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Full Length Article

Addition of Consortium of Lactic Acid Bacteria Inoculants Improved Fermentation Quality and Microbial Communities of Whole Corn Silage

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Abstract

This study aimed to explore how a consortium of lactic acid bacteria (LAB) inoculants impacted fermentation quality and microbial communities of whole corn silage. Corn forage was chopped and ensiled with distilled water (CON) or a consortium of LAB inoculants [1×10^6 cfu of *Lactobacillus plantarum* and 5×10^5 cfu of *Lactobacillus buchneri* per gram of fresh material (FM)] for 2, 4, 7, 15, 30 and 60 days. The results showed that the dry matter (DM), neutral detergent fiber (NDF), and water-soluble carbohydrate (WSC) contents decreased following 60 days of ensiling. During the initial 7 days of ensiling, LAB silages showed a faster decrease in pH and rise in lactic acid content. The DM loss, ammonia-N concentration, and yeast count were lower in LAB silages than in CON silages during the ensiling process. The dominant phylum and genus in FM were Proteobacteria and *Rosenbergiella*, respectively. In both silage types, *Lactobacillus* was the genus present with the highest abundance. Interestingly, undesirable microorganisms, including *Paenibacillus* and *Klebsiella*, were significantly decreased in LAB silages. In conclusion, the addition of a consortium of LAB inoculants can enhance fermentation quality and reduce population of undesirable microorganisms in corn silage.

Keywords: Corn; Fermentation quality; Microbial community; Silage; Lactic acid bacteria

Introduction

Ensiling can effectively preserve forage crops (Dunière *et al*. 2013), and access to high-quality silage feed for ruminants is the goal of preparing silage. A wide variety of silage additives has been developed to enhance the quality of silages, including fermentation stimulants and inhibitors, chemicals, and enzymes (Muck *et al*. 2018). Among these, lactic acid bacteria (LAB) inoculant is one of the most used additives for preparing silage (Muck *et al*. 2018). The interaction between LAB inoculants and epiphytic microbial communities in fresh material (FM) is critical to the overall fermentation process, and the fermentation products of these microorganisms directly affect the silage quality (Xu *et al*. 2019). However, fermentation is a complex process regulated by myriad different microbes. Thus, profiling of the microbial community in ensiled forages might offer valuable insight into the development of LAB inoculants and regulation of fermentation.

Previous studies have mainly focused on the effect of LAB inoculants on silage fermentation and animal production (Rossi and Dellaglio 2007; Muck *et al*. 2013; Li *et al*. 2015). A recent meta-analysis of 130 articles revealed that LAB inoculants can improve silage fermentation by reducing the butyric acid and ammoniacal nitrogen (NH_3-N) levels and increasing the lactic acid concentrations (Oliveira *et al*. 2017). Furthermore, A recent meta-analysis of 31 lactating dairy cattle studies indicated that silage inoculated with LAB inoculants enhanced milk production (Oliveira *et al*. 2017). However, Muck *et al*. (2013) reported an improvement in silage characteristics owing to LAB inoculants but could not explain the magnitude of the increase in milk production. In another study, although inoculated silage increased animal productivity, the inoculants did not affect silage fermentation (Kung and Muck 2015). Ellis *et al*. (2016) suggested that these inconsistent results may be due to variations in microbial communities between silages and interactions with microbes in the rumen, implying that profiling of the silage microbial community might improve our understanding of silage formation.

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Denaturing gradient gel electrophoresis (Li and Nishino 2011), real-time PCR (Stevenson *et al*. 2006), and ribosomal intergenic spacer analysis (Brusetti *et al*. 2011), have been used to assess the microbial community in silages. However, these approaches offer limited insight regarding the overall properties of microbial communities. More recently, next-generation high-throughput sequencing technologies have been used to investigate microbial communities in many flower silver grass (*Miscanthus floridulu* (Labnll.) Warb) (Li *et al*. 2015), soybean (*Glycine max* Merr.) (Ni *et al*. 2017), corn stalk (Zhang *et al*. 2018) and corn silages (Keshri *et al*. 2018). However, climatic conditions of different regions affect the fermentation quality of corn silage by influencing microbial community (Guana *et al*. 2018). Therefore, it is valuable and worthwhile to combine microbial community and fermentation in local corn silage with silage chemical analysis. LAB inoculants used to improve the quality of silages can be divided into homofermentative and heterofermentative based on their fermentation pattern. Homofermentative LAB decrease silage pH during early ensiling stages by fermenting water-soluble carbohydrates (WSC) to lactic acid, which inhibits molds, yeasts, pathogenic bacteria, and other detrimental microbes, thus enhancing silage fermentation (Weinberg *et al*. 1993; Keshri *et al*. 2018). In contrast, heterofermentative LAB can convert lactic acid into acetic acid during later ensiling stages, thus improving the aerobic stability of silages during feeding out (Hu *et al*. 2009).

Thus, consortium of inoculants is commonly used in the production of silage to exploit the benefits of both homo- and heterofermentative LABs (Muck *et al*. 2018). The most commonly used homo- and heterofermentative LABs are *Lactobacillus plantarum* and *Lactobacillus buchneri*, respectively (Elferink *et al*. 2001; Koc *et al*. 2017; Blajman *et al*. 2018). Therefore, the objective of this study was thus to understand how the consortium of *L. plantarum* and *L. buchneri* affect fermentation products during ensiling in corn silage and further to investigate the variation in microbial community after ensiling using Illumina MiSeq sequencing.

Materials and Methods

Silage preparation

A corn hybrid (Denghai 605; Shandong Denghai Seeds Co., Ltd., Shandong, China) was planted on July 12, 2018 in the experimental field at Shandong Academy of Agricultural Sciences (117°58′E, 37°63′N). The crop was harvested on September 16, 2018 and cut to 20 mm long segments using a chopper (FS-690; Zili, Guangdong, China).

Experimental details

Chopped forage was mixed together prior to equal division into portions for the following two treatments: distilled water (CON) and consortium of LAB inoculants (LAB). The consortium of LAB inoculants was applied at 1×10^6 colony-forming units (cfu) of *L. plantarum* and 5×10^5 cfu of *L. buchneri* per gram of fresh material (FM) by mixing 5 mL of *L. plantarum* (10^9 cfu/mL cell suspension) with 5 mL of *L. buchneri* (5×10^8 cfu/mL cell suspension) and spraying the suspension on 5 kg of chopped corn. The two LAB species were isolated from whole-plant corn silage and identified using approaches previously detailed by Zhang *et al*. (2016). These two LABs were selected based on their rapid rates of growth and significant acid production capabilities. The *L. plantarum* and *L. buchneri* strains used herein were closely related to *L. plantarum* and *L. buchneri*, with 100 and 99.79% 16S rDNA gene sequence identity, respectively. Sequences for the *L. plantarum* and *L. buchneri* strains used in the present study were deposited in GenBank with the accession numbers MN701197 and MN700263, respectively. Approximately 1000 g of pre-ensiled sample was placed into plastic bag (20 cm × 30 cm; Deli Group Co., Ltd., Shanghai, China), which was vacuum sealed (Deli 14886; Deli Group Co., Ltd.). Experiment was laid out following completely randomized design (CRD) and a total of 60 bags (2 treatments × 6 ensiling periods × 5 replicates) were made and maintained at the ambient temperature (23–28°C). Five bags for each treatment were opened for analyzing microbial community, pH value and organic acid contents after 2, 4, 7, 15, 30, and 60 days of ensiling, respectively.

Microbial and chemical analyses

Bags were unsealed on a clean workspace, after which 20 g of silage sample was mixed with 180 mL of sterile water, and the sample then underwent serial dilution from 10^{-1} to 10^{-9}. Numbers of LAB and *Clostridia* were determined using the plate count method on de Man, Rogosa, and Sharpe agar (CM361; Oxoid Ltd., Waltham, MA, USA) and Clostridia Count Agar (11045; Qingdao Rishui Bio-technologies Co., Ltd., Qingdao, China), respectively. The plates were anaerobically incubated at 37°C for 48 h using AnaeroPack-Anaero (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan). The yeast count was determined on potato dextrose agar (HB0233-12; Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) following a 30°C incubation for 48 h. Colony counts is presented as the number of viable microorganisms in cfu/g FM.

To determine the pH and organic acid content, 180 mL of sterile water was mixed with 20 g of fresh samples for 1 minute in a blender, followed by sample passage through a 0.22 μm membrane filter. Hundred milliliters of sample were used to immediately determine the pH using a glass electrode pH meter (HI991000; Hanna Instruments, RI, USA). Five milliliters of sample were stored in 1 mL of 25% (w/v) metaphosphoric acid at –20°C to determine the organic acid content. Five milliliters of sample were stored in 1 mL of 1% H_2SO_4 at -20°C to measure NH_3-N levels. The content of organic acids (lactic, acetic, butyric, and

propionic acids) was measured *via* HPLC using a Shodex RSpak KC-811S-DVB gel C column (8.0 mm × 30 cm; Shimadzu, Tokyo, Japan), under the following conditions: oven temperature, 50°C; mobile phase, 3 mmol/L $HClO_4$; flow rate, 1.0 mL/min; injection volume, 5 mL; and detector, SPD-M10AVP (210 nm). The NH_3-N content was assessed *via* phenol-hypochlorite assay as previously detailed in a study conducted by Weatherburn (1967).

A 65°C forced air oven was used to dry samples over a 48 h period, after which a Wiley mill (A. H. Thomas, PA) was used to grind sampled through a 1-mm screen. The analytical dry matter (DM) content was analyzed by weighing the sample after drying at 105°C for 3 h. Crude protein (CP), ether extract (EE), and acid detergent lignin (ADL) were measured *via* standardized protocols produced by the Association of Official Analytical Chemists (AOAC 1990). Levels of both neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the methods reported by Soest *et al*. (1991), with NDF analyses relying on amylase and sodium sulfite. The WSC content was measured *via* the anthrone method (Murphy 1958). DM recovery was calculated based on the initial and final forage weights and the DM contents of the fresh and ensiled forage.

Microbial DNA extraction and PCR amplification

Approximately 50 g per silage sample was immediately frozen with liquid nitrogen. A subsample of 5 g was ball-milled at 25–28°C for 1 min, and an EZNA Soil DNA kit (Omega Bio-tek, GA, USA) was used to isolate microbial DNA. DNA purity and quality were checked using a UV-vis spectrophotometer (NanoDrop 2000; Thermo Scientific, USA) and by 1% agarose gel electrophoresis, respectively.

The 16s rRNA V3-V4 hypervariable regions were amplified using the 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers in a GeneAmp 9700 thermocycler system (ABI, USA). Triplicate reactions were conducted, with each reaction having a 20 μL total volume containing 10 ng of DNA along with 4 μL of 5 × FastPfu Buffer, 0.4 μL of FastPfu Polymerase, 2 μL of 2.5 mM dNTPs, and 0.8 μL of the forward and reverse primers (5 μM). Thermocycler conditions were: 3 min at 95°C; 27 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; and 10 min at 72°C. Next, 2% agarose gel electrophoresis and an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA) were used to isolate PCR products, while a QuantiFluor™-ST (Promega, USA) was used for their quantification.

Sequencing and microbial diversity analysis

Equimolar concentrations of PCR amplicons were pooled prior to paired-end sequencing (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). The raw data were quality-filtered using Trimmomatic and merged with FLASH (Magoč and Salzberg 2011) based upon the criteria that follow: (i) all sequences were truncated at any ste that had a < 20 average quality score over a sliding 50 bp window; (ii) any reads were removed if they contained ambiguous bases or were exact matches to primer sequences with up to a two-nucleotide mismatch; and (iii) any sequences that had > 10 bp of overlap were merged. UPARSE (version 7.1) was used for operational taxonomic unit (OTU) clustering based upon a 97% similarity threshold, with UCHIME (Edgar 2013) used for chimeric sequence identification and removal. To analyze the taxonomy, the Silva (SSU123) 16S rRNA database was used for alignment of 16S rRNA sequences based upon the RDP classifier algorithm (version 2.2) (Wang *et al*. 2007) with a 70% confidence threshold. Alpha diversity indices (Shannon index, Chao richness estimator, and Good's coverage estimator) and beta diversity indices were determined using QIIME (v. 1.7.0). R (v. 2.15.3) was used for principal component analysis (PCA).

Statistical analysis

The data of this study were analyzed using one-way Analysis of Variance (ANOVA) analysis and Duncan's multiple range tests based on the general linear model procedure (PROC GLM) of SAS (version 9.1, SAS Institute Inc., Cary, NC, USA). All data are presented as least-squares mean. The effects of the factors were considered significant at $P \leq 0.05$ and trends were recognized at $0.05 < P \leq 0.10$.

Results

Chemical composition and microbial population before and after ensiling

Chemical composition and microbial count as determined by plate culture prior to and after 60 days of ensiling is shown in Table 1. Relative to values within the FM, DM, NDF, and WSC levels were reduced after 60 days of ensiling ($P < 0.05$), while the CP, ADF, ADL and EE were similar among the treatments ($P > 0.10$). The DM of LAB silages was higher than that of CON silages ($P < 0.05$), whereas other chemical parameters were similar between LAB and CON silages ($P > 0.10$). Compared with those in the FM, the LAB count was increased, and yeast and Clostridia counts were decreased after 60 days of ensiling ($P < 0.01$). The LAB count was higher, and the yeast count was lower in LAB silages than in CON silages, whereas the Clostridia count was similar between CON and LAB silages.

Effect of LAB inoculants on DM loss, pH, and the microbial population in corn silages

As shown in Fig. 1, following 60 days of ensiling, the DM loss from LAB silage was lower than from CON silage. The pH of CON and LAB silages decreased during the ensiling

process (Fig. 2), but LAB silage showed a fast pH decrease from the beginning of ensiling and thus, its pH was lower than that of CON during the initial 7 days of ensiling ($P < 0.05$). The populations of LAB and yeast in all treatments increased initially, and then decreased during the ensiling process, but LAB silage had a higher LAB count and lower yeast count than CON silage throughout the ensiling process ($P < 0.05$). The Clostridia count decreased with increasing ensiling time in all treatments ($P < 0.05$). The Clostridia count in LAB silage was lower than that in CON silage during the initial 7 days of ensiling, while prolonged ensiling time induced no other changes.

Impact of LAB inoculants on the content of organic acids and NH_3-N/total nitrogen (TN) in corn silages

As shown in Fig. 3, the lactic acid content was higher in LAB silages than in CON silages during the first 15 days of ensiling ($P < 0.05$), whereas no difference was observed with prolonged ensiling time ($P > 0.05$). The acetic acid content was similar between CON and LAB silages during the first 15 days of ensiling, but it was higher in LAB silage than in CON silage after 30 days of ensiling. The lactic to acetic acid ratio in LAB silages was higher at 2 days of ensiling and lower after 60 days than that in CON silages ($P < 0.05$). The NH_3-N to TN ratio was higher in CON silages than in LAB silages during the entire ensiling period (Fig. 3).

Effect of LAB inoculants on bacterial communities following 60 days of ensiling

Bacterial alpha diversity index values were assessed (Table 2). Good's coverage was approximately 0.99 for all treatments, indicating that most of the bacteria were detected. The number of OTUs was increased after 60 days of ensiling when compared to that in the FM; however, it did not significantly differ between CON and LAB silages. Other richness bacterial community indices, Chao and Shannon, showed trends similar to that of the OTUs.

Shifts in the bacterial community under different treatments can be demonstrated by PCA. As shown in Fig. 4, principal components 1 and 2 explained 50.84 and 33.98% of the total variance, respectively. The FM was well separated from the silage samples and CON silages were well separated from LAB silages.

Phylum and genus level bacterial community structures of pre-ensiled and ensiled samples are presented in Fig. 5 and 6, respectively. The dominant phylum in FM was Proteobacteria (69.94–98.51%), whereas that in silages after 60 days of ensiling was Firmicutes (66.12–95.15%; Supplementary Table S1). The dominant genera in FM were *Rosenbergiella*, *Klebsiella*, and *Pantoea*, with relative abundances of 9.7–58.5%, 11.3–29.9%, and 8.8–20.9%, respectively (Supplementary Table S2). However, the abundances of these genera were significantly reduced after ensiling. After ensiling, *Lactobacillus*, *Paenibacillus*, and *Klebsiella* were the dominant bacteria, with relative abundances of 45.7–86.5%, 2.5–24.4%, and 1.7–9.3%, respectively.

As shown in Fig. 7, differences in the bacterial microbiota among treatments and the specific bacterial microbiota in each treatment were analyzed using the linear discriminant analysis (LDA) effect size method (LDA score > 4.0). *Paenibacillus*, *Klebsiella*, and *Leuconostoc*, which were abundant in CON silages, and *Lactobacillus*, which was abundant in LAB silages, were the primary genera resulting in differences between CON and LAB silages.

Table 1: Chemical composition and microbial count determined by plate culture before and after 60 days of ensiling

	Treatments				
Item	FM	CON	LAB	SEM	*P* value
DM (% FM)	32.74^{a}	29.78^{c}	30.66^{b}	0.34	< 0.01
CP (% DM)	8.28	8.33	8.28	0.03	0.81
NDF (% DM)	39.86^{a}	38.65^{ab}	37.98^{b}	0.32	0.04
ADF (% DM)	21.18	21.32	21.58	0.20	0.74
ADL (% DM)	2.78	2.53	2.57	0.07	0.36
EE (% DM)	3.08	2.93	3.03	0.09	0.83
WSC (% DM)	10.61^{a}	4.66^{b}	4.50^{b}	0.78	< 0.01
LAB (log cfu/g FM)	5.47^{c}	6.27^{b}	6.78^{a}	0.16	< 0.01
Yeast (log cfu/g FM)	6.73^{a}	4.52^{b}	4.11^{c}	0.29	< 0.01
Clostridia (log cfu/g FM)	8.40^{a}	3.03^{b}	2.91^{b}	0.62	< 0.01

Values in the same row with different superscript letters differ at $P < 0.05$.
FM, fresh material; CON, corn silage ensiled without inoculant; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; EE, ether extract; WSC, water-soluble carbohydrate

Table 2: Alpha diversity indices of bacterial diversity in fresh material and silages after 60 days of ensiling

	Reads	OTUs	Shannon	Chao1	Good's coverage
FM	30772	105.20^{b}	1.94^{b}	142.88^{b}	0.99
CON	30772	209.40^{a}	2.23^{a}	231.46^{a}	0.99
LAB	30772	202.20^{a}	2.20^{a}	217.73^{a}	0.99

Values in the same row with different superscript letters differ at $P < 0.05$
FM, fresh material; CON, corn silage ensiled without inoculant; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*

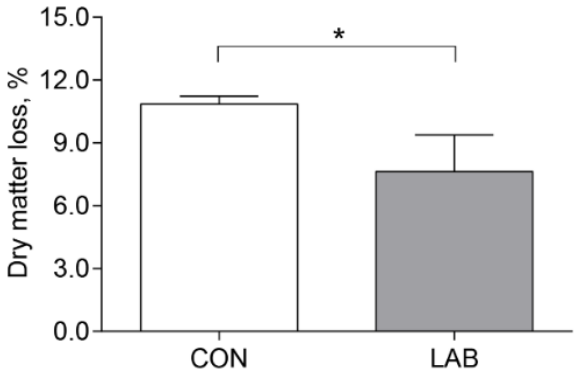

Fig. 1: Effect of consortium of LAB inoculants on dry matter loss from corn silage after 60 days of ensiling
CON, corn silage ensiled without inoculants; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*; * indicates that the dry matter loss between CON and LAB silages differed at $P < 0.05$

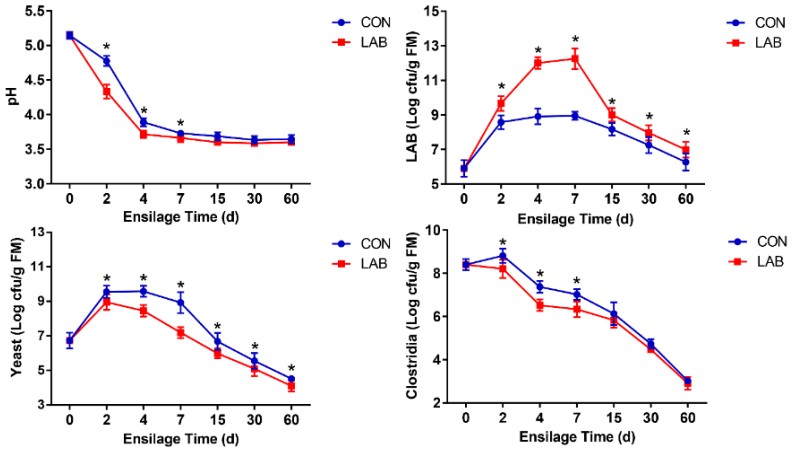

Fig. 2: Changes in pH and microbial population during the ensiling of corn silage
*$P < 0.05$ *vs*. CON

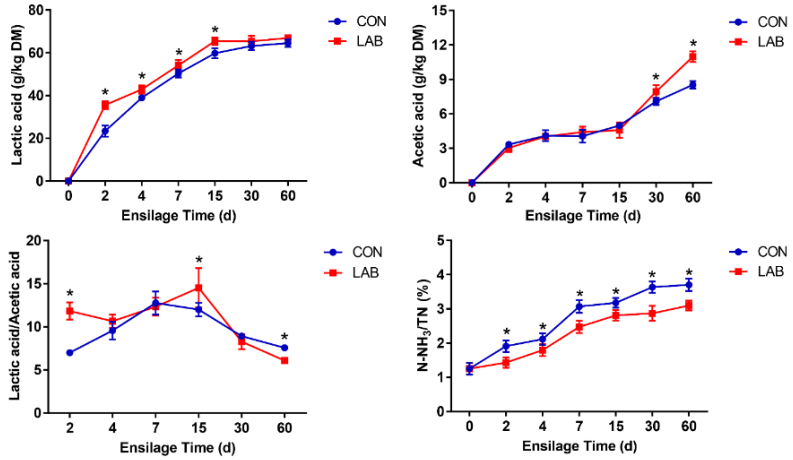

Fig. 3: Changes in organic acid and NH_3-N/TN contents during the ensiling of corn silage
*$P < 0.05$ *vs*. CON

Discussion

The LAB cell number and WSC content in FM are considered crucial factors in determining the adequacy of silage fermentation. A LAB cell count of more than 10^5 cfu/g FM (Cai *et al*. 1998) and a WSC content higher than 6% DM (Woolford and Pahlow 1984) have been reported to be adequate for ensuring acceptable fermentation quality. In this regard, the LAB count and content of WSC in the FM were sufficient for adequate fermentation during ensiling. This might explain the similar chemical compositions of LAB and CON silages. As expected, the DM and WSC content decreased after ensiling, mainly because plant cells continue to consume the oxygen entrapped in the silage material during early ensiling stages and then, microorganisms ferment the WSC mainly into lactic acid. The decreased NDF content in silages compared with FM may be the result of hydrolysis of the digestible cell wall fraction by organic acids produced during ensiling (Larsen *et al*. 2017).

One of the purposes of using silage inoculants in silage production is to reduce the loss of DM. One limitation of using *L. buchneri*, an obligate heterofermentative LAB, as a silage inoculant is that it can result in higher DM loss. Kleinschmit and Kung (2006) reported that *L. buchneri* treatment of silages alone led to higher DM loss than that in non-treated silages as indicated by a meta-analysis. However, in the present study, inoculation of *L. buchneri* combined with the homofermentative *L. plantarum* reduced DM loss when compared with that in CON silages. Driehuis *et al*. (2001) and Arriola *et al*. (2011) have reported similar results. These results indicate that inoculants that combine homo- and heterofermentative LABs can exploit the benefits of both types of inoculants in silages.

The pH fluctuation is a key indicator of microbial activity and of the process of silage fermentation (McDonald *et al*. 1991). Consistent with findings in previous studies (Desta *et al*. 2016; Ni *et al*. 2017), we found that pH fell mainly during the first 7 days of ensiling. This decreased pH observed in CON and LAB silages can

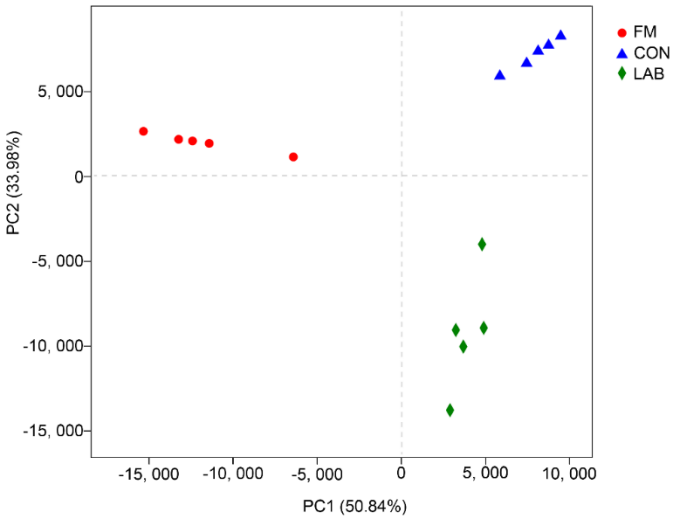

Fig. 4: Principle component analysis (PCA) of samples
PC1, principal component 1; PC2, principal component 2; FM, fresh material; CON, corn silage ensiled without inoculants; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*

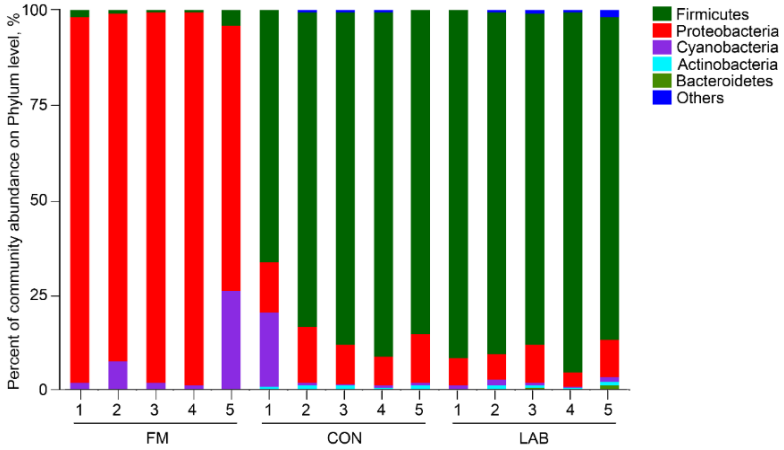

Fig. 5: Relative abundances of bacteria at the phylum level
FM, fresh material; CON, corn silage ensiled without inoculants; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*

be mainly attributed to the increased lactic acid content, which is the main organic acid throughout the ensiling process and in the final silage. In the present study, the lactic acid content considerably rose over the initial 15 days of ensiling, and then plateaued. However, Ni *et al.* (2017) reported plateauing on days 7 and 14 in soybean silage without and with molasses (2% FM), respectively. Similarly, Desta *et al.* (2016) reported plateauing on days 7 and 60 in Napier grass silage without and with molasses (4% FM), respectively. The discrepancy probably is due to differences in WSC content and buffering capacity among these treatments. The WSC content in corn silage was higher than that in soybean silage and Napier grass, resulting in more soluble carbohydrate supply for LAB metabolism. Meanwhile, the added molasses could compensate for the lack of soluble carbohydrates in soybean silage and Napier grass.

The rapid decrease in pH and increase in lactic acid content in LAB silages observed herein can be mainly linked to *L. plantarum* activity, which can minimize the activity of other microorganisms, such as enterobacteria and bacilli, in the early phase of fermentation (Muck 2010). Herein, acetic acid levels were higher in LAB silages relative to CON silages following 30 days of ensiling, implying that *L. buchneri* mediated anaerobic lactic to acetic acid conversion. Similarly, Driehuis *et al.* (1999) applied *L. buchneri* at 5×10^5 cfu/g to corn silage and noted a difference in the acetic acid content between treated and non-treated materials on day 28. Furthermore, the higher content of acetic acid in LAB silages in this study implied an improvement in the aerobic stability of the silages (Muck *et al.* 2018). As expected, the NH_3-N content was lower in LAB silages than in CON silages in this study. A recent meta-analysis of 130 articles reported similar results

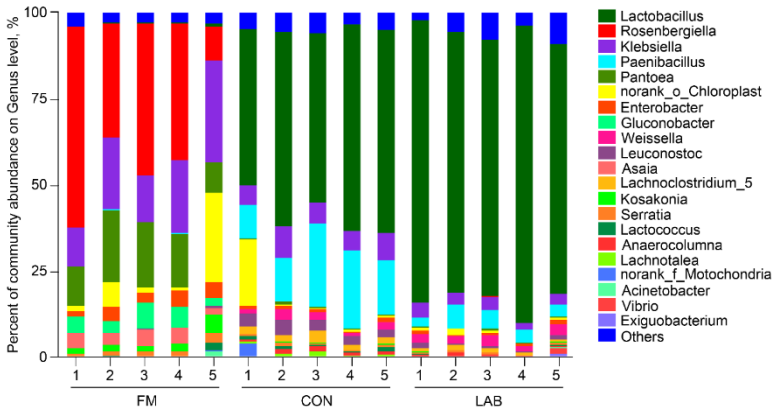

Fig. 6: Relative abundances of bacteria at the genus level
FM, fresh material; CON, corn silage ensiled without inoculants; LAB, corn silage ensiled with consortium of inoculants containing *Lactobacillus plantarum* and *Lactobacillus buchneri*

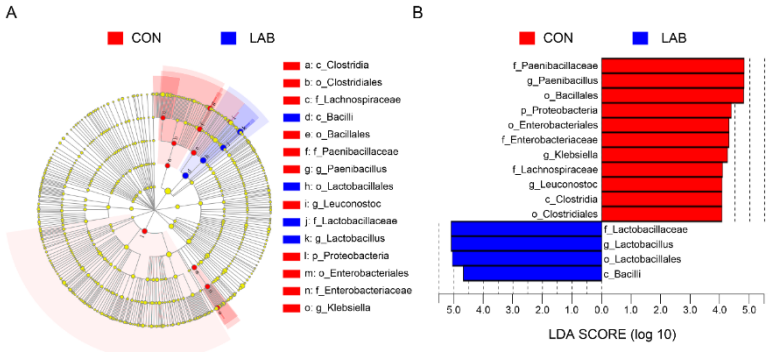

Fig. 7: Changes in the microbial community in LAB *vs*. CON silages as determined by the linear discriminant analysis effect size method (**A**) Cladogram of significantly differential bacteria. Differences are represented in the color of the most abundant taxa. (**B**) Histogram of the LDA scores for differentially abundant features among treatments. The threshold on the logarithmic LDA score for discriminative features was set to 4.0

(Oliveira *et al*. 2017). Yeasts are undesirable in silage because they cause aerobic deterioration either during the aerobic phase in the beginning of ensiling or during the unloading phase and thus reduce the nutritional value of silage. The yeast count increased on day 2 of ensiling, which might be due to the oxygen entrapped in the silage as the aerobic yeasts develop until the oxygen is exhausted (Dunière *et al*. 2013). After 2 days of ensiling, the yeast count decreased, mainly because of the decrease in pH (Ni *et al*. 2017). We found that LAB silages had lower yeast and Clostridia counts and NH_3-N content, but higher lactic acid and acetic acid contents relative to CON silages during the process of ensiling. This suggests that the addition of LAB improves the fermentation quality of silages.

Consistent with past findings (Ni *et al*. 2017; Zhao *et al*. 2017; Wang *et al*. 2018), the bacterial community was more diverse after ensiling. Proteobacteria was the dominant phylum in FM in this study. However, relative Firmicutes abundance rose during the ensiling process such that it became the predominant phylum after ensiling. Similar results had been reported in studies on silages of other materials, such as *Moringa oleifera* leaf (Wang *et al*. 2018), *Medicago* (Bao *et al*. 2016), and grass silages (Eikmeyer *et al*. 2013). We found that dominant genera in FM were mostly undesirable bacteria, such as *Rosenbergiella*, *Klebsiella*, *Pantoea*, and *Enterobacter*, which can ferment lactic acid to thereby drive nutrition loss (Ridwan *et al*. 2015). However, relative levels of these undesirable bacteria were substantially decreased after ensiling. This might be due to increasing lactic acid content accompanied by decreasing pH, resulting in growth inhibition of these undesirable microorganisms.

Lactobacillus (54.0%), *Paenibacillus* (17.1%), and *Klebsiella* (6.8%) were the dominant genera in CON silages. *Paenibacillus* is a gram-positive, facultative anaerobic, spore-forming bacterium. It has been detected in grass (Giffel *et al*. 2002) and corn (Rossi and Dellaglio 2007) silages. *Paenibacillus* spp. is undesirable microorganisms in silages. *Paenibacillus* in silages is one of the primary contamination sources of spore formers at the farm level (Coorevits *et al*. 2008), and its spores are heat-resistant and thus can survive pasteurization (Borreani *et al*. 2013). Spore formers can contaminate milk *via* the cow's diet and then produce toxins and spoilage enzymes, exerting harmful effects on food safety and product quality (Ivy *et al*. 2012). Pahlow *et al*. (2003) reported that the *Paenibacillus* spore count in silage determines the number of endospores that leave the animal and resultant milk contamination

magnitude. Therefore, silage additives that can directly inhibit or reduce the *Paenibacillus* count in silages should be developed in future. *Klebsiella* spp. is gram-negative, facultative anaerobes that can cause animal diseases and therefore also are undesirable in silages. Herein, relative *Lactobacillus* abundance in LAB silages increased to 78.2%, whereas that of *Paenibacillus* and *Klebsiella* decreased to 4.5 and 3.3%, respectively. These results corroborate that the addition of *L. plantarum* and *L. buchneri* improves silage quality.

Conclusion

Addition of a consortium of LAB inoculants led to a rapid pH reduction and elevated lactic acid contents in the early phase of ensiling. Following 60 days of ensiling, these LAB inoculants improved the LAB count and acetic acid content and decrease the yeast count and NH_3-N content. Moreover, it enhanced the abundance of desirable *Lactobacillus* while preventing undesirable *Paenibacillus* growth and reducing DM loss. In conclusion, addition of consortium of LAB inoculants addition can improve the fermentation quality and decrease undesirable microorganisms in corn silage.

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Author Contributions

FJ, HC and ES planned the experiments, QJ and CW interpreted the results, GZ, ZZ, WS and GS made the write up and FJ statistically analyzed the data and made illustrations.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics Approval

Not Applicable.

Data Availability

The data will be available upon reasonable requests to the corresponding author.

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