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Effect of Applying Molasses and Propionic Acid on Fermentation Quality and Aerobic Stability of Total Mixed Ration Silage Prepared with Whole-plant Corn in Tibet

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ABSTRACT: The objective of this study was to evaluate the effects of molasses and propionic acid on the fermentation quality and aerobic stability of total mixed ration (TMR) silages prepared with whole-plant corn in Tibet. TMR (354 g/kg DM) was ensiled with four different treatments: no additive (control), molasses (M), propionic acid (P), and molasses+propionic acid (PM), in laboratory silos (250 mL) and fermented for 45 d. Silos were opened and silages were subjected to an aerobic stability test for 12 days, in which chemical and microbiological parameters of TMR silages were measured to determined the aerobic deterioration. After 45 d of ensiling, the four TMR silages were of good quality with low pH value and ammonia/total N (AN), and high lactic acid (LA) content and V-scores. M silage showed the highest ($p<0.05$) LA content and higher dry matter (DM) recovery than the control and P silages. P silage had lower ($p<0.05$) LA content than the control silage. During aerobic exposure, lactic acid contents decreased gradually in the control and M silages, while that of P and PM silages increased, and the peak values were observed after 9 d. M silage had similar yeast counts with the control silage (>10^5 cfu/g FM), however, it appeared to be more stable as indicated by a delayed pH value increase. P and PM silages showed fewer yeasts (<10^5 cfu/g FM) ($p<0.05$) and were more stable than the control and M silages during aerobic exposure. It was concluded that M application increased LA content and improved aerobic stability of TMR silage prepared with whole-plant corn in Tibet. P application inhibited lactic acid production during ensiling, and apparently preserved available sugars which stimulated large increases in lactic acid during aerobic exposure stage, which resulted in greater aerobic stability of TMR silage. (**Key Words:** Molasses, Propionic Acid, Fermentation Quality, Aerobic Stability, Whole-plant Corn TMR)

INTRODUCTION

The Tibetan plateau located in southwest China with an average altitude of over 4,000 m (Duan et al., 2008), is regarded as the highest unique territorial unit in the world. Shortage of feedstuffs due to seasonal changes throughout the year, yak farmers do not know how to reasonably match roughage and concentrate to feed dairy cows resulted in the fluctuation of milk production, thus the development of animal husbandry is relatively backward in Tibet. In recent years, fermented total mixed ration is widely applied in feeding dairy cows in Tibet, and whole-plant corn silage usually used as roughage, since whole-plant corn silage is one of the most popular forages fed to dairy cows because it has good agronomic characteristics, yields high concentrations of nutrients, ensiles well, and incorporates easily into total mixed ration (TMR) (Neylon and Kung, 2003). Application of fermented total mixed ration could not only provide year-round and nutrition balance feed, could but also compensate for the inadequacy of roughage and concentrate.

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There were many researches which adding additives to enhance the acidification of ensiled forages (Alli et al., 1984; Wuisman et al., 2006). Molasses has been used extensively as a fermentation stimulant, since it could provide fermentable substrates for lactic acid bacteria. Adding molasses to materials before ensiling could decrease pH, volatile basic nitrogen and DM loss, and increase higher lactic acid and residual water-soluble carbohydrate contents (Alli et al., 1984). However, silages with such additive are prone to aerobic deterioration because it result in relatively greater levels of residual water-soluble carbohydrates (WSC) and lactic acid, which are used as substrates for spoilage-causing yeasts and molds.

Agricultural areas are far from pastoral areas in Tibet, long distance transportation is essential, and yak farmers do not know how to feed TMR silage scientifically. During the long-distance transport as well as feeding after opening silos periods, TMR silage is often exposed to air for a long time, thus it is susceptible to aerobic deterioration. Propionic acid was one of effectively antimycotic agents among the short-chain fatty acids (Woolford et al., 1975). It has been used as a forage preservative for many years when used at high rates (1.0% to 3.0% of the DM) were deemed to be effective inhibitors of aerobic deterioration of silage (Woolford et al., 1984). The antimycotic effect of propionic acid is inhibited undesirable microorganism to metabolism to improve the aerobic stability of corn silage (Woolford et al., 1975).

The objectives of this study were to determine the effect of molasses or/and propionic acid applied on the fermentation quality and aerobic stability of total mixed ration silage prepared with whole-plant corn in Tibet.

MATERIAL AND METHODS

Silage preparation

Whole-plant corn was cultivated in the experimental field of the Grassland Station of Rikaze (29.27 N, 88.88 E, Tibet, China), harvested at the one-half milk line stage (227 g/kg DM fresh weight) and prepared for ensiling. Forage was chopped with a conventional forage harvester to a length of 2 to 3 cm. As shown in Table 1, total mixed ration was formulated with whole-plant corn, cracked corn, rape cake meal, cotton seed, distiller dried grains with soluble (DDGS), wheat bran, and vitamin-mineral supplement at a ratio of 52:3.6:9.6:9.6:13.2:9.6:2.4 on DM basis. TMR (354 g/kg DM) was ensiled with four different treatments: no additive (control), molasses addition at 3% (M), propionic acid addition at 0.4% (P), and 3% molasses+0.4% propionic acid addition (PM) on a fresh matter basis of TMR. From each treatment, 190 g of TMR mixture was packed into a laboratory silo (250 mL capacity), followed by being sealed with a screw top and kept at the ambient temperature. The silos for each treatment were opened on 45 d after ensiling, and then subjected to an aerobic stability test for 12 days. Triplicates silos were made for each treatment and each sampling day.

Table 1. Ingredient and chemical composition of total mixed ration

Items	TMR
Ingredient (% DM)	
Whole crop corn	52
Mixed concentrate[1]	48
chemical composition (% DM)	
Dry matter	35.4
Crude protein	13.4
Ether extract	4.91
Neutral detergent fiber	47.9
Acid detergent fiber	22.3
Ash	6.75
NFC[2]	27.5

DM = Dry matter.
[1] Mixed concentrate: 7.5% cracked corn, 20% rape cake meal, 20% cotton seed, 27.5% DDGS, 20% wheat bran, 5% vitamin-mineral.
[2] NFC (non-fibrous carbohydrate) = 100−crude protein−neutral detergent fiber−ether extract−ash.

Chemical and microbiological analyses

Fresh forages, unensiled TMR and fermented TMR were analyzed for chemical and microbiological composition. To measure fermentation indices, 35 g of each silage was blended with 75 mL of deionized water left at 4°C for 24 h, the extracts were then filtered through 2 layers of cheesecloth and a filter paper (Xinhua Co, China). The filtrates were used for determining pH, buffering capacity ammonia-N (AN), lactic acid (LA) and volatile fatty acids (VFAs) contents. The pH of the silage was measured with a glass electrode pH meter (HANNA pH 211, Hanna Instruments Italia Srl, Italy). Buffering capacity (BC) was determined by the hydrochloric acid-sodium hydroxide method of Playne and McDonald (1966). The DM contents of unensiled forage samples and silage samples were determined in a forced-draft oven set to 60°C for 48 h. Dry matter recovery of d 45 silages was estimated by comparing the product of forage mass and forage DM contents before and after ensiling for each silo. Ash was determined by placing samples in a muffle furnace set at 500°C for 5 h. The WSC contents were determined by colorimetric after reaction with anthrone reagent (Thomas, 1977). Ammonia-N (AN) was determined using the phenol-hypochlorite reaction method (Broderick and Kang, 1980). The methods of Van Soest et al. (1991) were used for NDF and ADF analysis and the analyses were not sequential. Amylase and sodium sulfite were used in the NDF analysis and the results were expressed on a DM basis inclusive of ash. Total

nitrogen (TN) was analyzed by the Kjeldahl procedure (Krishnamoorthy et al., 1982), crude protein (CP) was determined as the TN multiplied by 6.25. Ether extract content was determined according to Horii et al. (1971). Non-fibrous carbohydrate (NFC) was calculated by the formula: NFC = 100−CP−NDF−EE−ash (NRC, 2001). The LA was determined by the method of Barker and Summerson (1941). VFAs were determined with gas chromatography (Shimadzu GC-17A, Japan, with 12 m capillary column, condition: column temperature 130°C, injection temperature 220°C. To assess the quality of the silage, we calculated the V-score from the AN/total N and VFA contents (Takahashi et al., 2005).

The TMR samples (10 g) were blended with 90 mL of sterilized water, and serially diluted in sterilized water. Enumeration of yeasts and lactic bacteria was done from the fresh TMR and silages (d 45). The number of lactic acid bacteria (LAB) were measured by plate count on Lactobacilli de Man, Rogosa, Sharpe (MRS) agar incubated at 30°C for 48 h under anaerobic conditions (Anaerobic box; YIHENG Technical co., Ltd., Shanghai, China). Yeast were counted on potato dextrose agar (Sincere Biotech co., Ltd., Shanghai, China), incubated for 24 h at 30°C. Colonies were counted as viable numbers of microorganisms from plates containing a minimum of 30 and a maximum of 300 colonies. All the microbiological data were log transformed.

Aerobic stability test

The aerobic stability was defined as the number of hours that the pH value of the silage remained stable before rising more than 0.5 above the initial pH value. During the aerobic exposure (0, 6, 9, and 12 days), the silages were sampled to determine pH value, AN/TN, LA, and WSC contents, yeast and lactic acid bacteria counts.

Statistical analyses

Analyses were performed using the general linear model procedure (SAS Institute, Cary, NC, USA). Data on Chemical composition and characteristics data of fresh and ensiled TMR were subjected to one-way analysis of variance (ANOVA) with treatment as factor. In aerobic conditions, the data on chemical composition of silages were 4 (treatments)×4 (deterioration periods)×3 (replicates) = 48 observations corresponding to each variable and were analyzed in a repeated measures analysis of variance using the PROC GLM. In General Linear Model, seven various covariance structures (CS, UN, HF, AR, ARH, ANTE) were applied. AIC and AICC criteria were used to determine the most appropriate covariance pattern for fitting data, it was determined that unstructured (UN) covariance structure gave the best fit to data set. The triplicate samples were considered as replicates, and treatments and deterioration periods were considered to be between- and within-subjects factor, respectively. All unstructured covariance matrix of the data were meet the assumption of sphericity. Statistical difference between means was determined by Tukey's multiple comparison. Differences were considered significant when probability was less than 0.05.

RESULTS

Chemical composition of materials

The chemical composition and microbial counts of whole-plant corn and TMR before ensiling are presented in Table 2. The DM content of TMR mixture was 354 g/kg FW. The WSC content was 223.54 g/kg DM. The buffering capacity and CP concentrations of TMR were 230.67 mE/kg DM and 13.40% DM respectively. Epiphytic LAB on TMR were more than 1.0×10^5 cfu/g FM, yeast were more than 1.0×10^4 cfu/g FM.

Fermentation quality of TMR silage after 45 days of ensiling

The fermentation quality and the microbial composition of TMR silages after 45 days of ensiling are presented in Table 3. DM recovery of TMR silages treated with M and PM were higher ($p<0.05$) than that of TMR silage treated with P, which was significantly higher than that of the control silage. All TMR silages showed lower pH, which were below 4.0. M silage had the highest lactic acid content followed by control and then P and PM silages. In contrast, treatment with P and PM resulted in a marked decrease ($p<0.05$) in the contents of acetic acid in silage. The addition of P and PM significantly decreased acetic acid concentrations compared with the control. P and PM silages showed higher propionic acid concentrations, while only small amount of propionic acid were found in the control

Table 2. Chemical and microbial composition of whole crop corn and total mixed ration before being ensiled

Items	Whole-plant corn	TMR
Dry matter (g/kg FM)	227	354
pH	5.58	5.29
Crude protein (% DM)	5.10	13.40
Water soluble carbohydrate (g/kg DM)	298.40	239.84
Buffering capacity (mE/kg DM)	222.06	230.67
Neutral detergent fiber (g/kg DM)	512	479
Acid detergent fiber (g/kg DM)	255	223
Lactic acid bacteria (log cfu/g)	6.60	5.56
Yeast (log cfu/g)	4.11	4.59

DM = Dry matter.

Table 3. Chemical and microbial composition of total mixed ration silages after 45 days of ensiling

Items	Treatments				p-value
	Control	M	P	M+P	
DM content (g/kg)	324.58±6.87^{b}	349.64±0.75^{a}	328.92±2.12^{b}	350.60±9.05^{a}	0.0275
DM recovery (g/kg)	917.33±1.53^{c}	973.33±1.52^{a}	924.33±3.06^{b}	975.67±2.08^{a}	<0.0001
pH value	3.90±0.06^{a}	3.89±0.02^{a}	3.93±0.05^{a}	3.87±0.03^{a}	0.373
Lactic acid (g/kg DM)	86.53±2.38^{b}	97.61±2.49^{a}	65.44±1.91^{c}	64.63±1.61^{c}	0.732
AN/TN (g/kg TN)	52.83±1.58^{a}	41.43±2.20^{b}	42.48±0.74^{b}	29.88±2.50^{c}	0.0163
WSC (g/kg DM)	39.99±0.28^{d}	44.71±0.94^{c}	88.92±1.52^{b}	130.2±4.12^{a}	<0.0001
Acetic acid (g/kg DM)	12.19±2.09^{a}	11.49±1.16^{ab}	10.04±1.11^{bc}	8.48±0.46^{c}	0.0028
Propionic acid (g/kg DM)	0.17±0.02^{b}	0.47±0.03^{b}	10.21±0.73^{a}	10.21±0.18^{a}	<0.0001
Butyric acid (g/kg DM)	0.19±0.02^{a}	0.13±0.01^{b}	0.04±0.01^{c}	0.02±0.00^{c}	<0.0001
NDF (g/kg DM)	475.32±9.62^{a}	448.38±10.34^{b}	458.54±4.84^{b}	412.90±2.49^{c}	<0.0001
ADF (g/kg DM)	232.20±5.54^{a}	208.54±1.39^{bc}	216.58±8.32^{b}	201.41±1.45^{c}	0.0004
V-score	97.36±0.16^{ab}	97.80±0.42^{a}	95.97±026^{c}	96.52±0.10^{bc}	0.0052
Lactic acid bacteria (log10 cfu/g)	6.30±0.20^{b}	7.23±0.38^{a}	7.23±0.38^{a}	7.2±0.25^{a}	0.0085
Yeasts (log10 cfu/g)	<2.00	<2.00	<2.00	<2.00	-

M = Molasses, P = Propionic acid, M+P = Molasses+propionic acid.
FM = Fresh matter, DM = Dry matter, NDF = Neutral detergent fiber, ADF = Acid detergent fiber.
Values in the same row ($^{a-d}$) with different following letters are significantly different ($p<0.05$).

and M. The concentrations of butyric acid in P and PM silages were nearly at undetectable levels, which were significantly lower than that in the control and M silages ($p<0.05$). The residual WSC contents in treated TMR silages were significantly higher ($p<0.05$) than that of the control silage, and P and PM silages showed doubled and thrice of residual WSC as well as the control. The AN/TN contents of treated TMR sikages were lower ($p<0.05$) than that of the control silage, and lowest AN/TN was found in PM silage (29.88 g/kg DM). In addition, silages treated with M and P had lower ($p<0.05$) concentrations of ADF and NDF compared with the control silage, and silages treated with PM had the lowest ($p<0.05$) concentrations of ADF and NDF than that of other silages. The counts of lactic bacteria in M silage were as high as 10^7 cfu/g, which was higher ($p<0.05$) than that in the control silage, while the counts of lactic bacteria in P and PM silages were lower than control. Yeasts populations in all TMR silage were reduced to below the detectable level ($<10^2$ cfu/g FM).

Effects of molasses and propionic acid on aerobic stability of TMR silages

Changes of pH and lactic acid contents of all TMR silages are shown in Table 4. Changes of pH value and lactic acid content with time of aerobic exposure in all silages are shown in Table 4. The lactic acid content decreased gradually at the beginning of the deterioration test until the end of aerobic stability test in the control silage, from 86.53 g/kg DM to 15.49 g/kg DM, thus quickly raising the pH from 3.9 to 7.7. On the ninth measurement of

Table 4. Values of pH and LA contents of total mixed ration silages treated with additives sampled in periods (days of aerobic exposure)

Items	Periods	Treatments				Mean	p-value		
		Control	M	P	PM		T	D	T×D
pH	0	3.90±0.06^{Ac}	3.93±0.05^{Abc}	3.89±0.02^{Aab}	3.87±0.03^{Aa}	3.90^{d}	<0.0001	<0.0001	<0.0001
	6	4.28±0.13^{Ac}	3.99±0.06^{Bb}	3.88±0.07^{Ba}	3.86±0.02^{Ab}	4.01^{c}			
	9	5.1±0.41^{Ab}	4.02±0.05^{Bb}	3.87±0.03^{Ba}	3.84±0.02^{Bab}	4.20^{b}			
	12	7.07±0.04^{Aa}	4.95±0.01^{Ba}	3.75±0.03^{Cc}	3.80±0.04^{Cb}	4.89^{a}			
	Mean	5.09±1.29^{A}	4.22±0.44^{B}	3.84±0.08^{C}	3.85±0.04^{C}				
Lactic acid (g/kg DM)	0	86.53±2.38^{Ba}	97.61±2.49^{Aa}	65.44±1.91^{Bc}	64.63±1.61^{Cc}	78.56^{a}	<0.0001	<0.0001	<0.0001
	6	66.95±2.67^{Bd}	74.32±2.00^{Bc}	83.34±0.70^{Aa}	78.09±1.94^{ABb}	75.67^{b}			
	9	38.63±2.55^{Cc}	75.06±2.18^{Bb}	83.42±1.41^{Aa}	80.22±1.59^{Aa}	69.34^{c}			
	12	15.49±2.75^{Dd}	32.45±1.94^{Cc}	67.09±0.76^{Bb}	75.47±1.28^{Ba}	47.63^{d}			
	Mean	51.90±28.34^{C}	69.86±24.66^{B}	74.82±9.02^{A}	74.61±9.43^{A}				

M = Molasses, P = Propionic acid, M+P = Molasses+propionic acid.
T = Effect of treatment, D = Effect of deterioration period, T×D = Effect of treatment×effect of deterioration period.
Values in the same row ($^{A-D}$) or in the same column ($^{a-d}$) with different following letters are significantly different ($p<0.05$).

Table 5. AN/TN and WSC contents of total mixed ration silages treated with additives sampled in periods (days of aerobic exposure)

Items	Periods	Treatments				Mean	p-value		
		Control	M	P	PM		T	D	T×D
AN/TN (g/kg TN)	0	52.83±1.58^{Abc}	41.43±2.20^{Bb}	42.48±0.74^{Bc}	29.88±2.5^{Cd}	41.65^{d}	<0.0001	<0.0001	<0.0001
	6	51.87±1.57^{Ac}	46.07±3.20^{Bb}	41.94±0.96^{Cc}	52.18±1.78^{Ac}	48.01^{c}			
	9	55.38±1.96^{Ab}	42.77±1.26^{Cb}	51.72±0.74^{Bb}	57.84±1.4^{Ab}	51.93^{b}			
	12	65.59±1.23^{Aa}	53.77±4.39^{Ca}	57.36±2.63^{BCa}	62.12±0.18^{ABa}	59.71^{a}			
	Mean	56.42^{A}	46.01^{C}	48.38^{B}	50.51^{B}				
WSC (g/kg DM)	0	39.99±0.28^{Da}	55.20±1.01^{Ba}	88.92±1.52^{Ba}	130.20±4.12^{Aa}	78.18^{a}	<0.0001	<0.0001	0.0001
	6	29.16±2.20^{Dc}	44.71±0.94^{Cb}	78.23±2.64^{Bb}	115.70±3.96^{Ab}	65.60^{b}			
	9	34.64±1.31^{Db}	45.55±2.64^{Cb}	72.23±1.28^{Bc}	105.66±2.21^{Ac}	64.52^{b}			
	12	29.56±0.65^{Cc}	39.32±1.57^{Cc}	54.23±1.00^{Bd}	106.10±3.46^{Ac}	57.30^{c}			
	Mean	33.34^{D}	46.20^{C}	73.40^{B}	114.42^{A}				

M = Molasses, P = Propionic acid, M+P = Molasses+propionic acid. AN/TN = Ammonia nitrogen/total nitrogen, WSC = Water soluble carbohydrate.
T = Effect of treatment, D = Effect of deterioration period, T×D = Effect of treatment×effect of deterioration period.
Values in the same row ($^{A-D}$) or in the same column ($^{a-d}$) with different following letters are significantly different ($p<0.05$).

aerobic exposure, the pH had already raised to 5.1, thus we deemed it as deterioration because it was more than 0.5 above the initial pH value (3.9). Similar to the control silage, the lactic acid content of M silage also decreased gradually with time of aerobic exposure, raising the pH from 3.93 to 4.22. Silage treated with M remained stable for less than 288 h but more than 216 h. Lactic acid contents increased in both P and PM silages until 9 days and then decreased, however, they were still above the initial value (at silo opening) until aerobic exposure was terminated after 12 days. The pH was thus maintained below the initial value until aerobic exposure was terminated after 12 days. Thus treatment with BP and BM markedly improved the ($p<0.05$) aerobic stability of the silages.

The ratio of AN/TN increased with time of aerobic exposure in all TMR silages, and reached highest On the twelfth measurement of aerobic exposure (Table 5). AN/TN of P and M silages significantly lower ($p<0.05$) than that of the control and PM silages after 6 d of aerobic exposure respectively. The WSC contents decreased with time of aerobic exposure in all TMR silages (Table 5). During the periods of aerobic exposure, addition of P and PM silages significantly increased ($p<0.05$) residual WSC content compared with the control and M silages.

Lactic bacteria and yeast populations varied with time of aerobic exposure (Table 6). The population of LAB in the control and M silages gradually decreased after exposure to air, while that in P and PM silages, increased until 9 days and then decreased below the initial value (at the begin of aerobic exposure). Treated silages showed significantly lower ($p<0.05$) populations of yeast as compared with the control silages. On the sixth measurement during aerobic exposure, yeast counts in the control silage increased steadily to a level of more than 10^8 cfu/g. On the ninth measurement during aerobic exposure, yeast counts in M silage were also more than 10^8 cfu/g, while yeast counts in P and PM silages were less than 10^5 cfu/g throughout the aerobic exposure time.

Table 6. Microbial composition of total mixed ration silages treated with additives sampled in periods (days of aerobic exposure)

Items	Periods	Treatments				Mean	p-value		
		Control	M	P	PM		T	D	T×D
Yeast (log10 cfu/g)	0	<2.00^{c}	<2.00^{d}	<2.00^{d}	<2.00^{c}	<2.00^{c}	<0.0001	<0.0001	<0.0001
	6	8.46±0.27^{Ab}	7.12±0.04^{Bc}	4.9±0.00^{Ca}	4.60±0.00^{Db}	6.27^{b}			
	9	8.75±0.03^{Aa}	8.09±0.13^{Bb}	4.55±0.09^{Cc}	4.60±0.00^{Cb}	6.50^{a}			
	12	8.58±0.07^{Aab}	8.5±0.05^{Aa}	4.71±0.32^{Bb}	4.74±0.12^{Ba}	6.63^{a}			
	Mean	6.94^{A}	6.42^{B}	4.04^{C}	3.98^{C}				
Lactic acid bacteria (log10 cfu/g)	0	6.30±0.20^{Ba}	7.23±0.38^{Aa}	5.6±0.00^{Cc}	5.59±0.14^{Cb}	6.17^{b}	<0.0001	<0.0001	0.0001
	6	6.41±0.20^{Ba}	7.11±0.07^{Aa}	6.54±0.53^{Bb}	5.6±0.00^{Cb}	6.42^{b}			
	9	6.03±0.64^{Bb}	6.61±0.32^{Bb}	7.14±0.23^{Aa}	7.2±0.25^{Aa}	6.74^{a}			
	12	6.09±0.08^{Ab}	5.79±0.13^{Bc}	5.47±0.23^{Cc}	5.16±0.04^{Dc}	5.63^{c}			
	Mean	6.21^{B}	6.68^{A}	6.19^{B}	5.89^{C}				

M = Molasses, P = Propionic acid, M+P = Molasses+propionic acid. AN/TN = Ammonia nitrogen/total nitrogen, WSC = Water soluble carbohydrate.
T = Effect of treatment, D = Effect of deterioration period, T×D = Effect of treatment×effect of deterioration period.
Values in the same row ($^{A-D}$) or in the same column ($^{a-d}$) with different following letters are significantly different ($p<0.05$).

DISCUSSION

Chemical composition

The DM content of a crop at ensiling strongly influence the rate and extent of the fermentation quality, and a low DM content at ensiling, with a low sugar content increases the risk of a clostridial fermentation and subsequent poor acceptance of the silage by the animals (Fraser et al., 2000). Weinberg (2008) reported that the requirements for successful ensiling include: WSC content of material before ensiling should be at least 30 to 50 g/kg DM, the buffering capacity of the ensiled biomass should be low, LAB populations should be more than 1×10^5 cfu/g. In our study the DM was 354 g/kg DM and the WSC concentration was 149.39 g/kg DM before ensiling, LAB populations was more than 1×10^5 cfu/g which is critical for a successful fermentation (Haigh, 1990).

Effects of molasses and propionic acid application on fermentation quality

After 45 days of ensiling, M addition significantly increased lactic acid content by 13% compared with the control, increased DM recovery and reduced acetic acid content. This may due to silage treated with M provided more fermentation substrates for lactic acid bacteria, thus lactic acid bacteria was the predominant bacterium and enhanced the metabolism to a more homofermentative fermentation process in M silage after 45 days of ensiling. Alli et al. (1984) reported that adding molasses to chopped whole-plant Leucaena increased rates of lactic acid production and reduced DM losses as compared with the control. In contrast, lower lactic contents in P and PM silages may due to inhibit lactic acid bacteria activity by propionic acid. Britt et al. (1975) reported that lactic acid contents of silages were decreased ($p<0.01$) by addition of propionic acid, which suggests an inhibition of microbial activity. P and PM silages also showed significantly ($p<0.05$) lower acetic acid (AA) concentrations as compared with the control silage. This may be explained as propionic acid application suppressed AA-producing bacteria activity during fermentation.

AN is related to degradation of CP and amino acids, which has been taken as an indicator of extent of proteolysis in silage. In this study, silages treated with additives contained lower AN/TN than the control silage, and PM silage showed the lowest AN/TN. Alli et al. (1984) reported that adding molasses to chopped whole-plant Leucaena reduced levels of AN/TN as compared with the control silage. Stallings et al. (1981) reported that the ammonia-N concentrations of silages treated with propionic acid were lower than that in untreated silages due to a quick propionic acid-induced decreased pH and consequently suppressed proteolysis by microbial hydrolytic activities, this study is agreement with these results.

Additives significantly increased the contents of residual WSC compared with the control silage, P silage contained higher residual WSC content than M silage, and PM silage showed the highest content of residual WSC. Adding molasses provides additional WSC for lactic bacteria fermentation, thus more WSC were residual in M silage than the control silage. Water-soluble carbohydrates are the main source of food for microorganisms during silage fermentation. Propionic acid is a potentiality antimycotic agent, during the early stage of ensiling, propionic acid could effectively inhibit the undesirable microorganisms activity, resulting in minimize the consumption of WSC by undesirable microorganisms (Woolford, 1975; Moon, 1983).

Both of NDF and ADF contents in M and P silage were significantly lower than the control silage ($p<0.05$), and PM silage had the lowest content of NDF and ADF. The decreases of NDF and ADF content in M silage may be due to the effect of molasses on promoting silage fermentation (Mcdonald et al., 1991; Baytok et al., 2005). The decreases of NDF and ADF in P silage may be attributed to acid hydrolysis. It appears that a reduction in the NDF content of corn silages was due to partial acid hydrolysis of hemicellulose (Muck and Kung, 1997).

Ensiling had significant affect on the population of yeasts, in all silages yeasts was inhibited to below the detectable level which may due to low pH (<4.00) value in all silages after 45d ensiling which restrained yeasts growth.

Effects of molasses and propionic acid application on aerobic stability

Large quantities of air often entry to silos bescuse of plastic sheet damage. Sometimes silos are disproportionately large in relation to the size of the herd being fed, and considerable quantities of silage may not be removed from the silos between feedings (Ranjit and Kung Jr, 2000). Therefore, evaluating the chemical and microbiological changes is important when silages were exposed to air, the TMR silages were sampled and analyzed with time of aerobic exposure.

Yeasts have long been considered to be responsible for the aerobic deterioration of silage, and silage with yeasts in excess of 1×10^5 cfu/g are prone to aerobic deterioration (Mcdonald et al., 1991). In the present research, the control and M silages with more than10^5 yeast/g after 6 days of aerobic exposure, and pH of the control and M silages increased gradually and reached the highest value at the end of aerobic exposure, which may be due to decrease of lactic acid contents with time of aerobic exposure. The pH is an indicator of aerobic deterioration of the silage because the

lactic acid is consumed by yeasts during aerobic exposure, and the silage becomes favourable to the growth of other undesirable microorganisms such as molds and bacteria (Basso et al., 2012). Hara S et al. (1978) also reported if silage deteriorated, the content of lactic acid would be lowered coupling with pH increasing. However, lactic acid content of M silage was always higher than that of the control silage during the time of aerobic exposure, which may result in a more stable than the control silage. While yeasts populations in P and PM silages maintained below 1×10^5 cfu/g during the aerobic exposure periods. This may be due to propionic acid is a antimicrobial agent, and high content of propionic acid could inhibit yeast growth which is consistent with findings of Huber and Soejono et al. (1977). During the 12 days of exposure to air, lactic acid in P and PM silages gradually increased until 9 d and then decreased below the initial value (at silo opening). This increase might be attributed to the volatilization and/or metabolism of propionic acid which resulted in lactate producers use the residual readily-available carbohydrate during the normal fermentation periods. The pH value of P and PM silages decreased with time of aerobic exposure, and maintain to below 4.0 throughout the whole stage of aerobic exposure, which may be due to the increase of lactic acid content. Thus P and PM silages showed the longer stage of aerobic stable. The ratio of AN/TN in all silages increased gradually, while the contents of residual WSC in all silages decreased gradually with time of aerobic exposure. One possible explanation for this result is related to an increase of CP degradation to AN in all TMR silages by aerobic bacteria utilizing residual WSC during aerobic exposure stages which is in agreement with the Literature (Bayatkouhsar et al., 2011).

CONCLUSIONS

The results of this study showed that adding molasses increased the LA content and DM recovery during ensiling, and tended to improve aerobic stability of whole-plant corn TMR silage. While applying propionic acid decreased LA content during ensiling, and preserved more WSC which stimulated LA production during aerobic exposure stage, thus applying propionic acid significantly improved aerobic stability of TMR silage.

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