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A comparison of buffered propionic acid and *Propionibacterium acidipropionici* as additives for high oil maize stover silage*

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ABSTRACT

High oil maize stover upon removal of ears was ensiled in 3 L volume glass jars for 1, 3, 6, 9 weeks to investigate the effects of buffered propionic acid and propionic acid bacteria on fermentation and aerobic stability of the silage. Treatments were: 1. control (no additives added), 2. buffered propionic acid added at 5 L/t fresh high oil maize stovers, and 3. propionic acid bacteria from *Propionibacterium acidipropionici* added at 10^5 cfu/g of fresh high oil maize stovers. The addition of buffered propionic acid and propionic acid bacteria had little effect on final pH or concentrations of total lactic acid and ammonia N. Final concentrations of residual water soluble carbohydrate and propionic acid and the percentage of D-lactic acid of total lactic acid were higher in buffered propionic acid treated silage. Aerobic stability of the silages was investigated after opening the laboratory silo jars at 9 wk postfilling. Silage treated with propionic acid bacteria had more yeasts than other silages after 7 d of aerobic exposure. Molds were only found at 5 d of aerobic exposure in control. Butyric acid was not detected with exception of untreated silage at the end of 7 d of exposure. After 7 d of aerobic exposure, pH of control increased from 3.39 to 5.30, while the corresponding pH values were increased from 3.5 to 4.11 in propionic acid bacteria and from 3.56 to 3.65 in silage treated with buffered propionic acid. Both buffered propionic acid and propionic acid bacteria used as silage additives improved the aerobic stability of high oil maize stover silage, but buffered propionic acid was more effective.

KEY WORDS: high oil maize stover, silage, ensiling gas, *Propionibacterium acidipropionici*, buffered propionic acid, aerobic stability

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INTRODUCTION

Recent studies at China Agricultural University showed that high oil maize (HOM) stovers had a higher content of water-soluble carbohydrate (WSC), fat, protein and phosphorus, and lower content of NDF and lignin compared with other typical maize stovers at the same grain maturity stage (Zhao, 2003; Yan, 2005). These traits make HOM stovers a preferable source of maize silage. It was reported that most HOM stover silage had good fermentation traits during anaerobic fermentation phase (Zhao, 2003). However, the aerobic stability of HOM silage may be a problem because of its high contents of lactic acid and WSC in silage. Upon exposure to the air, the introduction of oxygen will stimulate the growth of yeasts, molds and aerobic bacteria resulting in a rapid increase in temperature and pH, and a decrease in WSC and fermentation end-products that adversely affect silage quality.

Many silage additives including chemical reagents (e.g., organic acids and ammonia) and microbial inoculants have been extensively used to preserve forages during ensiling (Bolsen et al., 1996). Organic acids, such as propionic acid, formic acid and acetic acid, have been applied for many years in silage making. Of these acids, propionic acid has the greatest antimycotic activity, making it an ideal candidate to improve the aerobic stability of maize silage (Woolford, 1975). It was reported that the addition of propionic acid or formic acid to crop silages alone prior to ensiling improved the fermentation and aerobic stability (Kung et al., 1998, 2003). However, the disadvantages of unbuffered organic acids are their corrosive properties toward harvesting equipment, health risks to the user when not handled properly, and increased effluent production (Wilkinson, 1990). In recent years, some new preservative products that contain buffered propionic acid (BPA) have been designed by commercial companies for addition to silages and the corrosive nature of propionic acid has been reduced by buffering. Although the positive role of buffered propionic acid-based additives alone or combined with microbial inoculation in the fermentation of high moisture maize and whole-crop barley has been investigated (Kung et al., 2003), no information in the literature is available concerning such additives with buffered ingredients functioning in improving the aerobic stability of HOM silages.

Inoculation of forages with selected strains of bacteria at ensiling has been recognized as the means to improve the fermentation and aerobic stability of silage (Bolsen et al., 1996). Although several strains of lactic acid bacteria are commonly used as microbial inoculants of silages, most of them only function during anaerobic fermentation phase of ensiling and have minimal impact on the silage upon aerobic exposure (Wohlt, 1989). Propionic acid bacteria can ferment lactate to propionate and acetate; these short-chain aliphatic acids inhibit yeasts and moulds (Moon,

1983). Bolsen et al. (1996) observed that aerobic stability was improved by using *Propionibacterium acidipropionici* to preserve maize silage. However, other studies showed that Propionibacteria were less effective on the fermentation and aerobic stability of silage (Weinberg and Aashbell, 1995; Higginbotham et al., 1996). However, literature is lacking on the effect of Propionibacterial inoculant on the fermentation and aerobic stability of HOM stover silage.

The purpose of the present study is to compare the effects of buffered propionic acid-based preservative and propionic acid bacteria on chemical composition, fermentation end-products and aerobic stability in high oil maize stover silage.

MATERIAL AND METHODS

High oil maize (variety BHO #1) plants were harvested at the full milk-line stage of maturity. Upon removal of ears, the maize stover was chopped into an average length of 9 mm and ensiled in 3 L volume laboratory glass jars (average 750 g silage DM) with a special gas collection device. Treatments were: 1. control (no additives added), 2. buffered propionic acid (BPA), and 3. propionic acid bacteria (PAB). The BPA product, containing 75% propionic acid, 10% ammonium salt and rest water, was supplied as a non-commercial product by an international business company. The product was sprayed in liquid form onto the HOM stovers at the rate of 5 L/t fresh weight. Propionic acid bacteria revived from freeze-dried strain of *Propionibacterium acidipropionici* CAU 05 (stored in this lab), were applied as water solutions at 10^5 cfu/g fresh weight of HOM stovers. Either chemical agent or bacterial inoculant was then mixed thoroughly with the fresh maize stovers by gloved hands. In order to keep the equal moisture content of the stovers among treatments, the same volume of distilled water was added into the HOM stovers of the control. Twelve laboratory glass silo jars were used per treatment, and triplicate jars were opened at 1, 3, 6 and 9 weeks postfilling. All the jars were filled and sealed within 20 min after being chopped, and stored in an environment with ambient temperatures ranging between 22 and 25°C. During the silage fermentation, gas volume produced from fermentation was read from the calibrated scale on the glass syringes connected to the gas collection device. Dry matter recoveries during the fermentation and storage phases were calculated by measuring the silage DM loss before and after 9 wk postfilling. The jars on week 9 were subsampled for standard chemical analysis and then the remainders of the material were used for the aerobic exposure study. Silages in these containers were kept at room temperature for 7 d to monitor aerobic stability. Samples were collected from each of silages before exposure to air and after 1, 3, 5 and 7 d of aerobic exposure, and then microorganisms were enumerated using selective media.

Dry matter (DM) determination was conducted in a forced-air oven at 55°C for 72 h. Representative samples of fresh silage or fresh HOM stovers were freeze-dried and ground through a 1-mm screen prior to analyses for total nitrogen (TN), neutral detergent fibre (NDF). The NDF content was determined using the methods of Van Soest et al. (1991) without addition of amylase. Total N was determined using a Nitrogen Analyzer (Model Rapid N III, Elementar, Germany) based on the Dumas combustion method (AOAC, 990.03). Twenty grams of silage samples from each treatment at each fermentation or storage period and on d 2, 3, 5 and 7 post aerobic exposure was homogenized in 100 ml of distilled water, and were measured for pH values using a pH meter connected with a glass probe (Model PHS-3C, Shanghai Leici Equipment Co., Shanghai, China). The slurry was filtered through filter paper (Xinhua Quantity Filter Paper; Hangzhou Xinhua Filter Paper Inc., China), and the water extract was analysed for water-soluble carbohydrates (WSC) by colorimetry (Nelson, 1944) and ammonia N by colorimetry (Broderick and Kang, 1980). One ml of water extract was combined with 200 µl of 25% meta-phosphoric acid containing 2-ethyel butyric acid as an internal standard. Samples were centrifuged for 15 min at 10,000 × *g* and analysed for acetic, propionic, butyric, valeric, isobutyric and isovaleric acids by gas chromatography (6890 N, Agilent) with a 530-µm Carbowax 20 M column (Supelco, Bellefonte, BPA). The chromatograph oven was programmed as follows: 70°C for 1 min, 5°C/min increment to 100°C, 45°C /min increment to 170°C, and a final holding time of 5 min. In addition, the water extracts of silages were analysed for L-lactic acid (LA) by an enzymatic procedure (kit 826-UV; Sigma, St. Louis, MO). For the analysis of D-lactic acid, L-lactic dehydrogenase was replaced by a similar amount of D-lactic dehydrogenase (Sigma L-9636). L-Lactic acid (Sigma L-2250) and D-lactic acid (Sigma L-1000) were used as standards for their respective assays. The sum of the L- and D-lactic acids was reported as the total lactate concentration.

Microbial enumeration was conducted in the aerobic stability trial. Twenty-five grams of the sample from each treatment on d 2, 3, 5 and 7 post aerobic exposure were diluted in 225 ml of sterile distilled water and homogenized. Ten-fold serial dilutions were made subsequently with sterile buffer for microbial analyses. Lactic acid bacteria were enumerated using Man, Rogosa and Sharpe (MRS) agar incubated at 37°C for 24-48 h. Yeasts and molds were enumerated using potato dextrose agar (no. 7149; Acumedia, Baltimore, MD) with 0.15% tartaric acid incubated aerobically at 37°C for 24-48 h.

Statistical analysis

Chemical compositions of silages were analysed as a completely randomized design separately for each individual sampling time by the general linear models procedure of SAS (1985). Mean comparisons were conducted by Tukey′s Test (Snedecor and Corchan, 1980). The experiment of aerobic stability followed a split-plot design with inoculant treatment as a main treatment and time period as

a subplot treatment. Data from the fermentation and aerobic stability study were analysed using the following statistical model:

$$Y_{ijk} = M + D_i + T_j + DT_{ij} + e_{ijk}$$

where Y_{ijk} is the observation, M is the overall mean, D_i is the day of aerobic exposure (0, 2, 3, 5 and 7), T_j is the treatment effect (control, BPA and PAB), DT_{ij} is interaction between day and treatment and e_{ijk} is residual error.

RESULTS

Chemical composition and fermentation

HOM stover was ensiled at 289.9 g DM/kg, and the contents of WSC, CP, EE, starch, NDF and ADF were 73.5, 71.0, 19.2, 9.1, 592.6 and 375.6 g/kg DM prior to ensiling, respectively. Similar analysis of fresh maize stovers among lots suggested that the material was adequately mixed prior to treatment.

Table 1. Effects of BPA[1] and PAB[2] on the chemical composition of HOM[3] stover silage

	Week of ensiling			
	1	3	6	9
DM, %				
Control	27.56	27.86	27.37	27.21
BPA	28.02	27.63	27.85	27.23
PAB	28.65	28.12	28.01	27.95
SEM[4]	0.56	0.43	0.37	0.26
WSC, %DM				
Control	3.20[b]	3.13[b]	3.04	2.32[c]
BPA	6.65[a]	6.75[a]	6.73	6.71[a]
PAB	3.26[b]	3.09[b]	3.06	2.95[b]
SEM	0.32	0.15	0.21	0.28
CP, %DM				
Control	7.11	7.22	7.35	7.58
BPA	7.03	7.24	7.43	7.61
PAB	7.10	7.3	7.51	7.65
SEM	0.35	0.36	0.25	0.39
NDF, %DM				
Control	62.12	64.21	64.45	65.68
BPA	61.73	63.25	63.89	65.22
PAB	62.21	64.38	64.87	65.38
SEM	1.18	0.76	0.58	1.34

[a-c] means with different letters in the same column differ significantly ($P<0.05$), [1]BPA - buffered propionic acid; [2]PAB - propionic acid bacteria; [3]HOM - high oil maize; [4]SEM - standard error of the mean

The contents of DM, NDF, WSC and CP in the HOM stover silages as affected by the three treatments during fermentation are presented in Table 1. The content of WSC was higher (P=0.02) for BPA treatment throughout the entire fermentation and storage period, while there was no significant difference between PAB treatment and control. Furthermore, compared with the fresh HOM stovers, more than half of WSC from control and PAB were utilized within 1 wk of ensiling (Table 1). With advanced fermentation period, CP, NDF continuously increased for all treatments; however, these chemical compositions did not differ (P=0.15) between treatments at any time point during ensiling. Compared with control, PAB and BPA did not improved (P=0.12) the DM recovery (DM recovery = 94.78, 96.91, and 95.12%, respectively).

Changes in silage pH and fermentation end product proportions during the fermentation period are presented in Table 2. The pH dropped to lower than 4.0 after 1 wk of ensiling for PAB and control silages. However, the pH was 4.4 for BPA which was greater than for the other treatments. The lower pH was maintained until the end of the 9 wk at which time point pH values did not differ (P>0.10) between three silages. As silage fermentation progressed, the concentration of lactate, acetate and ammonia N increased for all three silages. During the whole fermentation period, silages from control and PAB treatment had more than 67% of total lactate produced within 1 week, whereas the silage from BPA treatment required 3 wk to achieve the similar concentration. After 3 wk of fermentation, lactic acid (LA) concentrations of all three HOM stover silages did not differ. The percentage of D-lactate of total lactate was affected by treatment. Though similar percentages at 1 wk of fermentation were measured in all silages, the silage from BPA treatment had higher (P=0.03) percentages of D-lactate than control and PAB treatment after 1 wk of fermentation period. Furthermore, the difference of D-lactate percentages of total lactate between BPA and PAB or control silages were steadily broadened with advanced fermentation period. The acetic acid concentration was lower (P=0.02) for silage from BPA treatment than that of silage from control. However, no considerable difference between PAB and control were found throughout the fermentation period. As expected, propionic acid concentration during the fermentation period was much higher for BPA treated silage due to the addition of propionic acid. There were minor amounts of such acid produced in the silage from PAB or control, although the concentration was somewhat higher for PAB treatment than control. Butyric acid (including isobutyric acid) was undetectable in all treated silages.

Ensiling gas production was recorded during the silage fermentation (Figure 1). Not only the amount but also dynamics of gas production were different among the three treatments during the fermentation and storage period. The cumulative gas production during whole fermentation period was the highest (P=0.004) for

Table 2. Effects of BPA[1] and PAB[2] on the fermentation end product profile of three HOM[3] stover silages from laboratory silo jars

	Week of ensiling			
	1	3	6	9
pH				
Control	3.45[b]	3.40	3.38	3.39
BPA	4.40[a]	3.51	3.52	3.56
PAB	3.52[b]	3.48	3.49	3.50
SEM[4]	0.03	0.01	0.01	0.03
LA[5], %DM				
Control	2.36[a]	2.49	3.01	3.04
BPA	0.51[b]	2.38	3.04	3.06
PAB	2.12[a]	2.87	3.10	3.12
SEM	0.24	0.37	0.07	0.05
D-LA/total LA, %DM				
Control	55.56	49.76[b]	49.75[b]	49.27[b]
BPA	51.29	67.12[a]	71.43[a]	74.08[a]
PAB	58.48	50.00[b]	49.27[b]	47.85[b]
SEM	0.98	1.02	1.05	0.92
Acetic acid, %DM				
Control	0.86[a]	0.91[a]	0.92[a]	0.94[a]
BPA	0.35[b]	0.38[b]	0.41[b]	0.45[b]
PAB	0.95[a]	1.01[a]	1.15[a]	1.18[a]
SEM	0.08	0.04	0.02	0.01
Propionic acid, %DM				
Control	0.03[b]	0.03[b]	0.02[b]	0.02[b]
BPA	0.51[a]	0.49[a]	0.51[a]	0.50[a]
PAB	0.03[b]	0.06[b]	0.05[b]	0.05[b]
SEM	0.001	0.001	0.001	0.001
NH_3-N/TN[6], %				
Control	6.9	7.3	7.5	7.5
BPA	6.8	7.0	7.1	7.1
PAB	7.4	7.4	7.4	7.5
SEM	0.51	0.32	0.41	0.32

[a-c] means with different letters in the same column differ significantly ($P<0.05$); [1-4] see Table 1
[5] LA - lactic acid; [6]TN - total nitrogen

control (147 ml/100 g DM), followed by BPA (128 ml/100 g DM) and PAB (105 ml/100 g DM). It was interesting that almost all gases were produced within the first 3 days of ensiling in control and within the first 4 days of ensiling in PAB. In BPA, however, only small amount of gas was produced during the first 4 days of fermentation and a rapid increase of gas production was recorded until 5 d postfilling.

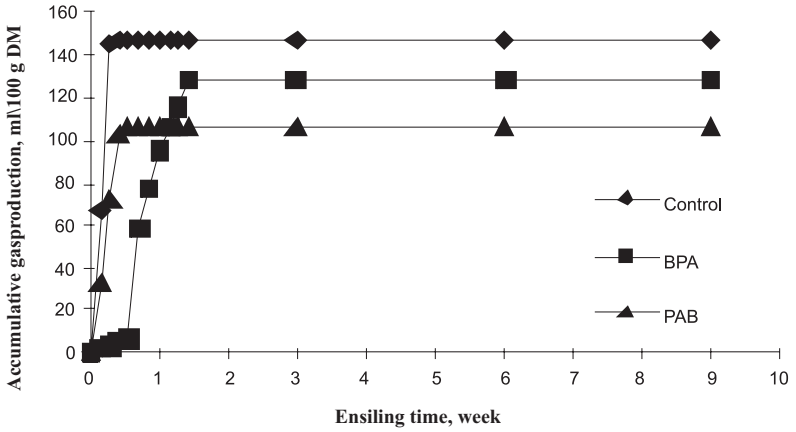

Figure 1. Effects of BPA[1] and PAB[2] on the ensiling gas production of HOM[3] stover silages during the fermentation phase. [1]BPA - buffered propionic acid, [2]PAB - propionic acid bacteria, [3]HOM = high oil maize

Aerobic stability

Data of yeast, mold and *Lactobacillus* counts of the HOM silages for 9 wk postfilling after aerobic exposure for 2, 3, 5 and 7 d are presented in Table 3.

Table 3. Effects of BPA[1] and PAB[2] on changes in yeast and *Lactobacillus* numbers in HOM[3] stover silages post aerobic exposure

	Days after aerobic exposure				
	0	2	3	5	7
Yeast, $\log_{10}$ cfu/g					
Control	2.88	2.80	2.19	2.97	3.02^{ab}
BPA	2.27	2.65	2.79	2.24	2.42^{b}
PAB	3.00	3.41	3.23	3.61	4.69^{a}
SEM[4]	0.25	0.50	0.47	0.29	0.42
Lactobacillus, $\log_{10}$ cfu/g					
Control	3.42	2.91	3.22	3.63^{a}	3.32^{ab}
BPA	3.10	3.23	3.5	3.02^{b}	3.98^{b}
PAB	4.24	3.12	3.76	3.60^{a}	4.48^{a}
SEM	0.32	0.18	0.32	0.24	0.35

[a-c] means with different letters in the same column differ significantly ($P<0.05$); [1-4] see Table 1

Yeast counts in all silages were low (less than 1×10^3 cfu/g of silage DM) upon aerobic exposure, and then increased dramatically during aerobic exposure. Slight molding was observed after 5 d of aerobic exposure in control silage (data not

shown). After 7 d of aerobic exposure, the numbers of *Lactobacillus* and yeast were lower (P=0.002) in BPA than those in control silage, while there was no remarkable difference between PAB treatment and control.

With the advance of aerobic exposure, NDF content increased, while the content of WSC and CP decreased in all three silages. Silage from BPA treatment showed a higher WSC content than did control and PAB. During 7 d of aerobic exposure, the WSC loss was the greatest in control (58.2%), followed by BPA (51.3%) and PAB (39.3%) based on the balance of residual WSC content between aerobic exposure on d 7 and initial d 0 (Table 4).

Table 4. Effects of BPA[1] and PAB[2] on chemical compositions of HOM[3] stover silages post aerobic exposure

	Days after aerobic exposure				
	0	2	3	5	7
NDF, %DM					
Control	65.68	69.64	70.61	73.54	72.33
BPA	65.02	66.63	69.65	67.74	69.23
PAB	65.38	66.81	66.47	69.58	70.70
SEM[4]	1.34	1.18	1.44	3.99	1.35
WSC, %DM					
Control	2.32^{c}	2.24^{b}	2.79^{b}	1.88^{b}	0.97^{c}
BPA	6.71^{a}	6.57^{a}	6.03^{a}	3.50^{a}	3.27^{a}
PAB	2.95^{b}	2.37^{b}	1.93^{c}	1.49^{c}	1.79^{b}
SEM	0.28	0.38	0.06	0.04	0.04
CP, %DM					
Control	7.58	6.94	6.82	6.93	6.38^{b}
BPA	7.61	7.67	7.55	7.66	7.45^{a}
PAB	7.65	7.59	7.76	7.84	7.13^{b}
SEM	0.39	0.27	0.25	0.25	0.23

$^{a-c}$ means with different letters in the same column differ significantly (P<0.05)
[1-4] see Table 1

Changes in pH and microbial metabolites including organic acid after aerobic exposure are shown in Table 5. The pH in control silage rapidly increased with aerobic exposure progressing and reached to 5.30 after 7 d of exposure. The pH was below 4.2 throughout the aerobic exposure period for BPA and PAB treatment silage. Lactate concentrations changed little in PAB or BPA silages, but a remarkable lactate decrease was observed in the silage from control during the 7 d of aerobic exposure. The percentage of D-LA of total LA changed little for all three HOM stover silages throughout the entire aerobic exposure period.

BPA treated silage had a higher concentration of propionic acid and a lower concentration of acetic acid than did control, but there was no significant difference between control and PAB treated silage. No butyric acid was detected in silage from BPA treatment. In contrast, butyric acid was detected in silages from control at 2 d of aerobic exposure and from PAB treatment after 7 d of aerobic exposure, respectively.

Table 5. Effects of BPA[1] and PAB[2] on pH values, lactic acid and VFA production of three HOM[3] silages aerobic exposure during 7 d

	Days after aerobic exposure				
	0	2	3	5	7
pH					
Control	3.39	3.66^{b}	3.79	3.88	5.30
BPA	3.56	3.74^{a}	3.78	3.55	3.65
PAB	3.50	3.63^{b}	3.69	3.93	4.11
SEM[4]	0.03	0.01	0.06	0.08	0.47
LA, %DM					
Control	3.04	2.96	2.68	1.48^{b}	1.22^{b}
BPA	3.06	3.07	3.03	3.06^{a}	2.99^{a}
PAB	3.12	2.91	2.84	2.63^{a}	2.18^{a}
SEM	0.05	0.41	0.37	0.17	0.47
Acetic acid, %DM					
Control	0.94^{a}	1.24^{a}	1.76^{a}	2.07^{a}	2.18^{a}
BPA	0.45^{b}	0.43^{b}	0.39^{b}	0.58^{b}	1.13^{b}
PAB	1.18^{a}	1.29^{a}	1.55^{a}	1.40^{a}	2.10^{a}
SEM	0.01	0.14	0.06	0.35	0.21
Propionic acid, %DM					
Control	0.02^{b}	0^{c}	0^{b}	0^{b}	0^{b}
BPA	0.50^{a}	0.55^{a}	0.51^{a}	0.53^{a}	0.59^{a}
PAB	0.05^{b}	0.12^{b}	0.15^{b}	0.04^{b}	0.03^{b}
SEM	0.002	0.01	0.05	0.04	0.06
Butyric acid, %DM					
Control	ND[5]	0.02	0.08	0.08	0.12
BPA	ND	ND	ND	ND	ND
PAB	ND	ND	ND	ND	0.17
SEM	ND	ND	ND	ND	0.09

$^{a-c}$ means with different letters in the same column differ significantly ($P<0.05$)

[1-4] see Table 1; [5] ND - not detected

DISCUSSION

Both buffered organic acids and microbial inoculants are desirable as silage additives since they are easy to handle and less or non-corrosive to farm machinery. They have also been used for the preservation of hay with a low moisture content (Woolford, 1975; Kung et al., 2003). However, no data were obtained on the effect of buffered propionic acid additives on the efficiency of fermentation in high oil maize stovers silage. Microbial inoculants containing propionic acid bacteria were also suggested to be used as silage additives (Pahlow and Honig, 1994; Bolsen et al., 1996). Some studies (Weinberg and Aashbell, 1995; Bolsen et al., 1996) showed improvements in the aerobic stability of silage inoculated with propionic acid bacteria prior to ensiling, but others showed no effect (Weinberg and Aashbell, 1995). Almost all studies (Weinberg and Aashbell, 1995; Higginbotham et al., 1996, 1998) showed propionic acid bacteria had no effect on the fermentation phase. Information was not available regarding the effects of buffered propionic acid additives and propionic acid bacteria on the efficiency of fermentation and aerobic stability in HOM stover silage.

Chemical composition and silage fermentation

As reported in a recent study (Yan, 2005), in comparison with other varieties of typical maize stover (e.g., hybrid Nongda 3138), fresh high oil maize stovers (e.g., hybrid HOM 647) have relatively higher contents of WSC, CP, EE, starch and P, but lower NDF, ADF and lignin at the same maturity stage (full maturity). In the present study, HOM stovers had chemical compositions (WSC, CP, EE, starch and NDF) consistent with the previously reported values (Zhao, 2003; Yan, 2005).

With advanced fermentation period, the content of CP and NDF continuously increased for all treatments (Table 1). The increased silage CP and NDF contents may be attributed to the reduction of WSC during silage fermentation. Compared with control and PAB treatment, BPA treatment silage had relatively higher residual WSC content during the entire fermentation period (Table 1). Reduced respiration of BPA may at least partly explain this beneficial effect. BPA treatment also resulted in lower fermentation end-products including ensiling gas production (Figure 1) and lactic and acetic acids (Table 2) than control, suggesting that the BPA could effectively inhibit the activity of certain microorganisms in the silage during the anaerobic fermentation phase.

The pH drop to lower than 4.0 only occurred after 1 wk of ensiling in control and PAB silages. However, the pH was higher and lactic acid content was significantly lower in BPA treated silage at 1 wk fermentation period (Table 2). This effect is also expected as direct addition of acid prevents the microbial

fermentation. This finding was consistent with the result of ensiling gas production of silage during fermentation. Compared with control, only small amount of ensiling gas was produced before 4 d fermentation and the peak of the gas production was achieved at 5 d postfilling for BPA silage, suggesting that BPA can delay fermentation of the HOM stover silage. Similar results were found in other typical maize silages (Huber and Soejono, 1977; Hara and Ohyfama, 1978; Stallings et al., 1981). After 6 wk postfilling, total LA content was above 3% (on DM basis) for all silages and was maintained until the end of 9 wk fermentation (Table 2). Earlier criteria for the effective preservation of an ensiled crop included a high degree of lactic acid production and a pH below 4.2 after the fermentation phase (Bolsen et al., 1996). In the present study, all three silages appeared to be of good quality, as exemplified by low pH (below 3.8), high concentrations of LA and undetectable butyric acid.

It is well-known that silage containing very large amounts of D-lactic acid may result in lactic acidosis in ruminant animals (Dunlop, 1972). Schaadt and Johnson (1968) found that the production of lactate in silage largely involved the D-isomer. Cai and Kumai (1994) reported that on dairy farms, the proportion of D-lactate to total lactic acids in silage was 62 to 68%. In the present study, however, the proportion of D-lactate decreased with advanced fermentation period of HOM stover silages and was below to 50% after 9 wk of fermentation for control and PAB treatments (Table 2). In contrast, the BPA treatment silage had increased D-lactate proportion with advanced fermentation period. Therefore, BPA addition to the HOM stover silage can change and influence the proportion of lactate isomers during silage fermentation. These results suggest that BPA addition fails to improve HOM stover silage quality although it is responsible for decreased loss of WSC.

Aerobic stability

Aerobic deterioration of maize silage appears to be predominantly initiated by yeasts and molds (Bolsen et al., 1996). Silages containing at least 1×10^6 cfu of yeast/g were prone to undergo aerobic deterioration once exposed to air (Higginbotham et al., 1998). In the present study, less than 1×10^3 cfu of yeast/g were detected in the silage after 9 weeks, therefore, the silages deteriorated slowly when the lab silo jars were opened. Higginbotham et al. (1998) found that inoculation with propionic acid bacteria did not affect the count of yeasts before or after aerobic exposure. Dawson et al. (1993) established that the use of a large number of propionic acid bacteria (10^6-10^7cfu/g of forage) at the time of ensiling gave them a competitive advantage over yeast and mold populations. However, the addition of PAB to HOM stovers at the time of ensiling did not reduce yeast growth and dramatically increase the propionic acid concentration in the present study (day 0 in Table 3). The fact would

also suggest that the bacterial inoculant did not grow well during the fermentation process. Slight molds were detected in control silage at 5 d aerobic exposure while no mold was found in BPA and PAB treated silages, suggesting BPA and PAB improve the aerobic stability of HOM stover silage. Under aerobic exposure, yeasts and molds hydrolyse sugars and lactic acid to produce CO_2 and release heat, resulting in aerobic deterioration of silages (Bolsen et al., 1996). In the present study, with the extension of aerobic exposure, WSC contents dropped and yeast counts increased drastically for all treatments (Table 4). The BPA treated silage had the highest content of WSC, but the lowest count of yeasts and undetectable mold counts, indicating improved aerobic stability in HOM stover silage. After aerobic exposure, control silage contained lower total LA content compared with BPA and PAB treatments. The pH remained substantially lower in PAB and BPA silages even after yeast populations increased. The rapid increase in pH and decrease in total lactic acid concentration after aerobic exposure in control silage (Table 5) most likely due to the metabolism of lactic acid by yeast and molds. Butyric acid was detected in PAB treatment at 7 d aerobic exposure and in control treatment at 2 d aerobic exposure, respectively. There was no butyric acid detected in BPA treated silage during the entirely aerobic exposure period (Table 5). These results suggested that BPA markedly improve aerobic stability. Next to BPA, PAB inoculant also improved aerobic stability in HOM stover silage. In other studies, aerobic stability was also found to be improved when buffered propionic acid (Kung et al., 1998) or propionic acid bacteria (Bolsen et al., 1996) were added to the typical maize silages.

CONCLUSIONS

The addition of propionic acid bacteria did not affect the quality of high oil maize stover silages, but the addition of buffered propionic acid delayed the silage fermentation. Both of propionic acid bacteria and buffered propionic acid improved the aerobic stability of high oil maize stover silages with buffered propionic acid being more effective. Additional pilot study is needed to further investigate the efficacy and economical feasibility of these additives.

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