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Short Communication

Assessing the fermentation quality and microbial community of the mixed silage of forage soybean with crop corn or sorghum

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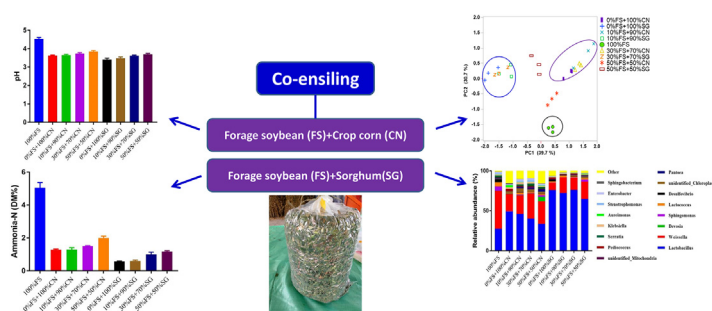
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GRAPHICAL ABSTRACT



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ABSTRACT

The silage quality of forage soybean (FS) rich in protein with crop corn (CN) or sorghum (SG) rich in water soluble carbohydrate was investigated, and microbial community after ensiling was analyzed. Results showed that pH in mixed silages dropped to 3.5–3.8 lower than 100%FS silage (4.5). Microbial analysis indicated that mixed ensiling could influence the microbial community. Although *Lactobacillus* and *Weissella* were the dominant genera in all silage samples, *Lactobacillus* abundance in mixed silages (33–76%) was higher compared with 100%FS silage (27%). In conclusion, FS ensiled with CN or SG could be an alternative approach to improve FS silage quality.

1. Introduction

FS (*Glycine max* Merr.) with abundant of protein and vitamin has been a promising source of green fodder for animal feed (Jahanzad et al., 2016). However, the harvest of FS is seasonal with high accumulation, which need suitable storage way for ruminants. Ensiling is a principle way to preserve wet biomass with very low fermentation loss.

Although FS contains more amount of protein than many other types of forage, the natural fermentation of FS usually leads to poor silage quality indicated by unpleasant odor and high butyric acid (Budakli, 2016). The reason is most possible due to low level of water soluble carbohydrate (WSC) in FS.

WSC can be supplemented by addition of molasses or forage rich in sugar, which not only improves silage quality, but partially reduces the

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content of cell wall constituent (Thompson et al., 2005). Ni et al. (2017) found that molasses addition could increase the content of lactic acid and enhance desirable *Lactobacillus* abundance during the ensiling process of soybean. Recently, some studies found that co-ensiling could be a feasible way to improve silage quality and enhance the stability of fermentation system compared with sole fermentation (Larsen et al., 2017; Jiang et al., 2018). CN and SG both contain high level of WSC and have been widely employed for making silage. Co-ensiling of FS with CN and SG may have potential advantages: (1) CN and SG can provide additional WSC to promote fermentation; (2) FS can increase protein content and balance nutrients; (3) co-ensiling might have synergistic effect on microorganism (Wu et al., 2016; Larsen et al., 2017). However, the mixed ensiling of FS with CN or SG should be evaluated to understand the effect of different mixed ratio on fermentation quality.

Ensiling process is involved in a variety of microorganism, and characterizing the microbial community is critical for further improving silage quality. To date, the microbial community of whole crop corn silage have been extensively studied, and Ni et al. (2017) also reported the microbial community of soybean silage. However, the microbial community related to mixed ensiling of FS with CN or SG have been rarely investigated.

Therefore, this study was to examine the fermentation quality and microbial community from co-ensiling of FS with CN or SG. We expected that the results obtained in this research could provide useful information for practical use of mixed ensiling.

2. Materials and methods

2.1. Materials and silage preparation

Forage soybean, crop corn and sorghum were collected from the experimental field of Shanxi Academy of Agricultural Sciences (111°47'E, 37°15'N) in China on September 24, 2017. Before ensiling, the harvested materials were cut into an approximate particle length of 20 mm using a crop chopper (model 9ZP-3.6, Kaiyue Machinery Company, China).

Experimental treatments included 6 mixtures and 3 treatments with the pure material. The chopped materials were mixed and compressed manually into plastic bags with a bag size of 55 by 95 cm and then vacuumed. Each bag contained 20 kg of fresh material (FM). 5 replicate bags were prepared for each treatment and ensiled for 60 days at room temperature (15–30 °C). Three bags per treatment were selected randomly for analyzing fermentation quality, chemical composition and microbial community, respectively. Five mixed ratios were applied: 100%FS, 0%FS + 100%CN/SG, 10%FS + 90%CN/SG, 30%FS + 70%CN/SG and 50%FS + 50%CN/SG.

2.2. Microbial analysis by culture-based method

Silage samples (30 g) per bag were blended with 270 mL of sterilized water, and serially diluted from 10^{-1} to 10^{-5} in a clean bench. The number of lactic acid bacteria were measured by plate count on Lactobacilli de Man, Rogosa, Sharpe (MRS) agar incubated at 30 °C for 48 h under anaerobic condition (DG 250/min MACS; Don Whitley Science; England). Yeast and molds were counted on potato dextrose

agar (Nissui), incubated at 30 °C for 24 h, and yeasts were distinguished from molds and other bacteria by colony appearance and the observation of cell morphology. Coliform was measured on nutrient agar (Nissui), incubated at 30 °C for under aerobic condition. Colonies were counted as viable number of microorganism in colony forming unit (cfu)/g of FM.

2.3. Analysis of fermentation quality

For pH determination, 30 g of fresh samples with 270 mL sterilized water was homogenized in a blender for 1 min, and then filtrated through 0.22 μ m membrane filters. The pH of the of this extract was immediately measured by a glass electrode pH meter (pH 213; HANNA; Italy). The concentration of ammonia-N was determined by using the indophenol-blue method. The dry matter (DM) content was determined by oven drying at 65 °C for 48 h. After drying, samples were ground through a 1-mm screen with a Wiley mill (ZM200, Retsch GmbH). Ether extract (EE) and crude protein (CP) were determined according to standard procedures detailed by the Association of Official Analytical Chemists (AOAC, 1990). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Van Soest et al. (1991).

2.4. Microbial diversity analysis

2.4.1. DNA extraction and PCR amplification

The procedures of DNA extraction and PCR amplification were followed by Ni et al. (2017).

2.4.2. MiSeq processing and data analysis

The DNA samples were sequenced at the Novogene Company using Paired-end sequencing with an Illumina MiSeq PE300 platform. To get high quality sequencing, their barcode and primers were discharged. And then Mothur (v.1.34.4) was used to discharge the sequences less than 200 bp whose maxhomop value is greater than 10. Remaining sequences were checked for chimeras in the de novo mode by USEARCH 8.0. After filtering process, the clean-tag remained for downstream analysis. The operational taxonomic units (OTUs) at 97% similarity level were clustered using QIIME (v1.8.0). OTUs file was used to calculate rarefaction (R (v.22)) and alpha diversity (Mothur (v1.34.4)). The weighted UniFrac distance matrix was employed to calculate the β diversity, and principle component analysis (PCA) was performed at 3% dissimilarity level.

2.5. Statistical analysis

The statistical analyses were performed using JMP software (version 10; SAS Institute, Tokyo, Japan) to examine the differences between different treatment. Tukey's HSD test was employed to the differences the treatment means.

Table 1

Chemical composition and microbial population of FM before ensiling.

	DM	%DM				Log cfu/g FM ⁻¹		
		EE	CP	NDF	ADF	WSC	LAB	Yeast
FS	34.45 \pm 1.22	3.91 \pm 0.12	17.26 \pm 0.56	46.69 \pm 1.25	34.46 \pm 1.22	1.23 \pm 0.05	3.66 \pm 0.25	6.54 \pm 0.89
CN	34.12 \pm 1.02	2.96 \pm 0.22	8.26 \pm 0.22	33.69 \pm 1.56	17.01 \pm 1.02	6.66 \pm 0.56	5.56 \pm 0.23	6.25 \pm 0.69
SG	24.17 \pm 0.99	2.75 \pm 0.09	6.12 \pm 0.15	44.69 \pm 2.02	25.51 \pm 1.05	16.61 \pm 0.59	5.23 \pm 0.56	6.65 \pm 0.36

FS, forage soybean; CN, crop corn; SG, sorghum.

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