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Effect of Sucrose and Lactic Acid Bacteria Additives on Fermentation Quality, Chemical Composition and Protein Fractions of Two Typical Woody Forage Silages

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Abstract: Paper mulberry (PM) and mulberry (MU) have been considered potential substitutes for traditional forages in response to the increasing demand for high-protein feed for livestock. To improve the utility of these two typical woody forages, our study investigated the effects of sucrose and lactic acid bacteria (LAB) additives on the fermentation quality, nutritive value, and protein fractions of their leaf silages. Collected leaves were separately subjected to ensiling treatments, either with or without sucrose (S), in combination with *Lactobacillus plantarum* (LP), or *Lactobacillus casei* (LC). The silage was sampled and analyzed for fermentation parameters, carbohydrates, and protein fractions after ensiling for 60 days. The pH value of paper mulberry silages with S was 19% lower than that without S, while LAB-treated mulberry silages showed decreased ammonia nitrogen (by 71%) and fraction A in crude protein (by 15%) compared with no LAB additives. In summary, adding S improved the fermentation quality, with no positive effect on protein fractions, in PM silage, whereas LAB additives improved the potential utilization of protein in MU silage.

Keywords: woody forage; mulberry; paper mulberry; silage additive; CNCPS

1. Introduction

A regional lack of green high-protein forage resources is a key reason to develop animal husbandry in the equatorial region. This shortage is caused by climatic and environmental factors [1,2]. In southern China, focus has been placed on the technology of processing and feeding of tropical and subtropical woody bioresources recently [3]. There are two typical woody forages with potential development value: paper mulberry (PM, *Broussonetia papyrifera* L.) and mulberry (MU, *Morus alba* L.), of which preliminary utilization by livestock has been reported.

Both PM and MU are multipurpose trees, belonging to two genera of Moraceae. The CP content of the leaves of PM can reach up to 24% DM, while MU has 22% DM, with a higher water-soluble carbohydrates (WSC) content [4]. Compared with MU, with its large amount of annual fresh aboveground biomass, cultivated PM is slightly lower in yield but could be used for more than ten years periodically. The shorter regeneration time of PM is one of the advantages for feeding; the growth rate from stubble to the appropriate height for the next harvest season (1.2–1.5 m) is around 35–45 days [5]. Another advantage of PM, and a reason for preference by livestock as well, is the soft texture of the leaves, with scarce

foliar villi by hybridization [6,7]. In contrast, MU is favored in plains areas because of inclined branches when planted in high density, which is more convenient for mechanized harvesting [8].

Due to the adaptability of woody forages to continuous growth and periodic harvesting in high temperature and rainy seasons, ensiling is the main approach to woody forage production when other processing technologies, such as hay-making, are costly or risky [3]. Studies on spontaneous fermentation dynamics and diversity of bacterial communities of woody forage silages have reported that most of the crude protein content could be properly preserved with effective lactic acid fermentation [4]. It has been also proven that lactic acid bacteria (LAB) and the nutrient substrates of the forage are crucial factors to improve the fermentation quality and nutritional value of high-protein forage silages in the early stage of ensiling [9]. Belonging to one category of directly fermented water soluble carbohydrates (WSCs), sucrose is often used in research to assist LAB to improve the quality of silages [10]. However, to our knowledge, few publications have focused on the characteristics, and especially protein fractions, of processed woody forage silages. Therefore, the goal of our study was to investigate the effects of *Lactobacillus plantarum* (LP) and *Lactobacillus casei* (LC), with or without sucrose, on PM and MU leaf silage.

2. Materials and Methods

2.1. Silage Preparation

Woody forages were harvested during the vegetative growth period after about three months of growth at the Zengcheng experimental field of South China Agricultural University (23.14 N, 113.32 E, elevation 250 m, annual mean temperature 15 °C, average annual precipitation 603 mm; Guangzhou, Guangdong Province, China). Both kinds of leaves were manually chopped into 1–2 cm pieces immediately after collection. The *Lactobacillus plantarum* (LP) and *Lactobacillus casei* (LC) strains were isolated and purified from silages studied earlier, and the additives for silage preparation were made via lyophilization according to the reported procedures [11]. Raw materials were separately subjected to ensiling treatments based on a 2 × 3 factorial arrangement in a completely randomized design, either with or without sucrose (20 g/kg on a fresh matter basis), dissolved in 10 mL sterilized deionized water containing nothing or cultured LP or LC (1.0×10^5 colony forming unit/g on a fresh matter basis). Six groups of treatments were labeled with a combination of S0 and S2 with CK, LC, and LP, respectively. The given solvent was sprayed with a disposable tiny sprayer onto minced leaves for every treatment. After mixing the ingredients thoroughly, four replicates (one for backup) of 200 g of each treated batch were packed into laboratory polyethylene bags (18 cm × 26 cm) and sealed with a vacuum sealing machine at a density of approximately 642 kg fresh weight (FW)/m^3 (DZ-280/2SE, Furuide Machinery Co., Ltd., Shandong, China). The silages were stored at ambient temperature conditions (25–28 °C), and opened after 60 days of ensiling for analysis.

2.2. Silage Fermentation

Silage samples were divided into samples of 20 g by the quartering method and then mixed in a blender with 180 mL sterilized distilled water for 1 min, and filtered through three layers of qualitative filter paper. The filtrate was collected for measuring the pH value, ammonium nitrogen (NH_3-N), lactic acid (LA), acetic acid (AA), propionic acid (PA), and butyric acid (BA). Specifically, the pH value was measured using a glass electrode pH meter (FiveEasy 20K; Mettler-Toledo International Inc., Greifensee, Switzerland). The NH_3-N concentration was determined with the phenol-sodium hypochlorite method [12], and the above four organic acids were analyzed using a high-performance liquid chromatography method, as previously described, with some adjusted operations (column, Shodex RS Pak KC-811; Showa Denko K.K., Kawasaki, Japan; detector, DAD, 210 nm, SPD-20A; Shimadzu Co., Ltd., Kyoto, Japan; eluent, 3 mmol L^{-1} $HClO_4$; flow speed, 1.0 mL min^{-1}; column oven temperature, 50 °C) [13].

2.3. Chemical Composition

Dry matter (DM) of woody forages and silages was measured after drying in a forced-air oven at 65 °C for 48 h, and then samples were ground in a hammer mill to pass through a 1 mm screen. The DM concentration was corrected for the loss of volatile compounds according to Porter and Murray [14], and the variables after ensiling were presented on the basis of corrected DM. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to the method of Van Soest et al. [15], using an ANKOM A2000 fiber analyzer. Furthermore, the contents of hemicellulose (HC) and cellulose (CE) were calculated using the difference between NDF and ADF, and the difference between ADF and ADL, respectively. The WSC content was determined using the improved anthrone colorimetric assay [16]. Crude protein (CP) was measured according to the Association of Official Analytical Chemists (AOAC) International (2000) procedures [17]. In addition, the buffering capacity (BC) of woody forage raw materials was analyzed by titration with lactic acid (0.1 mol L^{-1}) [18]. Specifically, 1 g of sample powder was suspended in 100 mL of distilled water for 30 min. The volume for lactic acid titration during the pH value of suspension down to 4.00 was recorded.

2.4. Protein Fraction

The protein fraction was calculated by the CNCPS (Cornell Net Carbohydrate and Protein System), and divided into three fractions: (1) non-protein nitrogen (NPN; fraction A, FA), (2) true protein (fraction B, FB), and (3) bound true protein (fraction C, FC). Based on the intrinsic rates of ruminal degradation, FB was further partitioned into three subsections, including the FB1, FB2, and FB3 fractions, representing rapidly degraded protein, intermediately degraded protein, and slowly degraded protein in the proper order. The NPN, soluble protein (SOLP), neutral detergent-insoluble protein (NDIP), and acid detergent-insoluble protein (ADIP) of the silages were determined as described by Licitra et al. [19]. The protein fraction was calculated according to Sniffen et al. [20].

$$\mathrm{FA(\%CP)} = \mathrm{NPN(\%SOLP)} \times 0.01 \times \mathrm{SOLP(\%CP)} \quad (1)$$

$$\mathrm{FB1(\%CP)} = \mathrm{SOLP(\%CP)} - \mathrm{FA(\%CP)} \quad (2)$$

$$\mathrm{FB2(\%CP)} = 100 - \mathrm{FA(\%CP)} - \mathrm{FB1(\%CP)} - \mathrm{FB3(\%CP)} - \mathrm{FC(\%CP)} \quad (3)$$

$$\mathrm{FB3(\%CP)} = \mathrm{NDIP(\%CP)} - \mathrm{ADIP(\%CP)} \quad (4)$$

$$\mathrm{FC(\%CP)} = \mathrm{ADIP(\%CP)} \quad (5)$$

2.5. Statistical Analysis

Data were analyzed using the software program JMP 14 (SAS Institute). The effects of treatment on the protein fractions were separately determined for each kind of woody forage silage by one-way analyses of variance (ANOVA). The fermentation quality and chemical composition parameters were determined according to the model for a factorial treatment design:

$$Y_{ij} = \mu + I_i + T_j + (I + T)_{ij} + e_{ij} \quad (6)$$

where Y_{ij} is the observed value; μ is the mean; I_i is the effect of adding sucrose (S); T_j is the effect of LAB additives (Ad); $(I + T)_{ij}$ is the effect of interaction between S and Ad; and e_{ij} is the residual error. Tukey's test was used for multiple comparisons, with differences declared significant at $p \leq 0.05$.

3. Results

3.1. Raw Material Characteristics Before Ensiling

Table 1 shows the characteristics of the two forages. The BC values of PM and MU were almost equal (83.54 g lactic $acid^{-1}$ kg DM and 83.55 g lactic $acid^{-1}$ kg DM). Significant differences ($p < 0.01$) were found in DM (28.74 % FW and 38.67 % FW). As for nutritional

content, PM leaves were significantly ($p < 0.01$) higher than MU leaves in CP (25.97% DM and 19.38% DM), NDF (34.24% DM and 18.43% DM), ADF (23.63% DM and 12.77% DM), ADL (10.28% DM and 2.62% DM), hemicellulose (10.60% DM and 5.65% DM), and cellulose (11.64% DM and 8.53% DM). In addition, the WSC content of PM leaves (3.12% DM) was significantly ($p < 0.01$) lower than that of MU leaves (10.72% DM).

Table 1. Characteristics of woody forage raw materials.

Items	PM	MU	SEM	*p*-Value
BC (g LA^{-1} kg DM)	83.54	83.55	0.00	NS
DM (%FW)	28.74 ^{b}	38.67 ^{a}	4.97	**
CP (%DM)	25.97 ^{a}	19.38 ^{b}	3.29	**
NDF (%DM)	34.24 ^{a}	18.43 ^{b}	7.90	**
ADF (%DM)	23.63 ^{a}	12.77 ^{b}	5.43	**
ADL (%DM)	10.28 ^{a}	2.62 ^{b}	3.83	**
HC (%DM)	10.60 ^{a}	5.65 ^{b}	2.47	**
CE (%DM)	11.64 ^{a}	8.53 ^{b}	1.55	**
WSC (%DM)	3.12 ^{b}	10.72 ^{a}	3.80	**

PM, paper mulberry; MU, mulberry; BC, buffering capacity; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; HC, hemicellulose; CE, cellulose; WSC, water-soluble carbohydrates; SEM, standard error of the mean; NS, not significant; **, Significant at 0.01; means in the row (a–b) with different superscript letters differ significantly from each other ($p < 0.05$).

3.2. Fermentation Quality of Silages

After ensiling for 60 days, all fermentation indicators, including pH value, LA, AA, PA, BA, and NH_3-N, were affected to varying degrees by different treatments (Table 2). Both S and Ad caused significant differences ($p < 0.01$) in the pH of the two woody forage silages, while significant interactions occurred only in MU silage ($p < 0.01$). Specifically, S2 resulted in a lower pH value in PM silage, and the lowest grade occurred in the S2+LP and S2+LC samples. Regardless of adding sucrose, silages inoculated with LP and LC had lower pH values for both PM and MU. LP caused a greater pH reduction than LC in PM, while the opposite was found in MU. Furthermore, the mean of the pH value in the PM group was higher than for MU. For the four organic acids, BA was not detected in the two kinds of woody forage silages, but there were significant differences in contents of LA, AA, and PA. The mean value of PM was lower than that of MU in LA and higher in AA and PA. Only S2-treated silages had lactic acid in PM, while the lactic acid content in the S2 group was higher than that in the S0 group of MU (except S2+LC). In PM samples, the S2+LC-treated silages contained the most LA, and S2+LP-treated silages had lowest AA and PA concentrations. For the MU samples, LP and LC-treated samples did not contain PA, and the AA concentration of S2+LP-treated silages was strikingly higher than that of S2+CK.

Compared with MU silages, the NH_3-N concentration of PM silages was significantly higher ($p < 0.01$). S reduced the NH_3-N concentration of PM and MU silages significantly ($p < 0.01$), while Ad exerted a significant effect only in the MU samples ($p < 0.01$). The production of NH_3-N in LP and LC-treated PM samples with sucrose was inhibited compared to those without sucrose. LC resulted in a greater decrease of the NH_3-N concentration than LP in S0-treated silages of MU. However, the reduction caused by various LAB additives did not show differences in the S2-treated silages of MU and all samples of PM.

3.3. Chemical Nutrition of the Silages

Additional sucrose in the PM samples resulted in differences in DM, CP, ADF, ($p < 0.05$) and CE ($p < 0.01$), and adding sucrose or additives affected ($p < 0.01$) the contents of CP, NDF, ADF and WSC of MU silages, respectively (Table 3). Silages of PM were higher ($p < 0.05$) in CP, NDF, ADF, HC, and CE compared with MU silages (Table 3). Meanwhile, no difference ($p > 0.05$) was found in the ADL and WSC from two woody forage silages. Sucrose application increased the DM content of PM silage inoculated with LP, while

the DM content of S2+LC-treated MU samples was higher than the other treatments. Inoculation of LP and LC with sucrose reduced the CP content of MU silage, but LC led to a lower loss than LP. Interestingly, there was no significant difference between each treatment in CP content in PM silages ($p > 0.05$). S2+CK-treated silage had lower contents of NDF, ADF, ADL, HC, CE, and WSC than S0+CK in PM. LP or LC-treated MU silages also showed lower contents of NDF, ADF, ADL, and HC than CK. It is worth emphasizing that the combination of LC or LP with S2 led to a lower WSC content than S0 in PM silages, while the opposite occurred in MU silages.

Table 2. The effect of sucrose and additives on fermentation quality of woody forage silages.

		S0			S2					*p*-Value		
Items	**Species**	**CK**	**LP**	**LC**	**CK**	**LP**	**LC**	**Mean**	**SEM**	**S**	**Ad**	**S × Ad**
pH value	PM	6.89 ^{a}	6.46 ^{c}	6.71 ^{b}	5.71 ^{d}	5.47 ^{e}	5.50 ^{e}	6.13 ^{A}	0.25	**	**	NS
	MU	5.06 ^{a}	4.33^{c}	4.15 ^{d}	4.73^{b}	4.14 ^{d}	3.99 ^{e}	4.40 ^{B}	0.17	**	**	**
LA (%DM)	PM	ND	ND	ND	0.76 ^{b}	3.14 ^{a}	4.58 ^{a}	1.41 ^{B}	0.80	**	NS	NS
	MU	2.52 ^{b}	2.17 ^{b}	3.51 ^{ab}	3.69 ^{ab}	6.11 ^{a}	2.10 ^{b}	3.35 ^{A}	0.62	NS	NS	*
AA (%DM)	PM	3.02	2.84	2.10	4.00	2.01	2.17	2.69 ^{A}	0.31	NS	NS	NS
	MU	0.46 ^{ab}	ND	ND	0.24 ^{b}	0.83 ^{a}	ND	0.26 ^{B}	0.14	NS	*	**
PA (%DM)	PM	2.27 ^{ab}	1.76 ^{ab}	1.66 ^{ab}	3.52 ^{a}	0.53 ^{b}	1.00 ^{ab}	1.79 ^{A}	0.43	NS	NS	NS
	MU	0.5	ND	ND	0.48	ND	ND	0.16 ^{B}	0.10	NS	NS	NS
BA (%DM)	PM	ND	ND	ND	ND	ND	ND	ND	ND	NS	NS	NS
	MU	ND	ND	ND	ND	ND	ND	ND	ND	NS	NS	NS
NH_3-N (%TN)	PM	16.05 ^{ab}	17.88 ^{a}	17.02 ^{a}	12.42 ^{bc}	11.57 ^{c}	11.82 ^{c}	14.44 ^{A}	1.16	**	NS	NS
	MU	6.68 ^{a}	2.86 ^{b}	1.82 ^{c}	5.91 ^{a}	1.65 ^{c}	1.02 ^{c}	3.32 ^{B}	0.98	**	**	NS

PM, paper mulberry; MU, mulberry; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; NH_3-N, ammonia nitrogen; TN, total nitrogen; ND, not detected; SEM, standard error of the mean; NS, not significant; S0, ensiling without sucrose; S2, ensiling with 20 g/kg sucrose on a fresh matter basis; CK, ensiling without lactic acid bacteria additives; LP, ensiling with *Lactobacillus plantarum* at the level of 10^6 cfu/g of fresh matter; LC, ensiling with *Lactobacillus casei* at the level of 10^6 cfu/g of fresh matter; S, sucrose; Ad, lactic acid bacteria additives; *, significant at 0.05; **, significant at 0.01; means in the same row (a–e) or column (A–B) with different superscript letters differ significantly from each other ($p < 0.05$).

Table 3. The effect of sucrose and additives on chemical composition of woody forage silages.

Items	**Species**	S0			S2					*p*-Value		
		CK	**LP**	**LC**	**CK**	**LP**	**LC**	**Mean**	**SEM**	**S**	**Ad**	**S × Ad**
DM (%FW)	PM	27.0 ^{ab}	25.7 ^{b}	26.9 ^{ab}	27.4 ^{ab}	28.9 ^{a}	28.6 ^{a}	27.4 ^{B}	0.48	*	NS	NS
	MU	32.8 ^{b}	33.4 ^{b}	33.5 ^{b}	33.7 ^{b}	34.7 ^{b}	41.3 ^{a}	34.9 ^{A}	1.31	NS	NS	NS
CP (%DM)	PM	23.5	23.4	23.7	22.4	23.2	22.7	23.1 ^{A}	0.20	*	NS	NS
	MU	19.1 ^{a}	18.9 ^{a}	19.1 ^{a}	19.1 ^{a}	17.6 ^{c}	18.3 ^{b}	18.7 ^{B}	0.26	**	**	*
NDF (%DM)	PM	29.6 ^{a}	22.0 ^{b}	25.4 ^{ab}	20.6 ^{b}	27.0 ^{ab}	23.3 ^{ab}	24.7 ^{A}	1.36	NS	NS	*
	MU	21.1 ^{a}	18.8 ^{bc}	18.9 ^{bc}	20.1 ^{ab}	16.9 ^{d}	17.4 ^{cd}	18.9 ^{B}	0.64	**	**	NS
ADF (%DM)	PM	18.7 ^{a}	17.4 ^{ab}	18.4 ^{ab}	16.4 ^{b}	17.3 ^{ab}	17.5 ^{ab}	17.6 ^{A}	0.33	*	NS	NS
	MU	15.0 ^{a}	14.2 ^{ab}	13.5 ^{bc}	14.1 ^{ab}	12.8 ^{cd}	12.3 ^{d}	13.6 ^{B}	0.41	**	**	NS
ADL (%DM)	PM	4.0	2.9	4.0	3.2	3.2	4.1	3.6	0.21	NS	NS	NS
	MU	4.5 ^{a}	3.3 ^{ab}	4.0 ^{ab}	4.5 ^{a}	2.8 ^{b}	3.1 ^{ab}	3.7	0.30	NS	*	NS
HC (%DM)	PM	10.8 ^{a}	4.6 ^{c}	7.1 ^{abc}	4.2 ^{c}	9.7 ^{ab}	5.8 ^{bc}	7.0 ^{A}	1.12	NS	NS	**
	MU	6.2 ^{a}	4.6 ^{bc}	5.4 ^{abc}	6.0 ^{ab}	4.2 ^{c}	5.2 ^{abc}	5.3 ^{B}	0.32	NS	**	NS
CE (%DM)	PM	14.0 ^{a}	12.9 ^{ab}	13.3 ^{a}	11.8 ^{b}	12.8 ^{ab}	11.7 ^{b}	12.8 ^{A}	0.36	**	NS	NS
	MU	9.2 ^{ab}	9.5 ^{a}	8.5 ^{ab}	8.6 ^{ab}	8.3 ^{b}	8.2 ^{b}	8.7 ^{B}	0.21	*	NS	NS
WSC (%DM)	PM	0.6 ^{ab}	0.7 ^{a}	0.6 ^{bc}	0.6 ^{bc}	0.6 ^{bc}	0.5 ^{c}	0.6	0.02	NS	NS	NS
	MU	1.0 ^{b}	1.3 ^{b}	1.1 ^{b}	1.0 ^{b}	2.5 ^{a}	1.5 ^{b}	1.4	0.23	**	**	*

PM, paper mulberry; MU, mulberry; DM, dry matter; FW, fresh weight; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; HC, hemicellulose; CE, cellulose; WSC, water-soluble carbohydrates; SEM, standard error of the mean; NS, not significant; S0, ensiling without sucrose; S2, ensiling with 20 g /kg sucrose on a fresh matter basis; CK, ensiling without lactic acid bacteria additives; LP, ensiling with *Lactobacillus plantarum* at the level of 10^6 cfu/g of fresh matter; LC, ensiling with *Lactobacillus casei* at the level of 10^6 cfu/g of fresh matter; S, sucrose; Ad, lactic acid bacteria additives; *, significant at 0.05; **, significant at 0.01; means in the same row (a–d) or column (A–B) with different superscript letters differ significantly from each other ($p < 0.05$).

3.4. Protein Fractions of the Silages

The two woody forages showed similar profiles of protein fractions (Figure 1). The contribution of FB was at least 47.90% in PM and 55.19% in MU. Differences caused by treatments classified PM silages significantly ($p < 0.05$) only in terms of the FA, FB2 and FB3 fractions (Figure 1a), while they were found for MU silages in all fractions (Figure 1b). Comparing S0-CK samples of PM, S0-LP and S2-CK increased in the FA fraction and decreased in the FB3 fraction. S2-LP had a lower FB2 fraction and higher FA fraction in PM. Regardless of adding sucrose or not in MU silages, the FA fraction was decreased by LP and LC, and furthermore, the FC fraction was decreased by mixing with sucrose concurrently. Except for S0+LP treated samples in MU, the FB3 fraction of all treatments was higher than that in S0+CK. Moreover, LP caused MU silages to contain a lower FB1 fraction and higher FB2 fraction than LB.

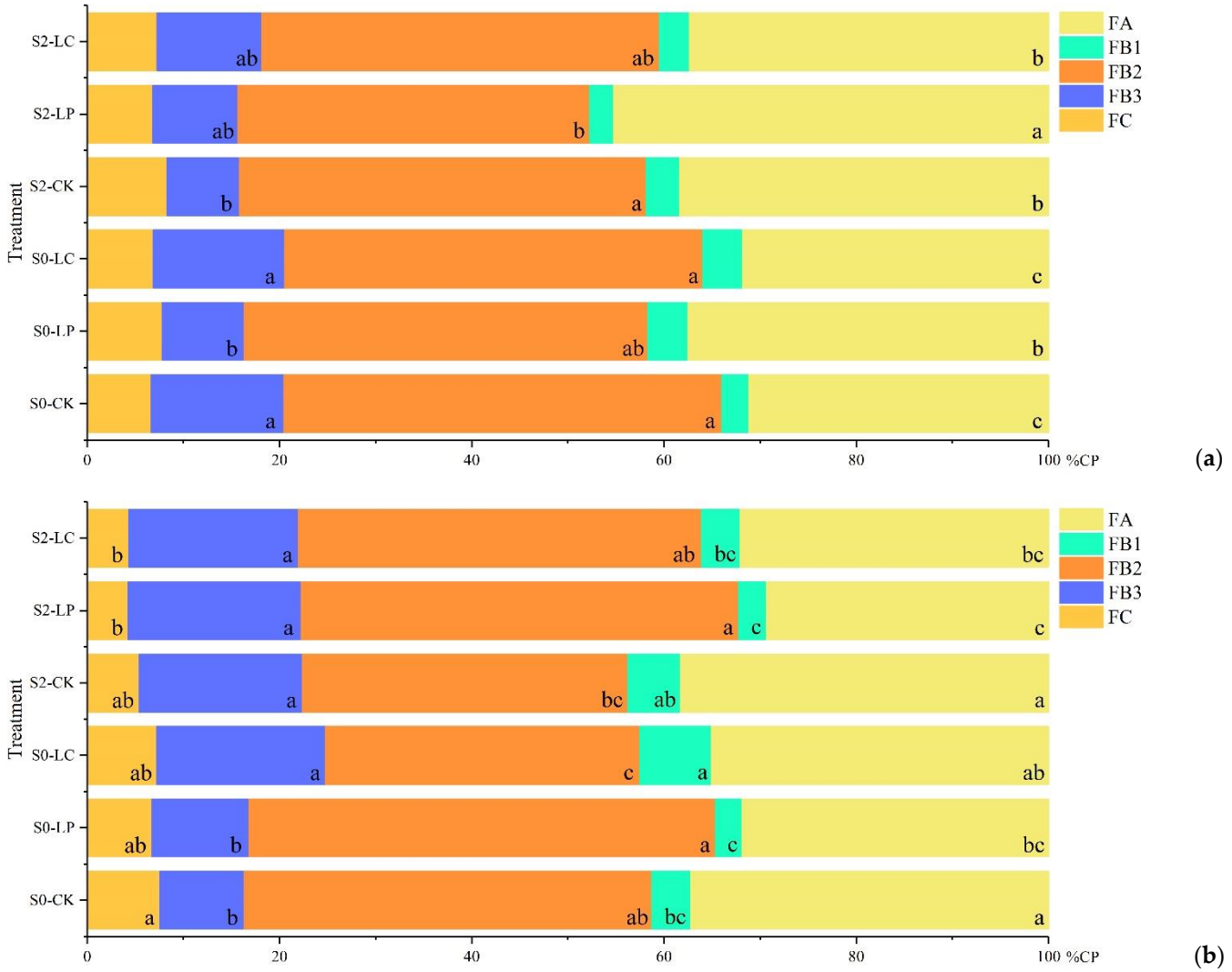

Figure 1. Protein fractions of leaf silage in paper mulberry (**a**) and mulberry (**b**). Different letters differ significantly from each other ($p < 0.05$) for the same color patch in each kind of silage. S0, ensiling without sucrose; S2, ensiling with 20 g /kg sucrose on a fresh matter basis; CK, ensiling without lactic acid bacteria additives; LP, ensiling with *Lactobacillus plantarum* at the level of 10^6 cfu/g of fresh matter; LC, ensiling with *Lactobacillus casei* at the level of 10^6 cfu/g of fresh matter; FA, fraction A; FB1, fraction B1; FB2, fraction B2; FB3, fraction B3; FC, fraction C.

4. Discussion

In our present study, two indigenous woody forages with the advantages of good resistance, convenient seedling sources, and uncomplicated management were planted. The results demonstrated that, for the leaves from PM and MU, we can reduce nutrient loss by ensiling, and the quality of silages may be improved by adding fermentable substrates and LAB additives. Both PM and MU are suitable for ensiling and have potential as high-protein forage silages for livestock.

All collected raw material samples showed a high CP content (25.97% DM and 19.38% DM), which was even higher than the content of common alfalfa varieties in southern China (from 16.5% DM to 20.4% DM) [21]. The carbohydrate components, including NDF, ADF, and ADL, were similar to the previously reported range [22,23]. In addition to the value of BC, the sharp gaps of WSC content might also be the reason for the difference of fermentation quality. PM and MU are deciduous broad-leaved species, and the wide variation in the WSC content of leaves was attributed to varieties and cycles of growth [24]. The WSC content of mulberry leaves from 45 germplasms and varieties ranged from 3.99% DM to 17.44% DM [8]. As a previous study showed, BC value and WSC content indicate the start of continuous fermentation, and endow silages with acceptable quality [3]. Thus, sucrose was reported as a supplement in the evaluation of the protein composition of silages, to balance the substrate limitations at early ensiling stages [25].

Previous studies have also attributed successful ensiling to the contribution made by LAB in anaerobic environments [26]. The low pH value and high LA content caused by enrichment of LAB are generally indicators of excellent silage quality. As the results showed, although PM silages only inoculated with LP or LC had a lower pH than the control, they were still over 6.00, while adding S (no matter whether in combination with LP or LC) would lead to an even lower pH of around 5.40–5.70. There is no significant difference of AA content between S0-CK and S2-CK, but trends of increasing AA by adding S may indicate that offering more WSC for PM may promote fermentation by the microorganisms attached to the plant surface. It should be noted that the contents of AA and PA in PM silage were higher than those in MU silage. Moreover, S2-treated PM silages showed lower PA contents than S0 with LAB, but the opposite occurred without LAB additives. This is likely because yeast seized more fermentation substrates without enough LAB load, resulting in the production of PA, which has been reported in high-moisture silage [27]. An analysis pointed out that AA and PA concentrations were significantly increased only when heterofermentative LAB was applied [28], suggesting another possibility: the majority of LAB in the surface microorganisms of PM might be heterofermentative bacteria, and may occupy a dominant position during ensiling. Our present study showed that exogenous sucrose improves LA content and decreases the pH value in silages of woody forage, in line with studies on alfalfa [29], kenaf [30] and king grass [10].

It has been reported that all epiphytic microorganisms on raw materials involved in the metabolic process are present at the beginning of fermentation, but LAB ensured that the fermentation would go in a positive direction [31]. Once the LAB load from the initial microbial population was greater than 1.0×10^5 colony forming unit/g of fresh weight, spoilage organisms could be inhibited [32]. The dominant LAB population provided by additives, both in PM and MU leaves, assisted the silages to show a lower pH value and higher LA content than CK. For MU silage, the pH value after ensiling was lower than PM silage. Adding different additives could drop the pH value to around 4.00. Furthermore, adding LC caused the silages show a lower pH value than LP, which might suggest that LC is a better additive for MU compared to LP. However, the high content of ammonia nitrogen may indicate that these LAB additives are not the best choice for PM ensiling, and we need to focus more on isolation of LAB specific to use with PM.

The high content of moisture in the silage was proven to be a possible condition for the activity of undesirable microorganisms, such as *Clostridium perfringens*, *Clostridium sporogenes*, *Clostridium ghonii*, and *Clostridium sartagoforme* [33], leading to spoilage more frequently in low DM silages. The DM contents both of raw materials and silages in

our study were from 25.72% FW to 41.32% FW, which are higher than adequate values (25% FW) considered by some researchers, and could inhibit the decomposition of the carbohydrate components [34]. However, the significant difference of DM content might still be an important factor leading to the different ensiling difficulty of the two woody forages. Lower NDF and ADF concentrations were observed in silages of MU than in PM. Carbohydrate components were reported to be disintegrated to a certain extent by LAB in the early ensiling stage, which might accelerate the domination of LAB. One kind of LAB has been proven to decrease the NDF content of alfalfa silage, because it produces ferulate esterase to assist degradation of the plant cell wall, and was aimed to release substrates and promote the reproduction of lactic acid bacteria [35]. The high ADL content of PM leaves might be due to the vigorous metabolic pathway in phenylpropanoid biosynthesis [7].

Limited proteolytic processes caused by the plants and microorganisms led to changes of protein fractions and the use of protein grading, presented as CNCPS, which could simulate and evaluate the digestion and utilization of protein in silages. According to previous studies, the protein degradability values significantly differed among forages. FA and FB2 were reported as the main fractions in alfalfa silage, comprising 46.2% CP and 36.5% CP, respectively [36]. However, for the silages of *Moringa oleifera* leaves, FB2 (55.2 % CP) was more than twice as abundant as FA (26.65% CP). In addition, the LAB additives have been reported to contribute to proteolysis inhibition by creating an acidic environment, resulting in a decrease of the FA concentration [37]. Generally, true protein is decomposed into peptides, free amino acids, ammonia, and other non-protein nitrogen by the action of plant proteases and microbial enzymes during silage production [38]. Most of the ammonia and amines are produced by microbial enzymes, not by plant proteases [39]. These results may be the main reason for the differences in enzyme activity between plant proteases and microbial enzymes. Alfalfa silages inoculated with LAB had a smaller proportion of FA than the control [40]. The pH value was negatively correlated with the fermentation time, and the enzyme activity decreased at a low pH value. In woody forage silages, when the LAB became the dominant bacteria, the decline rate of pH slowed. This process took 30–60 days in PM and MU silages, and 15 days in silages of *Moringa oleifera*. In the present study, the LC-treated silages showed a lower FA than LP in PM after ensiling for 60 days [4]. Furthermore, silages treated by S2 and LAB in MU showed a lower FC and higher FB3. A possible explanation for this shift is that the inoculants broke part of the chemical bonds during ensiling, untying the structure of some polyphenol-protein compounds [41]. Further research is needed, with more evaluations of the digestibility of the protein and carbohydrate components in PM and MU silages, including changes over time, which may provide more information for feeding livestock.

5. Conclusions

The present study showed that S2-treated PM silages had a lower pH value and higher LA content than S0, and LAB-treated MU silages had lower NH3-N and FA concentrations than CK. In summary, adding S improved fermentation quality with no positive effect on protein fractions in PM silage, while LAB additives improved the potential utilization of protein in MU silage.

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