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# Effect of Sugarcane Molasses and Whey on Silage Quality of Maize

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**Abstract:** The potential of cane molasses and whey as additives to ensile maize plants (*Zea mays* Linnaeus) was investigated. Maize stem plus leaves were chopped, mixed with cane molasses and whey, placed in cylindrical plastic containers, hermetically closed and characterized. The pH of the silage decreased significantly in each of the treatments with a faster decrease found when whey was added. The lactic acid concentration was > 60 g kg<sup>-1</sup> in silage amended with molasses and/or whey and 41 g kg<sup>-1</sup> in the control treatment after 15 days. Acetic acid was the only volatile fatty acid detected in the silage of maize and its concentration was 7.3 g kg<sup>-1</sup> when whey was added, but 16.2 g kg<sup>-1</sup> in the control treatment. In conclusion it was shown that maize plants can be effectively ensiled with whey in combination with sugarcane molasses as additives inducing a faster production of lactic acid and resulting in a better silage product.

**Key words:** Sugarcane molasses, ensiling, maize (Zea mays Linnaeus), mini-silos, whey

# INTRODUCTION

Maize (Zea mays Linnaeus) originates from Mexico and is still the main staple crop in large parts of the country. Cultivation of maize has increased in the world and the value of maize as a cost-effective ruminant feed is one of the main reason farmers grow it (McKendrick et al., 2003). Ensiling maize plants gives silage suitable for feeding while preserving its moisture content (Asbell et al., 2001). Water-soluble carbohydrates (WSC) are fermented to lactic acid by epiphytic lactic acid bacteria (LAB), which decreases pH, inhibits the activity of plant enzymes and reduces pathogenic or spoilage bacteria that could decrease the nutritive value of the silage however, it is recommended to add supplements (Davies et al., 2000). The main objective to apply additives for ensiling is to reduce pH more rapidly so as to preserve carbohydrates and proteins and inhibit the growth of microorganisms that might deteriorate the silage (Weinberg and Muck, 1996; Zhang et al., 2000a). Silage additives can be divided into two major groups: fermentation inhibitors (e.g., organic acids) and fermentation stimulators (e.g., strains of lactobacilli or readily degradable sugars) (Hetta et al., 2003; Meeske et al., 2002). The cost and availability of commercial silage additives are often a limiting factor and waste materials can then serve as an alternative. Use of waste materials depends on availability and their possible nutrimental value for cattle. The composition of the waste is also important as plant residues

might contain polyphenols and tannins that are known to inhibit microbial fermentation during ensiling (Kondo *et al.*, 2004). Two additives for the ensiling of maize were selected that are easily available in Chiapas (Mexico), that have a nutrimental value for cattle and do contain little or no fermentation inhibitors, i.e., cane molasses and whey. Cane molasses is a waste product of sugar production, an important industry in Chiapas. It has been used as a supplement for cattle manure silage with or without corn stover (Cobos *et al.*, 1997; Martínez-Avalos *et al.*, 1998). Whey is a cheap residue of cheese production and available to most farmers in Chiapas and it has been added to wheat straw and rice bran silage (Daniels *et al.*, 1983). A combination of molasses and dehydrated whey have been used as supplements to ensile fish waste, but little is known about their potential to ensile maize (De Lurdes *et al.*, 1998). The objective of this study was to evaluate the potential of cane molasses and whey as silage additives to whole maize plants.

### MATERIALS AND METHODS

This study was conducted in research laboratory from Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Chiapas, Chiapas, México (lat. 16045'0" N, long. 9307'0" W). Volatile fatty acids were determined in Colegio de posgraduados, Estado de México, México. The experiments were realized during the months of February to June 2006.

### Maize Genotype and Silage

Maize Maya-2002 hybrid seeds were obtained from PROASE (Productores Asociados de Semillas) Chiapas, México. Seeds were submerged in water for one day, cultivated in the field and harvested after 75 days. The whole plants (stem plus leaves) were chopped in 0.5 cm, mixed with cane molasses (100 g kg $^{-1}$ ) and whey (20 g kg $^{-1}$ ) and weighted (Table 1). A sub-sample of 5 g was taken to determine the moisture content, which ranged between 600-700 g kg $^{-1}$ . One kilogram of each mixture was placed in a cylindrical plastic container (15 cm height  $\times$  12 cm diameter) and closed airtight with a plastic lid, i.e., mini silos. Each mini silo was an experimental unit and implemented in triplicate. Mini silos were kept at 30°C for 15 days. A 10 g sub-sample was taken after at 0, 3, 7 and 15 days, weighted and characterized (Megías *et al.*, 1999).

# **Chemical Analysis**

Samples of pre- and post-ensiled mixtures were analysed for pH and dry matter, organic matter, total nitrogen and ash content (AOAC, 1980). The neutral detergent fiber content was analyzed as described by Goering and Van Soest (1970). Lactic acid was determined colorimetrically using standard solutions (0-30 µg mL<sup>-1</sup> in 5 µg increments) (Madrid *et al.*, 1999).

Volatile fatty acids, i.e., butyric acid, acetic acid and propionic acid, were measured on a Perkin Elmer Clarus 500 gas chromatograph (USA) (Cobos *et al.*, 1997). Samples were acidified with 250 g kg<sup>-1</sup> metaphosphoric acid, placed in 2 mL bottles and centrifuged at 15,000 rpm for 5 min. Samples were stored at -4°C and analyzed. A reference sample containing butyric, propionic and acetic acid in 4:1 (v/v) was used as a standard.

All results were subjected to a one-way analysis of variance to test for significant differences between the treatments using PROC GLM (SAS statistical package) with the Tukey's Studentized Range test (p<0.05) (SAS, 1989).

### RESULTS AND DISCUSSION

The pH of the silage decreased significantly over time in each of the treatments with a faster decrease found when whey was added (p<0.05) (Table 1). The drop in pH at the onset of the

Table 1: Changes in pH and lactic acid production and fiber, total nitrogen, ash, dry matter, carbon and total carbon content (g kg<sup>-1</sup> dry matter) of maize plants ensiled with cane molasses (100 g kg<sup>-1</sup>) and whey serum milk (20 g kg<sup>-1</sup>)

(20 g kg	pH					Lactic acid (g kg <sup>-1</sup> dry matter)				
Treatments	0 days	3 days	7 days	15 days	MSD <sup>a</sup>	0 days	3 days	7 days	15 days	MSD
Control	6.9 <b>a</b> A <sup>b</sup>	4.5aB	3.9aB	3.9aB	0.6	0aB	14bAB	34aA	41aA	32
Molasses	6.9aA	4.3aB	3.7abC	3.9aC	0.2	0aB	31aAB	55aA	61aA	32
Whey	6.2bA	3.9aB	3.5bB	3.8aB	0.4	0aB	41aA	65aA	69aA	23
Molasses+Whey	6.3bA	3.9aB	3.8aB	3.8aB	0.2	0aB	37aA	50aA	60aA	26
MSD	0.4	0.6a	0.2	0.1			10	35	43	
	Neutral detergent fiber (g kg <sup>-1</sup> dry matter)					Total nitrogen content (g kg <sup>-1</sup> dry matter)				
Control	12aA	12aA	12aA	13aA	1	1.8aA	1.8aA	1.9aA	1.9 <b>a</b> A	0.6
Molasses	9bA	9bA	9bA	10abA	2	2.1aA	1.9aA	2.1 aA	2.3aA	0.4
Whey	12aA	11aA	11aA	12aA	2	2.1aA	2.3aA	2.0aA	2.2aA	0.7
Molasses+Whey	9bA	8bA	7cA	8bA	3	2.0aA	2.2aA	1.9aA	2.1 aA	0.5
MSD	2	2	1	2		0.7	0.5	0.6	0.4	
	Ash content (g kg <sup>-1</sup> dry matter)					Dry matter content (g kg <sup>-1</sup> dry matter)				
Control	2.2aA	2.4aA	2.4aA	2.6aA	0.4	28aA	32aA	33aA	34aA	7
Molasses	2.0aAB	2.3aA	2.2aAB	1.9aB	0.3	30aA	32aA	33aA	33aA	4
Whey	2.4aA	2.4aA	2.4aA	2.6aA	0.4	31aA	32aA	31aA	33aA	7
Molasses+Whey	2.6aA	2.1aA	2.1aA	2.3aA	1.3	29aB	33ABa	31aAB	34aA	4
MSD	0.7	0.7	0.7	0.9		7	5	5	3	
	Total carbohydrates (g kg <sup>-1</sup> dry matter)					Organic carbon content (g kg <sup>-1</sup> dry matter)				
Control	11.4aA	11.8aA	11.6aA	11.9aA	1.3	18cA	19cA	21bA	22bA	5
Molasses	10.1aB	11.3aAI	3 11.6aA	11.5aA	1.3	21bcA	21cA	23bA	23bA	3
Whey	11.3aA	11.5aA	11.9aA	11.7aA	1.6	23bA	25bA	25bA	27abA	4
Molasses+Whey	9.9aA	9.8bA	10.8aA	10.8aA	1.5	28aA	29a A	30aA	30aA	2
MSD	1.5	1.1	1.3	1.7		3	2		6	

 $^{\circ}$ MSD: Minimum significant difference (p<0.05),  $^{\circ}$ Values with a different small letter(s) are significantly different between the treatments, while values with a different capital letter(s) are significantly different over time (p<0.05)

incubation was presumably related to lactic acid production. At the end of the ensilage, however, the pH was similar in all treatments. The pH of 3.8 to 3.9 in the final product is typical for lactic fermentation and indicated that the silage mixtures were well fermented (Davies *et al.*, 2000).

The lactic acid production was significantly larger in silage with molasses and molasses plus whey than in the control treatment after three days, with the largest increase found in the latter one (p<0.05). Higher lactic acid concentrations when molasses were added might be due to the rapid degradation of the WSC in the molasses. Those WSC were used immediately by lactic acid bacterium forming lactic acid thereby reducing pH (Weinberg et al., 1988). Additionally, the lower pH helps to hydrolyze polysaccharides in maize leaves so that they become available for LAB (Zhang et al., 2000b). Whey increased acidity and contains components, such as lacto albumins and lacto globulins rich in sulfur amino acids (cysteine and methionine) and minerals (Ha and Zemel, 2003), creating better conditions for growth of homo-fermentative LAB increasing lactic acid production (Zhang et al., 2000a). Whey also contains LAB, such as Lactobacillus delbrueckii, L. helveticus and Streptomices thermophilus used as inoculum in cheese production (Mannu et al., 2002), but presumably also other LAB that induced a rapid acidification of the forage during the early stages of ensiling (Weinberg and Muck, 1996). Fast production of lactic acid is important to obtain high quality silage because lactic acid is responsible for inactivation of plant enzymes and death of undesirable microorganism that might inhibit fermentation or lead to silage deterioration even after that ensiling was over, i.e., silage with a low stability (Opitz Von Boberfeld, 2001). After seven days, the lactic acid production was similar in all treatments due to the degradation of polysaccharides, i.e., (hemi) cellulose, of the maize leaves. Asbell et al. (2001) found that lactic acid was only produced after nine weeks in a maize ensiling and after one month for wheat ensiling, while it was already produced after 15 days in the study reported here.

Table 2: Changes in acetic acid concentration (g kg<sup>-1</sup>) in maize plants ensiling with cane molasses (M), whey (W) (serum milk) and cane molasses plus whey (M+W) additives

Treatments	0 days	3 days	7 days	15 days	MSD <sup>a</sup>
Control	0aC⁵	0aC	8.9aB	16.2aA	4.6
Molasses	0aB	0 <b>a</b> B	2.7bB	11.1bA	5.6
Whey	0aB	0 <b>a</b> B	0.0dB	7.3 <b>d</b> A	2.4
Molasses+Whey	0aB	0 <b>a</b> B	1.8cB	8.9cA	4.3
MSD	0	0	0.8	1.5	

 $^{\circ}$ MSD: Minimum significant difference (p<0.05),  $^{\circ}$ Values with a different small letter(s) are significantly different between the treatments, while values with a different capital letter(s) are significantly different over time (p<0.05)

The N concentration was not significantly different between the mixtures at all times tested for (Table 1). Losses of nitrogenous compounds were thus negligible and indicative of a suitable fermentation, although changes in the distribution of the nitrogenous components might have occurred during the ensilage (Driehuis and van Wikselaar, 2001).

Addition of molasses reduced significantly the neutral detergent fiber content in maize silage compared to the control treatment or maize silage added with whey (p<0.05) (Table 1). The fiber content decreased because cellulolytic microorganisms degraded (hemi)cellulose (Lin *et al.*, 1992). Ash and dry matter contents were not significantly different between treatments (Table 1). This is important because a key factor in ensiling is to preserve the dry matter content thereby reducing effluent production (Haigh, 1999). A negative relationship has often been found between the dry-matter content of the ensiled crop and the amount of effluent produced and silage effluent is a major source of agricultural pollution (Haigh, 1999; Megías *et al.*, 1999). The use of the additives did not significantly improve the fermentation stability of the silage, but the total production of effluents with cane molasses was significantly lower than that of the control without additives (data not showed) (p<0.05). Organic matter was significantly larger in maize silage added with whey and whey plus cane molasses (p<0.05). The carbohydrate content was not different between treatments.

Acetic acid was the only volatile fatty acid detected in the silage of maize and decreased significantly with the addition of whey and/or molasses (p<0.05) (Table 2). The acetic acid concentration was significantly lower in maize added molasses plus whey than in maize added with cane molasses but significantly larger than in maize added with whey (p<0.05). It has been reported that addition of molasses and lactobacilli reduced the concentration of acetic acid in red clover silage (Hetta *et al.*, 2003). Palatability of the forage increases with lower acetic acid concentrations. However, the presence of some acetic acid is required as it inhibits fungal growth and thus preserves silages susceptible to spoilage upon exposure to air (Weinberg *et al.*, 2003).

# CONCLUSIONS

It was shown that maize plants can be effectively ensiled with whey and molasses inducing a higher and faster production of lactic acid. Whey in combination with sugarcane molasses can be used to obtain maize silage suitable for ruminants. Further field experiments will be required to determine whether similar results can be achieved in commercial silos.

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# REFERENCES

AOAC., 1980. Official Methods of Analysis. 14th Edn. AOAC, Washington DC.

- Asbell, G., T. Kipnis, M. Titterton, Y. Hen, A. Azrieli and Z.G. Weinberg, 2001. Examination of a technology for silage making in plastic bags. Anim. Feed Sci. Technol., 91 (3-4): 213-222.
- Cobos, P.M., S.M. González, G.D.M. Mendoza, C.B. García and R.G. Barcena, 1997. Nutritional evaluation of cattle manure, molasses and corn stover silage for lambs. Small Rum. Res., 25 (1): 33-38.
- Daniels, L.B., M.J. Smith, O.T. Stallcup and J.M. Rakes, 1983. Nutritive value of ensiled broiler litter for cattle. Anim. Feed Sci. Technol., 8 (1): 19-24.
- Davies, Z.S., R.J. Gilbert, R.J. Merry, D.B. Kell, M.K. Theodorou and G.W. Griffith, 2000. Efficient improvement of silage additives by using genetic algorithms. Applied Environ. Microbiol., 66 (4): 1435-1443.
- De Lurdes, M., E. Dapkeviius, I. Batista, M.J.R. Nout, F.M. Rombouts and J.H. Houben, 1998. Lipid and protein changes during the ensilage of blue whiting (*Micromesistius poutassou* Risso) by acid and biological methods. Food Chem., 63 (1): 97-102.
- Driehuis, F. and P.G. Van Wikselaar, 2001. Determination of microbial protein in perennial ryegrass silage. Grass Forage Sci., 56 (1): 75-83.
- Goering, H.K. and P.J. Van Soest, 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and some Applications). USDA Agricultural Handbook No. 379, Washington, DC., pp. 1-37.
- Ha, E. and M.B. Zemel, 2003. Functional properties of whey, whey components and essential amino acids: Mechanisms underlying health benefits for active people (review). J. Nutr. Biochem., 14 (5): 251-258.
- Haigh, P.M., 1999. Effluent production from grass silage treated with additives and made in large-scale bunker silos. Grass Forage Sci., 54 (3): 208-218.
- Hetta, M., J.W. Cone, A.M. Gustavsson and K. Martinsson, 2003. The effect of additives in silages of pure timothy and timothy mixed with red clover on chemical composition and *in vitro* rumen fermentation characteristics. Grass Forage Sci., 58 (3): 249-257.
- Kondo, M., N. Naoki, K. Kazumi and H.O. Yolota, 2004. Enhanced lactic acid fermentation of silage by the addition of green tea waste. J. Sci. Food Agric., 84 (7): 728-734.
- Lin, C., K.K. Bolsen, B.E. Brent and D.Y.C. Fung, 1992. Epiphytic lactic acid bacteria succession during pre-ensiling and ensiling periods of alfalfa and maize. J. Applied Bacteriol., 73 (5): 375-387.
- Madrid, J., A. Martínez-Teruel, F. Hernández and M.D. Megías, 1999. A comparative study on the determination of lactic acid in silage by colorimetric, high-performance liquid chromatography and enzymatic methods. J. Sci. Food Agric., 79 (12): 1722-1726.
- Mannu, L., G. Riu, R. Commian, M.C. Fozzi and F. Scintu, 2002. A preliminary study of lactic acid bacteria in whey starter culture and industrial pecorino sardo ewes milk cheese: PCR-identification and evolution during ripening. Int. Dairy J., 12 (1): 17-26.
- Martínez-Avalos, A.M.M., G.D. Mendoza, M.A. Cobos, S. González, C.M. García-Bojalil and R. Bárcena, 1998. Nutritional evaluation of cattle manure silage with molasses for ruminants. Anim. Feed Sci. Technol., 70 (3): 257-264.
- McKendrick, E.J., D.J. Roberts and N.W. Offer, 2003. The value of malt distillers's grains ensiled with molasses sugar beet pellets as a feed for dairy cows. Grass Forage Sci., 58 (3): 287-294.
- Meeske, R., G.D. van der Merwe, J.F. Greyling and C.W. Cruywagen, 2002. The effect of adding an enzyme containing lactic acid bacterial inoculant to big round bale oat silage on intake, milk production and milk composition of Jersey cows. Anim. Feed Sci. Technol., 97 (3-4): 159-167.
- Megías, M.D., A. Martínez-Teruel and M.R. Hernández, 1999. Potential environmental impact of effluents from the artichoke (*Cynara scolymus* L.) byproduct ensiling process using additives. J. Agric. Food Chem., 47 (6): 2455-2458.

- Opitz Von Boberfeld, W., 2001. Effect of homofermentative lactic acid bacteria concentrates on the aerobic stability of grass silage. German J. Agron., 5 (1): 7-14.
- SAS, 1989. Institute Inc., Statistic Guide for Personal Computers. Version 6.04. SAS Institute, Cary. Weinberg, Z.G., A. Ashbell and A. Azrieli, 1988. The effect of applying lactic acid bacteria at ensilage on the chemical and microbiological composition of vetch, wheat and alfalfa silages. J. Applied Bacteriol., 64 (1): 1-7.
- Weinberg, Z.G. and R.E. Muck, 1996. New trends and opportunities in the development and use of inoculants for silage. FEMS. Microbiol. Rev., 19 (1): 53-68.
- Weinberg, Z.G., R.E. Muck and P.J. Weimer, 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. J. Applied Microbiol., 94 (6): 1066-1071.
- Zhang, J.G., Y. Cai, R. Kobayashi and S. Kumai, 2000a. Characteristics of lactic acid bacteria isolated from forage crops and their effects on silage fermentation. J. Sci. Food Agric., 80 (10): 1455-1460.
- Zhang, J.G., O. Tanaka, R. Uegaki, Y. Cali and R. Kobayashi, 2000b. The effect of inoculation and additives on D(-) and L(+) lactic acid production and fermentation quality of guineagrass (*Panicum maximum* Jacq) silage. J. Sci. Food Agric., 80 (15): 2186-2189.