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Chemical Composition and *In Vitro* Gas Production from Different Varieties of Native and Hybrid Maize Silage with the Addition of Acetic Acid or Enzymes#

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ABSTRACT

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The aim of this study was to evaluate and compare the chemical composition and *in vitro* gas production of corn white local native (WLN) corn yellow local native (YLN) and the hybrids H-51EA and CL080001 as silage, preserved by three treatments, control (CTR), the addition of acetic acid (AAC) or enzymes (ENZ). Samples were prepared in microsilos and analysed in 4x3 factorial design with three replicates of each one. The dry matter content (g/kg) was higher ($P<0.01$) for CLO80001 and lower for YLN (222 vs 176); organic matter (OM) content was higher ($P<0.01$) CLO80001 compared with the natives. Regarding treatments, OM in ENZ were higher ($P<0.01$) with respect to AAC and CTR; crude protein (CP) content differs by variety and treatments, WLN variety was higher ($P<0.01$) and the lowest CP was for CLO80001. CTR and AAC were higher in CP ($P<0.01$) than ENZ treatment. Neutral detergent fiber and acid detergent fiber content was higher ($P<0.01$) for WLN than YLN and the hybridsThe highest gas production (ml gas/g DM) ($P<0.01$) was for hybrids compared with local corn natives. There were no differences ($P>0.05$) for rate c and lag time between varieties. Dry matter disappeared (DMd) was higher ($P<0.01$) for CLO8001 and WLN than H51EA. ME (MJ/kg DM) was higher ($P<0.01$) for CLO80001 < H51EA < YLN < WLN. ME were higher ($P<0.01$) in ENZ and AAC than CTR. The WLN variety proves to be the best option for feeding cattle, as it turned out better than the rest of the varieties tested. Addition of corn silage with acetic acid or enzymes increased NDF digestibility and ME availability.

Key words: Enzymes, Acetic acid, Chemical composition, *In vitro* gas production, Silage.

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INTRODUCTION

Corn silage is the main source of livestock feed in the center of Mexico; this has led to implement programs for choosing corn varieties with higher forage production (SIAP-SAGARPA, 2010). The increased demand for animal feed and the low availability of land for cultivation has necessitated the search for new varieties of hybrid maize (Johnson *et al.*, 2003; Ivan *et al.*, 2005), which implies the need for new alternatives with heterosis for increased nutritional value in both forage and grain. The method of silage preservation is based on converting the soluble carbohydrates in organic acids, mainly lactic acid, under anaerobic conditions by lactic acid bacteria (McDonald *et al.*, 1991). The technique of *in vitro* gas production (Menke and Steingass, 1988), or the modifications by Theodorou *et al.* (1994) in simulating the digestive processes generated from microbial production (Getachew, 1998), allows us to know the fermentation and degradation of food according to the nutritional quality and availability of nutrients for ruminal bacterias. The objectives of this study were to determine the chemical composition and *in vitro* gas production of corn silage, preserved without additive or the addition of enzymes or chemical acids.

MATERIALS AND METHODS

Experimental site

The study was conducted in Toluca, State of Mexico (99° 39'14" West and 19° 37'32" North). Four varieties of corn were evaluated, White local native (WLN), Yellow local native (YLN), and the hybrids H-51 AE and CL080001, which were grown in the spring-summer 2009.

Three samples (10 kg Fresh matter) were taken from each variety, grounded (General Electric mill, 390N 5KH Mod 5525; length 5 cm) and kept in microsilos by triplicate, as untreated control (CTR), with a bacterial-enzymatic compound, 10 g/ton (ENZ) (Sil All$^{4x4®}$, Alltech, which contains: *Streptococcus faecium, Lactobacillus plantarum, Pediococcus acidilactici* and *Lactobacillus salivarius* and enzymes cellulase, hemicellulase, pentosanase and amylase, and to another microsilo (n=3) was added acetic acid (AAC) 1%. The microsilos were prepared by placing 1.5 kg of corn in a PVC tube (13x25 cm) and covering them with a polyethylene bag, compacting and sealing them well and eliminating most of oxygen present in the sample. After two months samples were opened and the pHs were determined (Conductronic model pH130). A part of the samples were dried (60°C, 48 h) and ground (1 mm diameter) to determine the dry matter (DM) and organic matter content (OM) (AOAC, 1991). The concentration of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin was determined by infrared spectrophotometry using a spectrophotometer (Buchi NIR FLEX N400) NIRCal software version 4.01 (Buchi); metabolizable energy (ME, MJ/kg DM) was determined by the equation proposed by Menke and Steingass (1988):

ME (MJ/kg DM)= 14.51- (0.143xADF); *where* ME= (MJ/kg DM) and ADF= (g/kg DM).

For *in vitro* gas production technique, we used three rumen fistulated non lactating dairy cattle (LW 450±20 kg) as donors of rumen fluid. The animals received a diet of oat hay and alfalfa hay (ratio of 50:50), formulated to meet all of their nutrient requirements (NRC, 2001), and were supplied twice a day (8:00 and 16:00 hours). Fresh water was available to cows at all times.

In vitro gas production

Gas production was determined in 125 ml amber flask per triplicate and three series of incubation for each sample of forage conservation method, using the technique proposed by Theodorou *et al.* (1994). In each flask we introduced 0.8 g DM of each of the samples, then we added 90 ml of buffer solution (Menke and Steingass, 1998) gassed with CO_2 and stored (4°C for 12 h) until the next day. Then from the flasks we took 700 ml of ruminal fluid and 300 g of solid rumen contents of each donor cattle; subsequently, the homogenized mixture was filtered through four layers of gauze and glass wool; we maintained the rumen fluid at 39°C; it was gassed with CO_2, and subsequently, we added to each flask 10 ml of ruminal fluid. Finally, the flasks were introduced into a water bath at 39°C and we initiated the gas production record using a pressure transducer (DELTA OHM, Manometer, 8804). The volume of gas produced was recorded at 3, 6, 9, 12, 24 and 30 h incubation. Additionally, three blanks per series were used (as well with three repetitions).

After the incubation period (30 h), the accumulated gas was released and the fermentation residues of each flask were dried (60°C, 48 h) to calculate the proportion of dry mater disappeared (DMd, %) and relative gas production (RGP, ml gas g DMd) according to Gonzalez Ronquillo *et al.* (1998).The kinetics of gas production were determined by adjusting the model: $GP=b\ (1-e^{-ct})$ proposed by Krishnamoorthy *et al.* (1991); according to the model, *b* represents the total production of gas (ml gas/g initial DM); *c* the rate of degradation in relation to time (h); t represents the lag time (h), which is the time when the food begins to be degraded by micro-organisms in the rumen.

Statistical analysis

Samples were analysed in a completely randomized design with a 4 x 3 factorial arrangement, with varieties (4) and treatments (3), with three replicates each one (n=36), using an analysis of variance (SAS, 1999). The averages of each variable were compared with the Tukey test at $P<0.05$.

RESULTS AND DISCUSSION

There were no significant differences ($P>0.05$) for pH between varieties and treatments (Table 1); these results are different from those presented by Ruiz *et al.* (2009) and Filya *et al.* (2006), which evaluated corn silage inoculants, showing

differences in pH between treatments, with pH lower than the present study. Similarly Kristensen *et al.* (2010) found differences in pH by treatment, with similar values than those of the present study. Kung and Shaver (2001) found that corn silage rarely has a pH above 4.2, which can be associated with very dry silage (over 42% dry), and pH 3.7 to 4.2 propose a normal range of 30 to 40% dry matter. Colombatto *et al.* (2003) found pH values below 4.0 in corn silage treated with enzymes in relation to other untreated silages, due to increased substrate available for fermentation. DM content was higher ($P<0.01$) for CLO80001, and lowest for YLN; Ruiz *et al.* (2009) found no differences in DM content in different corn hybrids, with similar contents to those of the present study. Colombatto *et al.* (2003) found no differences in DM content of corn silage treated with enzymes with higher values than the present study (324 to 347 vs 176 to 222 g/kg DM). Ranjit *et al.* (2002) reported differences in DM content of corn silage with four doses of Lactobacillus buchneri 40788 compared with a control group. OM content was higher ($P=0.01$) for CLO80001 compared to corn natives; Ruiz *et al.* (2009) reported differences in OM content per treatment, with similar results to those obtained in the present study; Colombatto *et al.* (2003) found differences in OM content of corn silage treated with enzymes in relation to other corn untreated, but with higher values than the present study (943 to 952 vs 913 to 933, g/kg DM respectively). OM content was higher ($P<0.01$) for ENZ compared to AAC and CTR. CP content differs ($P<0.01$) by variety and treatments, with a higher WLN ($P<0.01$) and lower for CLO80001; CTR and AAC treatments were higher ($P<0.01$) than ENZ. Ruiz *et al.* (2009) reported differences in the CP content in treatments and varieties, with similar results to those obtained in the present study. The content of NDF and ADF was higher ($P<0.01$) for WLN compared with YLN, and higher in hybrids (CLO80001 and H51EA). Kung *et al.* (1993) show that CP, NDF and ADF were similar for all corn silage treated with two inoculants; on the contrary Ruiz *et al.* (2009) found differences in CP, NDF and ADF per variety and treatment, while Ranjit *et al.* (2002) founded differences in NDF and ADF content in corn silage with four doses of *Lactobacillus buchneri* 40788 compared with a control group. This is due to increased use of the more soluble fraction of the corn plant in relation to fibrous fractions by the ENZ.

Table 1. Chemical composition (g/kg DM) of corn silage (native and hybrid), preserved without additive (CTR) or with the addition of enzymes (ENZ) or acetic acid (ACC).

Item	VARIETY (*V*)				TREATMENT (*T*)			SEM	P value		
	YLN	CLO80001	H51EA	WLN	CTR	ENZ	ACC		*V*	*T*	*VxT*
pH	3.96	3.97	4.18	3.96	4.04	4.03	4.00	0.12	0.58	0.97	0.06
DM	176^{f}	222^{d}	185^{e}	185^{e}	171^{e}	203^{d}	202^{d}	0.52	0.01	0.01	0.01
OM	913^{e}	933^{d}	922^{de}	922^{e}	912^{f}	934^{d}	922^{e}	0.23	0.01	0.01	0.33
CP	90^{e}	82^{f}	104^{d}	106^{d}	97^{d}	90^{e}	99^{d}	1.57	0.01	0.01	0.01
NDF	554^{e}	534^{f}	522^{f}	574^{d}	558^{e}	532^{f}	548^{d}	4.33	0.01	0.01	0.01
ADF	340^{e}	317^{f}	321^{f}	355^{d}	348^{d}	322^{f}	332^{e}	3.33	0.01	0.01	0.01
Lignin	70^{de}	64^{e}	75^{d}	64^{e}	71	66	68	1.75	0.01	0.22	0.11
ME†	9.64^{e}	9.96^{d}	9.88^{d}	9.42^{f}	9.52^{e}	9.90^{d}	9.75^{d}	0.05	0.01	0.01	0.01

Values are expressed as means. Different letters in the same row indicate significant difference ($P<0.05$), YLN=yellow local native, WLN=white local native. †ME, MJ/kg DM (Menke and Steingass, 1988; where ME=14.51- (0.143xADF), where ME= (MJ/kg DM) and ADF= (g/kg DM).

The highest gas production (ml gas/g DM) ($P<0.01$) was for hybrids compared with local natives (Table 2, Fig. 1). There were no differences ($P>0.05$) for fractional lag time and c rate between varieties; the DMd was higher ($P<0.01$) for CLO8001 and WLN compared with H51EA. The RGP was higher for H51EA ($P<0.01$) compared to the rest. ME content was higher ($P<0.01$) for CLO80001>H51EA>YLN>WLN. Regarding the effect of treatment (Fig. 2), there were no differences ($P>0.05$), except for ME, which were higher ($P<0.01$) ENZ and AAC compared with CTR treatment; Corral-Luna *et al*. (2011) found values of ME from 9.62 to 10.46 MJ ME/kg DM in corn silage treated with additives, similar to in the present study. The results indicate that the addition of ENZ reduces the amount of CP, NDF and ADF; this may be due to the impact of enzymes in the inoculum, which may act to degrade the structural carbohydrates (Muck and Bolsen, 1991).

Table 2. *In vitro* gas production (ml gas/g DM) of corn natives and hybrids silage, preserved by the addition of additives

Item	Varieties (*V*)				Treatment (*T*)			SEM	P value		
	YLN	CLO8001	H51EA	WLN	CTR	ENZ	AAC		*V*	*T*	*VxT*
b	282^{e}	319^{d}	316^{d}	297^{e}	306	300	304	8.34	0.01	0.85	0.42
c	0.049	0.050	0.051	0.047	0.047	0.053	0.049	0.01	0.77	0.50	0.97
Lag time	1.36	1.40	1.42	1.34	1.44	1.43	1.28	0.13	0.70	0.98	0.96
DMd,%	63de	65^{d}	59^{e}	63^{d}	61	63	63	0.94	0.01	0.16	0.53
NDFd,%	52	51	51	50	49^{e}	52^{d}	52^{d}	0.59	0.30	0.01	0.01
RGP	340^{e}	362^{e}	414^{d}	341^{e}	367	366	360	8.56	0.01	0.81	0.29

Values are expressed as means of different literals indicate significant difference ($P<0.05$), YLN=local native yellow, white WLN=White local native, CTR=untreated, ENZ=treatment with enzimes (Sill All®), AAC= acetic acid treatment. b=total gas production (ml gas/g DM incubated), c=range of fermentation (h) Lag time (initial fermentation time); DMd%=percentage of DM disappeared; RGP=relative gas production (mL gas/g DMd%); NDFd%= percentage of NDF disappeared.

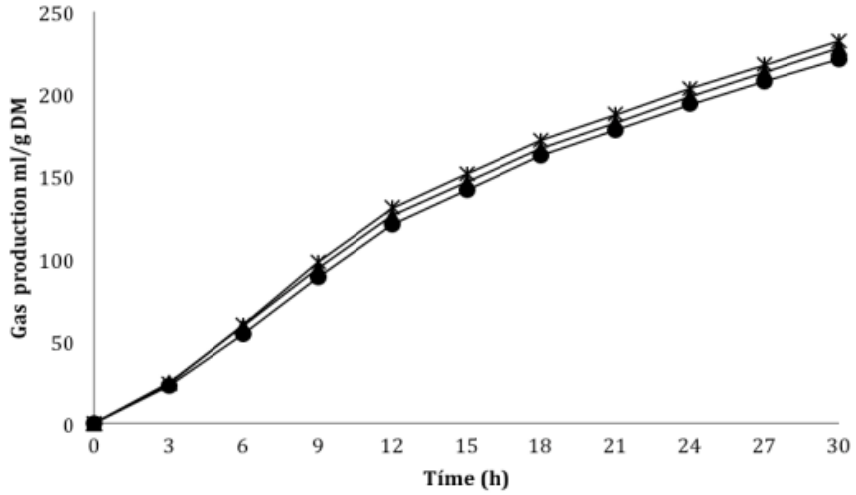

Fig. 1. *In vitro* gas production (ml gas/g DM) of corn silage by treatment (●, control-untreated; ⋆, enzyme (Sill All ®); and ▲, acetic acid).

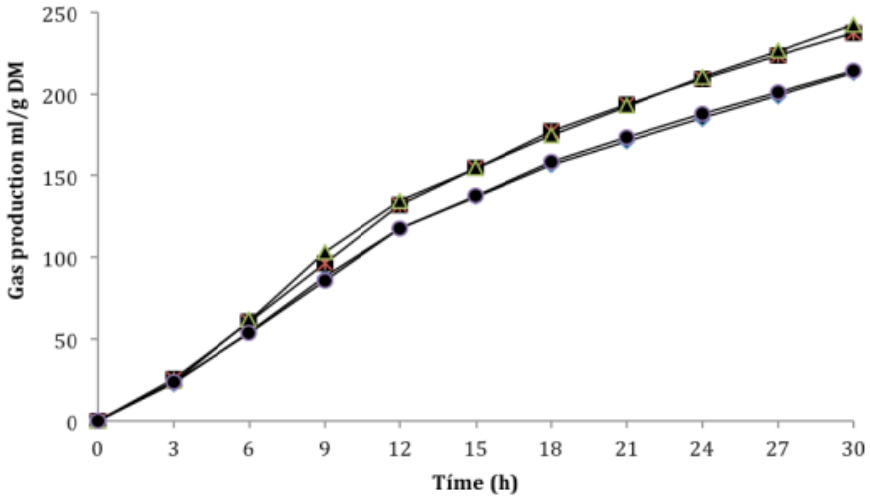

Fig. 2. *In vitro* gas production (ml gas/g DM) of silage corn variety (◆, Yellow local native; ■, CLO80001; ▲, H51EA and ●, White local native).

CONCLUSIONS

Nutritive value and fermentation of corn silage can be improved with treatment of acetic acid or enzyme inoculants. Addition of corn silage with acetic acid or enzymes increased NDF digestibility and ME availability.

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