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Full Length Article

Interrelationship between Nutrient and Microbial Constituents of Ensiled Whole-plant Maize as Affected by Morphological Parts

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ABSTRACT

Morphologically whole-plant maize contains various parts and those parts would influence ensiling process based on their current nutrient constituents. The objective of this study was to determine the interrelationship between microbial ensiling characteristics and nutrient constituents of whole-plant maize (WPM) parts. Whole-plant maize (*Zea mays* L.) was harvested at 2/3 milk-line maturity stage and ensiled as whole plant or separated and ensiled as ear, leaf and stalk, respectively. Microbial constituents of silages were performed during weeks 2 and 8 of ensiling period. Leaf silage had the lowest DM (25.5%) but the highest CP (13.9%), as compared to stalk silage having the highest DM (41.1%) and the lowest CP (5.4%). The lactobacilli (LAB) counts were higher for stalk (7.2 CFU g^{-1}) and WPM (7.1 CFU g^{-1}) but lower for ear (5.8 CFU g^{-1}) silages ($P<0.001$). The lowest yeast+mold (YM) count was observed in WPM silage (3.0 CFU g^{-1}) compared to leaf (6.4 CFU g^{-1}) and stalk (6.1 CFU g^{-1}) silages at week 8 ($P<0.001$). Results indicated that WPM compared to other morphological parts had a better microbial pattern for higher LAB and lower YM counts 2 and 8 weeks after ensiling. In addition, nutrient constituents can be an important parameter in determining the microbial pattern of ensiled whole-plant maize.

Key Words: Maize silage; Maize parts; Ensiling; Microbial constituents; Lactobacilli

INTRODUCTION

Several factors affect maize silage quality, such as genetic and agronomic, as well as harvest and preservation practices (Coors & Lauer, 2001). Hybrid selection is an important factor for animal performance. However, hybrid selection itself cannot determine the quality of silage without proper plant growth, harvest and ensiling practices. Ahsan *et al.* (2008) found that there were significant genotypic (r= 0.63) and phenotypic (r= 0.49) correlations between maize leaf area and whole-plant grain yield. Among the several preservation practices, ensiling of different whole plant parts and lengths of fermentation have been considered in previous researches (Verbic *et al*., 1995; Bal *et al*., 2000; Bal, 2006). Verbic *et al.* (1995) indicated that different whole-plant maize (WPM) parts varied in their effective dry matter (DM) degradability averaging 47.5% for ear, 41.1% for leaves and 41.6% for stalk, respectively. Besides stover and WPM had different DM (27.4 vs. 36.0%) and NDF (56.8 vs. 41.6%) contents at their ensiling periods (Bal *et al.,* 2000). Bal (2006) found a better silage fermentation based on pH of ensiling WPM for 8 and 16 compared to 1, 2 and 4 weeks of ensiling periods. Iqbal *et al.* (2005) also indicated that ensiling mott grass silage for 30 days had better pH (3.9 vs. 5.3 & 4.3) and lactic acid concentration (4.9 vs. 3.3 & 4.5%) compared to 10 and 20 days of ensiling periods. Although there has not been any data for predicting the microbial constituents of WPM by using nutrient constituents Russell *et al.* (1992) found a significant variables (DM & *in vitro* digestible DM) in regressions predicting lactic acid concentration of WPM silage (R^2= 0.36). Muck (1989) established a regression equation, which showed an increase of lactobacilli (LAB) counts as a function of increasing DM content of alfalfa silage. However Lin *et al.* (1992a) found no relationship between adequacy of WPM silage fermentation (pH, ammonia) and LAB counts. Therefore the objectives of this study were to determine the nutrient and microbial constituents of silages ensiled from WPM and their respective parts, as well as the interrelationship between these parameters during 2 and 8 weeks of ensiling periods.

MATERIALS AND METHODS

A commercially available maize hybrid (Bora®, May Agro) was harvested as WPM at 2/3 milk-line maturity stage. Three sets of 5 plants were randomly selected from 10 ha of maize field for ensiling each morphological part and

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WPM silages. The parts (ear, leaf & stalk) were separated from the whole plant and chopped using a commercial maize silage chopper set for a 0.93 cm theoretical length of cut. From each part and whole plant, duplicate 5-l capacity plastic jars were used for each treatment. Treatment silages were stored for 2 and 8 weeks periods. Stored jars were kept in a cold room until the respective weeks. Nutrient constituents of treatment silages were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash and crude fiber (CF) (AOAC, 1996) at week 8 only. For microbial constituents, 10 g of silage sample was diluted with 90 g of autoclaved distilled water and macerated for 30 s in a sterilized blender jar. A 0.1% proteaz-peptone (Difco-211684) solution was used for duplicate sets of serial dilutions from each sample (Muck *et al.*, 1992). A pour-plate method was used for populations of LAB (MRS broth, Merck 110661), total Enterobacteriaceae (EB; Nutrient broth, Merck 105443) and yeast+mold (YM; Malt extract broth, Merck 105397). Duplicate plates were used at each 10 x dilution between 10^1 and 10^5 so that there were 4 plates for dilution from treatment silages. The microbial constituent data at weeks 2 and 8 were analyzed in a completely randomized design for main effects of morphological parts, week and their interaction by GLM procedure of SAS® (1988). Correlation coefficients were determined between microbial and nutrient constituents of treatment silages at week 8.

Table I. Nutrient constituents of treatment silages at week 8

Treatment	Nutrient (%)				
	Dry matter	Crude protein	Ether extract	Ash	Crude fiber
Ear	35.4 ±1.4	8.6 ±0.4	2.2 ±0.1	2.0 ±0.3	14.4 ±1.0
Leaf	25.5 ±0.9	13.9 ±0.7	2.3 ±0.2	12.4 ±1.0	22.5 ±1.1
Stalk	41.1 ±1.7	5.4 ±0.2	0.1 ±0.02	5.1 ±0.5	37.6 ±1.6
WPM[a]	30.9 ±1.4	7.3 ±0.4	0.8 ±0.04	5.0 ±0.2	21.8 ±0.8

[a]**WPM**= Whole Plant Maize

Table II. Microbial constituents of treatment silages 2 and 8 weeks after ensiling

Treatment	Week	Microorganism ($\log_{10}$ CFU g^{-1} of fresh material)[a]		
		LAB	EB	YM
Ear	2	7.3	7.2	7.4
	8	5.8	6.1	5.6
Leaf	2	8.1	8.0	7.1
	8	6.8	7.2	6.4
Stalk	2	7.7	8.3	6.9
	8	7.2	8.9	6.1
WPM[b]	2	7.1	8.4	5.4
	8	7.1	5.3	3.0
SEM[c]		0.2	0.3	0.3
Effect (P≤)				
Treatment		0.001	0.001	0.001
Week		0.001	0.001	0.001
Treatment*Week		0.001	0.001	0.001

[a]**LAB**= Lactobacilli, **EB**= Enterobacteriaceae, **YM**= Yeast and Mold
[b]**WPM**= Whole Plant Maize
[c]**SEM**= Standard Error of Mean

Table III. Correlation coefficients and their significance between microbial and nutrient constituents of treatment silages 8 weeks after silage storage

Variables[a]	EB	YM	DM	CP	EE	Ash	CF
LAB	0.38	-0.21	0.02	-0.22	-0.71*	0.40	0.76*
EB		0.74*	0.49	-0.16	-0.39	0.25	0.84*
YM			0.12	0.36	0.32	0.36	0.25

[a]**LAB**= Lactobacilli, **EB**= Enterobacteriaceae, **YM**= Yeast and Mold, **DM**= Dry Matter, **CP**= Crude Protein, **EE**= Ether Extract, **CF**= Crude Fiber
*P≤ 0.05

RESULTS AND DISCUSSION

Nutrient constituents of treatment silages are presented in Table I. Highest and the lowest DM contents were observed for stalk (41.1%) and leaf (25.5%) portions of WPM, respectively. A similar DM pattern was observed for stalk (37%) and leaf (26%) of four different WPM hybrids harvested at physiological and blister stages of maturity before ensiling (Masoero *et al.*, 2006). A distinct CP difference was observed between leaf (13.9%) and other whole plant parts, averaging 7.1%. Lewis *et al.* (2004) also indicated that whole plant (7.5%) and stover (ear+stalk) portions (6.9%) had similar CP contents at physiological maturity. A highest CP content of maize leaf was also observed at tasseling (15.1%), blister (13.5%) and dough (11.4%) stages of maturity (Masoero *et al.*, 2006). This type of CP accumulation pattern on maize leaves could be explained by the plant's photosynthetic feature and the highest level and activity of nitrate reductase and glutamine synthetase enzymes, responsible for the flow of N from leaves to ear (Rizzi *et al.*, 1996). Similar to the present study, the lowest and the highest CP contents were observed for stalk (3.0%) and leaf (12%) of maize plant harvested during seven different dates within a 90 d period. Although the values in the latter study were based on fresh material, the ensiled treatment composition of the present study reflected the same pattern for CP. The highest ash was observed for leaf (12.4%) compared to other plant parts. Phipps and Weller (1979) also observed a greater mineral content for leaf (P: 0.4%, Mg: 0.3%, K: 1.9%, Ca: 1.0%) compared to stalk (P: 0.2%, Mg: 0.1%, K: 1.4%, Ca: 0.2%) and ear (P: 0.3%, Mg: 0.1%, K: 1.2%, Ca: 0.4%) portions of WPM. Similarly, Hunt *et al.* (1992) found a higher ash content for stover (3.2%) compared to ear (0.3%) of six different WPM hybrids. The stalk portion of treatment silage had the highest CF content (37.6%) compared to leaf and WPM, averaging 22.2%. This trend would be related to a greater extent of lignifications on the stalk portion as the plant grows and becomes more mature. This is especially true for the lower parts of stalk through the soil surface. Sanderson *et al.* (1995) indicated that cellulose content of stalk was greater than the WPM (33.7 vs. 23.7%). This was also confirmed by the lignin contents of stalk (4.8%) and WPM (2.5%). Although the starch content of treatment silages was not measured, lower CF along with ash and higher EE would result in greater energy content for ear silage.

Microbial constituents of treatment silages are presented in Table II. The highest LAB number was observed in leaf (8.1 CFU g^{-1}) compared to other parts at week 2, averaging 7.4 CFU g^{-1} ($P<0.001$). This could be related to the lowest DM content of leaf treatment (25.5%) compared to higher DM contents for other treatments. McEniry *et al.* (2007) demonstrated that when adequate anaerobic conditions are achieved, DM content of the herbage had a much greater impact on quality of silage fermentation than any other factor. O'Kiely and Muck (1998) also indicated that the large increase in the counts of LAB during the initial weeks of fermentation is related to lysis of plant cells under anaerobic conditions, thereby releasing readily fermentable substrate. The more distinct reduction on LAB count was observed for ear treatment (5.8 CFU g^{-1}) at week 8 (treatment*week interaction; $P<0.001$). However, WPM was the most stable silage for LAB counts without any reduction from week 2 to 8. This was somewhat surprising for maize silage, since LAB counts usually remain near maximum through 7 d of fermentation and then decrease throughout the remainder of the ensiling period (Lin *et al.*, 1992a). All EB counts were higher for stalk, WPM, ear and leaf silages at week 2. The EB counts at week 2 are especially higher than LAB counts for stalk and WPM silages. Lin *et al.* (1992b) indicated that EB were predominant species in both standing and chopped WPM silage at the initial phase of fermentation, regardless of LAB presence. This trend was changed at week 8 of fermentation for ear, leaf and WPM silages and the EB counts declined significantly from week 2 to 8 of fermentation ($P<0.001$). This reduction was more pronounced for WPM (3.1 CFU g^{-1}) compared to ear (1.1 CFU g^{-1}) and leaf (0.8 CFU g^{-1}) silages from weeks 2 to 8 (treatment*week interaction; $P<0.001$). In addition, the EB number was lower than LAB number for WPM silage only at week 8 of fermentation. The YM counts were higher for all treatments at week 2 of fermentation, averaging 6.7 CFU g^{-1}. This was also confirmed by Lin *et al.* (1992b) and the YM number for chopped WPM silage at initial phase of fermentation was higher (7.1 CFU g^{-1}). Similar to EB counts, the YM counts reduced at week 8 of fermentation ($P<0.001$). The lowest YM number was observed for WPM silage at both weeks 2 and 8 of fermentation (5.4 & 3.0 CFU g^{-1}; $P<0.001$). After 2 weeks of ensiling period, a stable LAB development and a possible decrease on pH proved an inhibitory effect on EB and YM activity for WPM silage at week 8 (McDonald *et al.*, 1991; Bal, 2006).

Although there has not been any data for explaining the relationship between LAB and EE, there was a negative relationship ($r= -0.71$; $P<0.05$) between LAB and EE in the present study (Table III). In addition, a positive relationship ($r= 0.76$; $P<0.05$) was found between LAB and CF of the treatment silages. Russell *et al.* (1992) found that DM was one of the best predictor for lactic acid concentration of the WPM silage ($R^2= 0.28$; $P<0.01$). In addition, they found that the *in vitro* DM degradability was one of the best predictor for lactic acid concentration of WPM silage ($R^2= 0.36$; $P<0.01$). Previous research (Russell *et al.*, 1992; Verbic *et al.*, 1995) also indicated that ADF was the best predictor of WPM silage nutritive value. There was no significant relationship between YM and any nutrient constituents of treatment silages.

CONCLUSION

Higher LAB but lower YM counts promoted the whole-plant maize silage over the leaf and ear silages. Eight weeks of ensiling period compared to 2 weeks eliminated a possible YM accumulation in all treatment silages. Nutrient constituents of WPM silage can be a determinant of silage microbial distribution. Besides any other nutrient constituents (i.e., sugar, starch), LAB count can be highly correlated with EE and fiber content of the WPM silage. Further research may be needed to test various parts of other available maize hybrids in terms of their specialty nutrient compositions (i.e., low fiber, high starch).

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