

Table 4. The effects of adding pineapple residue on the microbial analysis of corn straw silage.

Items [1]	**CON**	**P1**	**P2**	**P3**	***p*-Value**
Shannon	1.99 ± 0.11	1.58 ± 0.20	1.33 ± 0.73	1.87 ± 0.35	0.094
Simpson	0.23 ± 0.06	0.38 ± 0.08	0.47 ± 0.32	0.38 ± 0.10	0.233
Chao1	119.85 ± 11.35 [ab]	133.55 ± 20.54 [ab]	100.26 ± 33.09 [b]	167.37 ± 52.36 [a]	0.036
ACE	123.03 ± 13.56	133.81 ± 24.12	107.91 ± 37.23	172.56 ± 53.75	0.060
Coverage	0.9996 ± 0.00	0.9996 ± 0.00	0.9997 ± 0.00	0.9996 ± 0.00	0.788

[1] CON, 1[1] CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage. [a,b] Different letters in the same row indicate significant differences $p < 0.05$.

At the order level, *Lactobacillales* and *Enterobacterales* were the top two dominant genera in terms of relative abundance in the CON, P1, and P2 silages (Figure 1). However, the dominant genus in P3 was *Acetobacterales*.

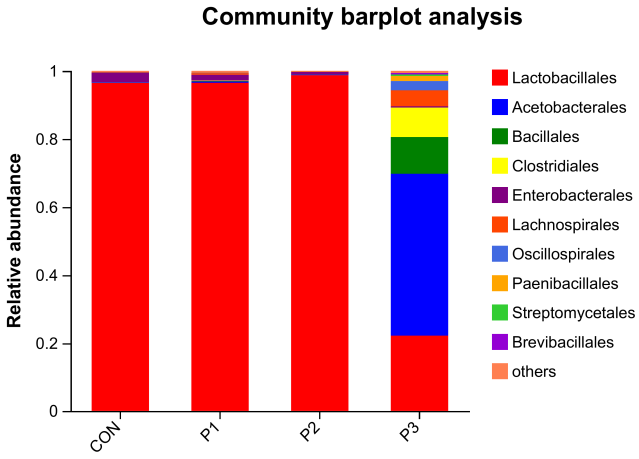

Figure 1. Effects of adding pineapple residue on the order level of microorganisms in corn straw silage. Abbreviations: CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage.

At the genus level, the top two genera in terms of relative abundance in CON, P1, and P2 were *Lactobacillus* and *Pediococcus* (Figure 2). The relative abundance of *Lactobacillus* in CON, P1, and P2 as a proportion of all genera was 87.47%, 90.75%, and 96.21%, respectively.

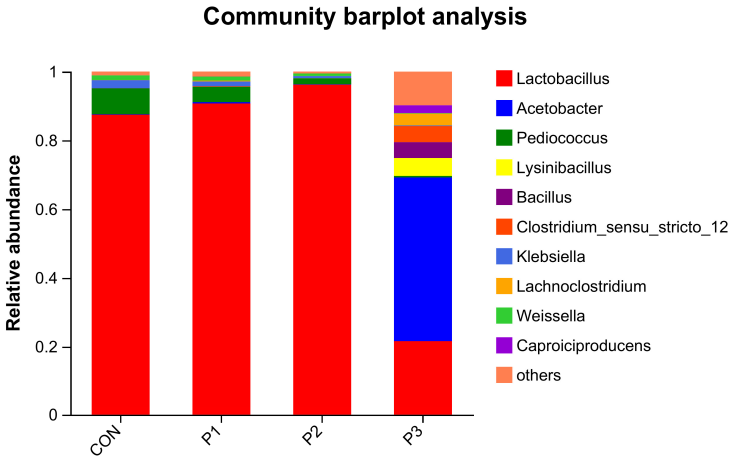

Figure 2. Effects of adding pineapple residue on the genus level of microorganisms in corn straw silage. Abbreviations: CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage.

The pH value and NH_3-N content were negatively correlated with *un-classified_f__Enterobacteriaceae*, *Weissella*, *Klebsiella*, and *Pediococcus* ($p < 0.05$), and positively correlated *unclassified_f__Clostridiaceae*, *Caproiciproducens*, and *Lysinibacillus* ($p < 0.05$) (Figure 3). MCP content was positively correlated with *unclassified_f__Clostridiaceae* and *Bacillus* ($p < 0.05$). LA and AA contents were positively correlated with *unclassified__f__Enterobacteriaceae*, *Weissella*, *Klebsiella*, and *Pediococcus* ($p < 0.05$), and negatively correlated with *unclassified__f__Clostridiaceae*, *Caproiciproducens*, and *Lysinibacillus* ($p < 0.05$).

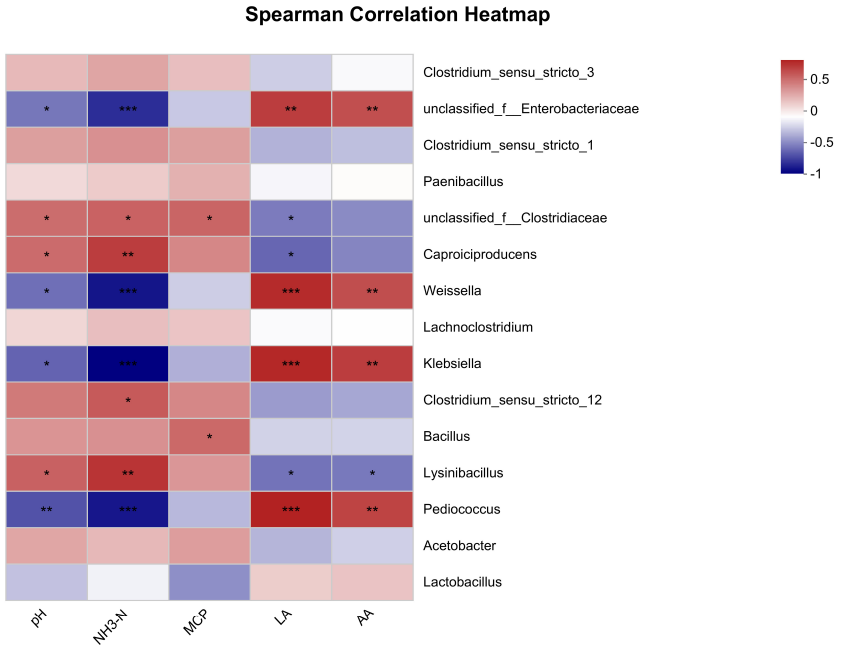

Figure 3. Correlation between relative abundance of bacteria and fermentation parameters at the genus level. Note: Columns in different colors indicate different subgroups; *p*-values are on the far right; "*" indicates $p \leq 0.05$, "**" indicates $p \leq 0.01$, and "***" indicates $p \leq 0.001$. Abbreviations: CON, 100% CS silage; P1, 85% CS + 15% PR silage; P2, 70% CS + 30% PR silage; P3 55% CS + 45% PR silage.

4. Discussion

Raw materials essential for producing high-quality silage should possess specific characteristics, including appropriate moisture levels and an environment that supports anaerobic conditions [27,28]. In this experiment, we observed that the lowest DM content occurred in P3, with sequentially higher levels in P2, P1, and CON, respectively; additionally, the CP content in P3 was noted to be lower than that in CON and P1. The reason for this result is due to the different chemical composition of the two feedstocks, with PR having lower DM content and CP content compared to CS. Consequently, an increase in the addition of PR to the silage mixture led to a decrease in both DM and CP contents. This is consistent with the findings of Muller et al., who reported that pineapple exhibits elevated levels of fiber and starch but a comparatively low content of CP [31]. According to research by Wang et al., CS can serve as a water absorbent in silage, mitigating the challenge posed by excessive water content in other materials [4]. As a result, the content of DM and CP declined concomitantly with the reduction in the proportion of CS and the increase in the proportion of the PR ratio in the silage. In the current study, the Ash content in P1 was lower than the P2 and P3, indicating that CS silage with 15% PR had the highest organic matter content. Ensiling CS with a certain proportion of PR can be modulated to produce a more nutritionally balanced feed.

Silage, an important nutritional source for herbivores, effectively guarantees a balanced supply of roughage throughout the year [32], which is the most commonly used technology for preserving forage [33]. The principle of silage fermentation is that the LAB attached to the materials, under the right temperature, moisture, and anaerobic conditions, ferments water-soluble carbohydrates into LA and other short-chain volatile fatty acids; as a result, the pH decreases, the growth of harmful microorganisms is inhibited, and the silage is preserved as long as it is not exposed to air [34–37]. The pH level of silage serves as a critical indicator for assessing its fermentation quality, where lower pH values are indicative of better fermentation quality and enhanced aerobic stability [38]. The value of pH is determined by organic acids, such as LA and AA, which are produced during the silage process. Research has shown that the content of LA is positively correlated with the overall quality of silage; similarly, AA also improves the aerobic stability of silage [39]. In this experiment, P1 exhibited a lower pH value but higher LA content and AA content compared to the other groups. This might be due to the fact that 15% PR creates a suitable environment for the growth and reproduction of benefit microorganisms, which utilize water-soluble carbohydrates to produce substantial amounts of LA under favorable conditions, causing the pH to drop. It indicates that ensiling CS with 15% PR can achieve the best fermentation quality. In this experiment, P3 had the highest pH value, the lowest lactic acid content, and poor fermentation quality, indicating that too much pineapple pomace should not be added to silage. NH_3-N content reflects the breakdown of proteins in the silage, with higher values indicating greater protein and amino acid breakdown and lower fermentation quality [40]. In this experiment, as the percentage of PR increased, the content of NH_3-N in silage increased, and P3 had the highest NH_3-N content. This is possibly due to the bromelain in PR, leading to protein breakdown and the production of NH_3-N [41].

LAB, recognized as beneficial bacteria, have the capacity to produce LA during the fermentation process. This production of LA leads to a decrease in the pH value, which effectively inhibits the growth of harmful bacteria [42]. Generally speaking, when the number of LAB reaches at least 10^5 (cfu/g FM), silage is considered to be well preserved [43]. In this experiment, the detected number of LAB in all groups reached 10^6 (cfu/g FM), which clearly indicates a successful and good fermentation process in all groups. The addition of an appropriate amount of PR enables the effective preservation of CS silage. Microorganisms play a pivotal role in silage fermentation and can affect silage fermentation through a series of metabolites [44]. Consequently, the structural composition and the abundance of different species of microorganisms present during the fermentation process are intricately linked to the overall quality of silage [45]. The Chao1 index and the Ace index are utilized to measure species richness, indicating the quantity of species [46]. In this

experiment, P3 exhibited the highest values for both the Chao1 index and the ACE index. This is possible due to the higher abundance of miscellaneous bacteria in PR, suggesting that an excessive addition (45%) of PR may have adverse effects on fermentation. Furthermore, the Shannon index and the Simpson index were employed to assess species diversity within the microbial community of the silage. These indices are influenced by both the richness and evenness of species within the community. Typically, larger values of these indices indicate a higher diversity of species [47]. In this experiment, the Shannon index and Simpson index were not different among the four groups, indicating that the addition of PR did not widely change the species' diversity in CS silage.

The most common genera of LAB in silage include *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, *Lactococcus*, *Leuconostoc*, and *Weissella* [48]. *Lactobacillus* can increase LA content, thereby inhibiting the growth and reproduction of harmful microorganism to ensure that the quality of silage is adequate. In this experiment, the relative abundance of *Lactobacillus* in the CON group was 87.47%, the relative abundance of *Lactobacillus* increased with the addition of 15% PR to a proportion of 90.75%, and the relative abundance of *Lactobacillus* reached 96.21% with the addition of 30% PR. This indicates that the addition of an appropriate amount of PR can increase the relative abundance of *Lactobacillus* and ensure that the quality of silage is adequate. *Enterobacterales* comprises Gram-negative, facultative anaerobic bacteria that are mainly found in poor silage, and it competes with LAB for the nutrients to grow and reproduce [49]. In this experiment, the relative abundance of *Enterobacterales* in P1 and P2 was reduced compared with CON. This suggests that the addition of moderate amounts of PR can reduce the relative abundance of harmful bacteria, such as *Enterobacterales*. In general, the surface of silage materials usually has a high number of microorganisms, such as aerobic bacteria, enterobacteria, yeast, and molds, attached to it. At the beginning, there is still oxygen present in the silage environment when aerobic bacteria grow vigorously. At the same time, *Streptococcus*, *Leuconostoc*, and *Pediococcus* are also active. After lactic acid fermentation, lactic acid bacteria begin to proliferate in large quantities and produce lactic acid; subsequently, with the formation of anaerobic and acidic environments, aerobic- and acid-intolerant microorganisms are gradually reduced, and they are steadily replaced by *Lactobacillus* and *Streptococcus lamellaris* together, which are the dominant bacteria in malolactic fermentation. In this experiment, *Acetobacter* was identified as a major genus in the P3 fermentation process, despite the anaerobic vacuum conditions typically being unsuitable for aerobic bacteria, such as *Acetobacter*. It is plausible that the presence of *Acetobacter* in P3 silage could be attributed to residual oxygen trapped within the biomass at the time of sealing. Additionally, the addition of high amounts of PR, which naturally carries *Acetobacter* as part of its flora, especially from the skin of the fruit, may introduce and support a larger population of these bacteria, even under less-than-ideal conditions. Because of the presence of *Acetobacter* and a high proportion of PR in P3 silage, the PR surface carries a high number of *Acetobacter*, which grows and multiplies in large quantities during the initial aerobic fermentation phase of silage fermentation, resulting in a large relative abundance of *Acetobacter*, which in turn affects the quality of the silage.

Change of the microbial genus and its abundance will affect the generation of fermentation products during ensiling [45]. In this experiment, LA and AA contents were positively correlated with *unclassified_f__Enterobacteriaceae*, *Weissella*, *Klebsiella*, and *Pediococcus*. However, the pH value and NH_3-N content were negatively correlated with *unclassified_f__Enterobacteriaceae*, *Weissella*, *Klebsiella*, and *Pediococcus*. This suggests that LA, AA, NH_3-N content, and pH value are simultaneously affected by them and that increasing their abundance improves fermentation quality.

5. Conclusions

Mixed CS with appropriate PR silage showed a lower pH value and higher LA and AA contents. Also, the addition of PR lead to an increase in the relative abundance of *Lactobacillus* in mixed silage. Consequently, the addition of PR could enhance CS silage quality, and the optimum addition ratio for PR was 15% on a FM basis.

Author Contributions: Conceptualization, C.Y. and X.W.; methodology, H.X. and F.Z.; software, D.L.; validation, L.P., C.Y. and D.L.; formal analysis, D.L. and X.L.; investigation, C.Y.; resources, C.Y.; data curation, D.L.; writing—original draft preparation, D.L. and X.S.; writing—review and editing, D.L. and H.X.; visualization, F.Z.; supervision, C.Y.; project administration, C.Y.; funding acquisition, C.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Guangxi Natural Science Foundation (Grant No. 2023JJA130137), the National Natural Science Foundation of China (Grant No. 32361143788), the Xinjiang Production and Construction Corps. Key Areas of Technological Research and Development (2023AB008), the Guangxi Dairy Buffalo Innovation Team of National Modern Agricultural Industry Technology System (Grant No. nycytxgxcxtd-2021-21), the Guangxi Science and Technology Major Project (Grant No. GuiKe AA22068099), the 8th Group Key Areas of Technological Research and Development (2023NY03), and the Scientific Research Project of Shihezi University (KJTP202316).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The sequence data reported in this study were archived in the Sequence Read Archive (SRA) under the accession number PRJNA1060478.

Conflicts of Interest: The authors declare no conflicts of interest.

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