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RESEARCH PAPER

Effect of xylanase, cellulase and natural maguey extract on the chemical composition of corn silage and *in vitro* rumen gas production

José Ramón Pascual Franco Martínez[1], Andrés González Huerta[1], Delfina de Jesús Pérez López[1], Rodolfo Serrato Cuevas[1], Abdelfattah Zeidan Mohamed Salem[2] , Lizbeth E. Robles-Jimenez[2], and Manuel Gonzalez-Ronquillo[2]

[1]Facultad de Ciencias Agrícolas-Universidad Autónoma del Estado de México, México.

[2]Facultad de Medicina Veterinaria-Universidad Autónoma del Estado de México, México. Instituto Literario 100. Toluca, Estado de Mexico. 50000

Abstract

J.R.P. Franco Martínez, A. González Huerta, D.J. Pérez López, R. Serrato Cuevas, A.Z.M. Salem, L.E. Robles-Jimenez, and M. Gonzalez-Ronquillo. 2020. Effect of xylanase, cellulase and natural maguey extract on the chemical composition of corn silage and in vitro rumen gas production. Int. J. Agric. Nat. Resour. This study considered the application of two exogenous enzymes, xylanase (XYL) and cellulase (CELL), and maguey extract (ME), (applying 1 ml per kg as fresh matter) and a control (without additive) in four maize silage varieties (San Diego, Cacahuacintle, P-1832 and Victoria), to investigate their effect on the chemical composition (CC), gas production (GP) and *in vitro* ruminal fermentation. The GP was measured at 0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 hours of incubation. Dry matter disappearance (DMD), organic matter disappearance (OMD), metabolizable energy (ME), and short chain fatty acids (SCFAs) were determined after 96 h of incubation. Data were analyzed using a completely randomized design with a 4×4 factorial arrangement with three replications. The CC showed a significant effect ($P<0.05$) for varieties with the exception of organic matter (OM), and the inclusion of additives increased the dry matter (DM) and crude protein (CP). A significant effect ($P<0.01$) was observed for the varieties in the GP parameters and ruminal fermentation. The addition of XYL, CEL and ME promoted dry matter degradation and increased energy availability, with increased *in vitro* gas production.

Keywords: Additives, enzymes, silage.

Introduction

Corn silage is most commonly used in dairy cow feed for its energy value and high dry matter (DM). Corn forage is a fibrous feed with high concentrations of cellulose and hemicellulose, which can create a structural complex of carbohydrates and lignin to reduce the digestibility of carbohydrates and thereby decrease the efficient utilization of feed by ruminants (Elghandour *et al*., 2014). Fibrolytic enzymes alter the structure of forages, making them more susceptible to ruminal hydrolysis (Nsereko *et al*., 2000). Cellulases, on the other hand, cause partial hydrolysis

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Corresponding author: mrg@uaemex.mx

of the plant cell wall during ensiling (Kuhad *et al*., 2011). Settimi *et al*. (2013) mention that direct application of enzymes to the substrate favors the formation of a stable enzyme-substrate complex, which increases the effectiveness of the exogenous enzymes, improving fiber solubility or availability for microbial attack in the rumen. Exogenous fibrolytic enzymes (EFEs) are most effective when applied in liquid form to food prior to ingestion, as they can help remove structural barriers that limit microbial digestion of the food in the rumen, encouraging the release of soluble carbohydrates (Beauchemin *et al*., 2004). EFEs such as xylanases and cellulases are synthesized by ruminal microorganisms (bacteria and fungi) and are added to feed to increase fiber degradation (Campioni *et al*., 2020); due to the high fiber content of fibrous feeds, ruminal enzymes sometimes cannot access the plant cell wall, and feed digestibility is reduced (Togtokhbayar *et al*., 2015). The use of EFEs could increase cellulose and hemicellulose degradation.

Cellulases are inducible enzymes synthesized by a wide range of microorganisms, including fungi and bacteria (Sang-Mok & Koo, 2001; Campioni *et al*., 2020). Microorganisms can be aerobic, anaerobic, mesophilic or thermophilic; among them, the genera *Clostridium, Cellulomonas, Thermomonospora, Trichoderma* and *Aspergillus* are the most widely studied cellulase producers (Sun & Cheng, 2002; Dhillon *et al*., 2011), of which the species *A. niger* is recognized as a cellulase producer (Onsori *et al*., 2005). Microbial cellulases have received attention as biocatalysts due to their complex nature and wide applications (Henrissat *et al*., 1998); for example, in animal feed, their addition to the diet of ruminants improves fiber digestion (Alsersy *et al*., 2015; Salem *et al*., 2015a, 2015b).

Xylanases, which are responsible for hemicellulose hydrolysis, include endoxylanases, which generate xylose oligomers, and β-1-4 xylosidases, which hydrolyze xylases. The most important enzymes involved in the degradation of hemicellulose are xylanases (E.C.3.2.1.8) and β-1-4 xylanases (E.C.3.2.1.37) (Bhat & Hazlewod, 2003). Xylan is the main component of hemicellulose, and by the action of a complex enzymatic system, it is hydrolyzed and converted into its constituent sugars (Breccia *et al*., 1998).

Currently, little attention has been paid to the use of "maguey pulquero" (*Agave salmiana*, A. *mapisaga,* and A. *atrovirens*), which has been reported from 136 of the 150 species of *Agave* in Mexico (Novel, 1998). These species are used in food, fiber, fodder, medicine, construction, and production of alcoholic beverages. Maguey extract is used for the preparation of "pulque" whose product of fermentation is a sweet, colorless, transparent liquid with a light herbal scent that is rich in carbohydrates and protein. This extract contains organic forms of nitrogen such as amino acids; while little attention has been paid to the organic forms of protein in maguey extract, all of the proteinogenic amino acids found in the phloem supplemented with the high content of sugars directly influence the fermentation process of the preserved organic materials, making the use of this extract interesting and necessary in order to analyze its effect, in comparison with those of other additives already tested, in improving the degradation of raw fiber and possible protein enrichment in forage feed for cattle.

Feed additives, such as exogenous enzymes (i.e., xylanases, cellulases, mannases), have been used to improve the degradation of carbohydrates and cell walls in ruminant animals (Vallejo *et al*., 2016; Salem *et al*., 2015a), but little is known about the use of maguey extract as a natural feed additive. Since the additives (i.e., maguey extract, Aguamiel, Salem *et al*., 2017) used have common amino acids, sugars, and other elements, the aim of this study was to compare the effects of xylanase and cellulose enzymes (synthetic origin) with those of maguey extract (natural organic compound) in terms of chemical composition, *in vitro* gas production and fermentation parameters when all are applied to four maize silage varieties.

Materials and methods

Material evaluated

Four genotypes of corn forage (*Zea mays)* were used: two Creole native breeds, Cacahuacintle (white grain) and San Diego (yellow grain) (the first one, a breed of Cacahuacintle, is a floury corn, with large and soft seeds that predominates in the high mountains of the State of Mexico; and the second one belongs to the breed Conico, has jagged grains and originated in the High Valleys of the transverse volcanic axis of Mexico. It was obtained from the cross between the breed Palomero Toluqueño with the genotype Cacahuacintle) and two hybrids, P-1832 (yellow grain) and Victoria (white grain), whose production characteristics are described by Franco *et al*. (2015). Cutting of the plants took place when the grains of corn reached a doughy state, at 155, 149, 176 and 168 days after planting for the four genotypes, respectively. Whole plants of the experimental plots of each variety were cut and then chopped in a Wiley mill (DPM Junior, Noriega) to obtain a particle size of 1.5 to 4.0 cm; subsequently, to form microsilos, 1 ml of enzyme (xylanase and cellulase) or maguey extract was used per kg of silage fresh matter per variety in triplicate. The xylanase (XYL) product consisted of 34.000 to 41.000 units xylanase g^{-1}, of 12,000 to 15,000 units of β-glucanase g^{-1} and 45,000 to 55,000 units of cellulose g^{-1}, cellulase (CELL) consisted of 30.000 to 36.000 units cellulose g^{-1} and from 7.500 to 10.000 units β-glucanase g^{-1} and the maguey extract (maguey extract) consisted of 8.62% sugars (fructose, dextrose, sucrose), 3% crude protein, 0.3% amino acids and 3% ash; the respective control groups received no additives. Subsequently, three cylinders were filled with 15×30 cm polyvinyl chloride with each of the varieties of maize and their respective treatments, leaving the respective controls without substrate, and each fodder cylinder was compacted, sealed with metal and finally covered with black plastic. The microsilos were stacked in a cool area and covered for six months at room temperature (Bartosik, 2012).

Chemical composition

Forage samples for post conservation chemical analysis were collected from silages (4 silages per sample) at opening. A minimum of 6 cores were taken from each micro silo and pooled to produce one sample per bag. Samples for chemical analysis were frozen at -18 °C before chemical composition analysis.

Pooled samples of feeds were ground to a 1 mm maximum size with a Wiley Mini Cutting Mill model 5KH39QN5525 and analyzed in duplicate following the procedures of AOAC (1997). The pH was measured immediately after opening the silages; dry matter (DM) was determined by oven drying at 105 °C to constant weight (ref. 934.01), organic matter and total ash by muffle furnace (ref. 942.05), and crude protein (CP) by the Kjeldahl method (ref. 976.05). The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (ADL) were determined according to Van Soest *et al*. (1991) using an Ankom 200 fiber analyzer (Ankom Technol. Co. Macedon, NY. USA). NDF was assayed without α-amylase. Both NDF and ADF are expressed without residual ash. The moisture content of the silages was determined through distillation with toluene (Haigh & Hopkins, 1977).

In vitro *gas production*

The corn silages previously inoculated with XIL, CEL and maguey extract were treated with the ruminal solution, collected during the first day of incubation from two dry Holstein cows (450 kg LW) fed with a diet based on corn silage/alfalfa hay (60/40 ratio) supplemented with commercial concentrate (Purina R), and water was given *ad libitum*. The ruminal fluid was extracted at 08:00 a.m. before the cows were fed, transported in a thermos to the laboratory, filtered using a triple layer of cheesecloth, then kept at a temperature of 39 °C and gassed with CO_2. Stirring solutions (Menke & Steingas, 1988) were maintained at 39

°C, and 10% ruminal fluid was added and mixed with buffer solution. A total of 800 mg of DM from each of the treatments was weighed and placed into 48 120-ml amber bottles (considering the 16 treatments in triplicate), and 90 ml of buffer solution was added. Finally, 101 ml of ruminal-buffer mixture solution was mixed with a 9:1 buffer/ruminal fluid ratio. After agitation, flasks were placed in a water bath at 39 °C. Subsequently, the pressure (PSI) was determined using a pressure transducer (DELTA OHM HD2124.1), and gas production was measured at 0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation. For gas correction, four flasks were used without substrate as blanks; moreover, four bottles with barley hay were used as standards (Theodorou *et al*., 1994).

The kinetic parameters of gas production (GP) were estimated through an iterative procedure of nonlinear regression analysis (PROC NLIN, SAS Institute 2002) according to Krishnamoorthy *et al*. (1991), calculated as:

$$GP = b\ (1\text{-}e^{-c(t-l)}) \quad (1)$$

where GP is the volume of GP at time (t); b is the asymptotic GP (ml g^{-1} DM); c is the rate of GP (g h^{-1}), and *l* (h) is the discrete lag time prior to gas production.

Ruminal fermentation in vitro

After the incubation period, the accumulated gas was released, and the DM residues of the fermentation were filtered using a 72-micron pore size deposited on crucibles. Then, the samples were dried using a stove at 110 °C for 24 hours. When the weight was calculated, the dry matter disappearance (DMD) was determined; then, samples were placed inside a muffle at 600 °C for 4 hours and weighed, and the OM disappearance (OMD) was determined.

At the end of the incubation, DM degradability (DMD, mg) and OM degradability (OMD, mg) were determined; gas yield (GY_{24h}) was calculated as the volume of gas (ml gas g^{-1} DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

$$\text{Gas yield } (GY_{24h}) = \text{ml gas. g } DM_{24h}\ g^{-1}\ \text{DMD} \quad (2)$$

Metabolizable energy (ME, MJ kg^{-1} DM) was estimated according to Menke *et al*. (1979), Theodorou *et al*. (1994) and Robles Jimenez *et al*. (2019) as:

$$ME = 2.20 + 0.136\ GP\ (\text{ml } 0.5\ g^{-1}\ DM) + 0.057\ CP\ (\text{g } kg^{-1}\ DM) \quad (3)$$

Relative gas production (RGP) was calculated as milliliters of gas per gram of DMD after

$$48\ \text{h incubation } (DMD_{48\ h)}. \quad (4)$$

Short chain fatty acid concentrations (SCFAs) were calculated according to Getachew *et al*. (2002) as (5)

where GP is the 24 h net gas production (ml 200 mg^{-1} DM).

Microbial CP biomass production was calculated according to Blümmel *et al.* (1997) as: (6)

where 2.2 mg ml^{-1} is a stoichiometric factor that expresses the mg of C, H and O required for the production of SCFA gas associated with the production of 1 ml of gas.

Statistical analysis

Data were analyzed as a completely randomized design with a 4×4 factorial arrangement (Steel *et al*., 1997), where the treatments were maize varieties (V) (n=4) and additives (Control, XYL, CELL and maguey extract) (A) (n=4), with three replications. Data were subjected to analysis of variance (ANOVA) using the general linear model procedure (GLM; SAS, 2002). The univariate

procedure of SAS was used to test for a normal distribution of the data.

The statistical model was as follows:

$$Yijk = \mu + V_i + A_j + (V * A)_{ij} + E_{ijk} \quad (7)$$

where Y_{ijk} = response variable of silages treated with xylanase, cellulase and maguey extract; μ = general mean; V_i = effect of the ith maize variety (i = 1-4); J = effect of the j^{th} additive (j = 1-4); $(V * A)_{ij}$ = effect of the interaction between the i^{th} variety and the j^{th} additive; E_{ijk} = experimental error of the i^{th} variety and the j^{th} and k^{th} additive repetitions. Differences among means were tested using Tukey's HSD (Snedecor and Cochran 1994), with significance declared if P<0.05.

Results

Chemical composition

The maize varieties showed a significant effect (P <0.05) on DM, CP, NDF and ADF, and the additives had a significant effect (P <0.05) on DM, OM, CP and ADF. The CP contents of corn silages from San Diego and Cacahuacintle were lower (P<0.05) than those of silages from the P-1832 and Victoria hybrids. The concentrations of NDF and ADF were higher (P<0.05) for the hybrids, especially for Victoria, compared with the native corns. The inclusion of maguey extract, CEL and XYL enzymes increased (P<0.05) the CP content compared to those of the control groups. The addition of maguey extract decreased (P <0.05) the content of ADF compared with the rest of the treatments (Table 1).

In vitro *gas production*

The application of enzymes and maguey extract increased the GP (ml gas g^{-1} DM) of the four genotypes, with higher (P<0.05) GP observed for Cacahuacintle and San Diego (227±2 ml gas g^{-1} DM) and the lowest for Victoria (198 ml gas g^{-1} DM); however, the greatest accumulation of gas was at 24 hours, and the fermentation rate "b" was higher (P<0.05) for San Diego and Cacahuacintle (0.0390 ±8) than for the corn hybrids (0.0308±4) (Figure 1, Table 2). The use of XYL increased gas production (229 ml gas g^{-1} DM) (P<0.05), followed by CEL and maguey extract (220±1 ml gas g^{-1} DM) (Figure 2). The addition of maguey extract, CEL and XYL resulted in a

Table 1. Chemical composition (g kg/DM) of silage from four corn varieties (V) treated with maguey extract, cellulose and xylanase

Item	Variety (V)				Treatment (Tx)				SEM V	P *value*		
	San Diego	Cacahuacintle	P-1832	Victoria	Control	Maguey extract†	Cellulose‡	Xylanase§		Tx	VxTx	
DM	229a	210a	203a	171b	180b	204ab	216a	213a	7.01	0.001	0.004	0.288
OM	920	913	921	913	903c	924ab	927a	913bc	3.18	0.143	0.001	0.001
CP	76bc	71c	80ab	83a	72b	82a	77a	78a	1.30	0.001	0.001	0.001
NDF	487b	495ab	548a	544a	546	514	489	525	15.23	0.010	0.080	0.308
ADF	207b	180b	203b	245a	206ab	190b	205b	234a	9.22	0.002	0.017	0.031

†Maguey extract had 8.62% sugars (fructose, dextrose, sucrose), 3% protein, 0.3% amino acids and 3% ash.

‡Cellulose had 30 000 to 36 000 units of cellulose per g^{-1} and 7 500 to 10 000 units of β-gluconase per g^{-1}.

§Xylanase contained 34 000 to 41 000 units of xylanase g^{-1}, 12 000 to 15 000 units of β-gluconase g^{-1} and 45 000 to 55 000 units of cellulose g^{-1}.

DM, dry mater; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

SEM, standard error of the mean. Different letters in the same row indicate significant differences (P<0.05).

linear increase in GP (gas ml hour^{-1}) over time, with a greater accumulation of gas than in the control silage (Table 2).

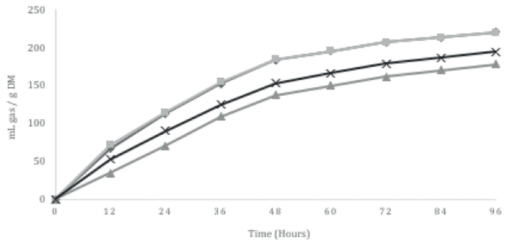

Figure 1. Cumulative gas production profiles (ml gas g^{-1} DM) from *in vitro* fermentation of silage from four corn forages (native San Diego -■-, and Cacahuacintle -♦-maize forage genotypes, and hybrids P-1832 -x- and Victoria-▲-) with different exogenous enzyme preparations (ENZ, g g^{-1} DM) in cows. S.E.M. is for the overall fit, and P' is for the effect of extract dose.

Ruminal fermentation in vitro

The ruminal pH was lowest (P<0.05) for the San Diego variety (4.31); likewise, maguey extract (4.87) decreased the pH of the silage (P<0.05), followed by XYL (4.70) and CEL (5.03). The addition of enzymes or maguey extract increased the concentration of ME (12.4 ±0.2 MJ ME) and OMD (745 ±10 mg g^{-1}) compared with the control treatment (10.1 Mj ME, 659 mg g$^{-1,}$ respectively), while the concentrations of SCFAs and MCP were higher (P <0.05) for the CEL and XYL groups than for the control. GY_{24h} was lower (P <0.05) for the control group (40.5 ml gas g^{-1}DM) than for the rest of the treatment groups (Table 2), with a higher XYL (74.9 ml gas g^{-1}DM).

Table 2. Gas production (ml gas g^{-1} DM) and ruminal *in vitro* fermentation parameters for silage from different corn varieties (V) treated with maguey extract, cellulose and xylanase (Tx).

Item	Variety (V)				Treatment (Tx)					P *value*		
	Cacahua cintle	San Diego	P-1832	Victoria	Control	Maguey extract†	Cellulose‡	Xylanase§	SEM	V	Tx	VxTx
A	229.0a	226.2a	209.8b	198.3c	192.5c	220.3b	221.4b	229.2a	2.021	0.001	0.001	0.001
b	0.0380a	0.0396a	0.0304b	0.0313b	0.0306b	0.0371a	0.0362a	0.0353	0.000	0.001	0.001	0.001
c	-0.0436a	-0.0407a	-0.0336a	-0.0589b	-0.0575b	-0.0522b	-0.0377a	-0.0294a	0.002	0.001	0.001	0.001
Lag time	1.29b	1.27b	0.96c	2.85a	2.25a	1.87b	1.20c	1.05c	0.066	0.001	0.001	0.001
Gas3	8.01a	6.74a	7.55a	2.50b	3.84c	5.97b	7.36ab	7.62a	0.367	0.001	0.001	0.002
Gas6	24.35a	24.45a	20.90b	10.12c	11.91d	19.66c	22.71b	25.54a	0.533	0.001	0.001	0.001
Gas9	45.44b	48.75a	36.63c	24.48d	23.63d	38.69c	43.64b	49.35a	0.678	0.001	0.001	0.001
Gas12	66.43b	71.21a	52.72c	34.72d	35.47d	57.03c	62.87b	69.71a	0.886	0.001	0.001	0.001
Gas24	112.88a	114.10a	90.13b	70.48c	69.81c	100.63b	106.30a	110.85a	1.433	0.001	0.001	0.001
Gas36	151.96a	154.25a	124.84b	108.72c	104.75c	141.15b	144.35ab	149.54a	1.659	0.001	0.001	0.001
Gas48	183.52a	183.65a	152.79b	137.26c	131.78c	172.28b	173.34ab	179.82a	1.763	0.001	0.001	0.001
Gas72	206.34a	206.28a	178.57b	161.59c	155.50c	196.25b	197.29ab	203.75a	1.811	0.001	0.001	0.001
Gas96	220.20a	219.45a	194.41b	177.77c	170.90c	209.22b	211.97ab	219.74a	2.037	0.001	0.001	0.001
pH	5.09b	4.31c	4.95b	6.37a	6.13a	4.87b	5.03b	4.70b	0.225	0.001	0.001	0.001
DMD	649.8b	676.5a	617.2c	553.4d	551.2c	613.5b	663.4a	668.9a	5.344	0.001	0.001	0.001
OMD	726.5ab	744.3a	718.6b	705.3b	659.5b	746.3a	734.5a	754.4a	6.630	0.002	0.001	0.001
ME	12.4a	12.7a	11.6b	10.7c	10.1b	12.3a	12.4a	12.7a	0.109	0.001	0.001	0.001
SCFA	0.496a	0.502a	0.395b	0.308c	0.305c	0.442b	0.467a	0.488a	0.006	0.001	0.001	0.001
MCP	165.4bc	193.7a	189.5ab	162.3c	175.2ab	153.2b	197.1a	185.5a	6.584	0.002	0.004	0.001
GY_{24h}	74.1a	77.3a	56.5b	40.7c	40.5d	62.5c	70.8b	74.9a	1.045	0.001	0.001	0.001

†Maguey extract had 8.62% sugars (fructose, dextrose, sucrose), 3% protein, 0.3% amino acids and 3% ashes.
‡Cellulose had 30 000 to 36 000 units of cellulose per g^{-1} and 7 500 to 10 000 units of β-gluconase per g^{-1}.
§Xylanase contained 34 000 to 41 000 units of xylanase g^{-1}, 12 000 to 15 000 units of β-gluconase g^{-1} and 45 000 to 55 000 units of cellulose. g^{-1}.
a: gas production (ml g^{-1} MS); b: fermentation rate (h^{-1}); c, fermentation rate (h$^{-1/2}$); lag time: (h^{-1}). DMD: Dry mater digestibility (mg g^{-1} DM); OMD: OM digestibility (mg g^{-1} DM); ME, Metabolizable energy (Mj kg^{-1} DM); SCFA: Short chain fatty acids (mmol g^{-1} DM); MCP: Microbial crude protein (mg g^{-1} DM); $GY_{24\,h}$ Gas yield at 24 h (ml gas g^{-1} DM).
SEM: standard error of the mean.
abc Different letters in the same row indicate significant differences (P <0.05).

Discussion

Chemical composition

The enzymes and maguey extract showed a greater contribution to dry matter in the native corns San Diego and Cacahuacintle than in the P-1832 and Victoria hybrids. This was attributable to the addition of exogenous fibrolytic enzymes, which altered the structure of the fiber and stimulated microbial colonization due to the strong relationship of the enzymes with the substrate when incorporated into the corns (Settimi *et al*., 2013). In the present study, the DM content was higher for CELL, XYL and ME than for the control treatment groups because the addition of exogenous fibrolytic enzymes (EFE) such as cellulase or xylanase increases the degradation and fermentation of maize fodder (Valdes *et al*., 2015; Vallejo *et al*., 2016). Kuhad *et al*. (2011) observed that the activity of cellulases can cause partial hydrolysis of the plant cell wall during ensiling; in addition to adding EM, which included 8.6% sugars, those spiked enzymes were actively involved in the fermentation of carbohydrates released by the feed enzymes and protein degradation to amino acids, stabilizing the silage. The highest content of CP was realized with the inclusion EM, which could have been due to the effect caused by the supply of 0.3% amino acids and 3% protein. The effect of adding enzymes rich in serine and threonine and composed of approximately 35 amino acids independent of the cellulase domain resulted in increases in the CP content of the silage when EM was added.

Corn stover can be used in feed for ruminants to improve its nutritional value (Elghandour *et al*., 2014) via the exogenous fibrolytic enzymes that enhance the degradation of carbohydrates and cell walls in the phase of ruminal preincubation, which is consistent with the decrease in NDF when applying CELL, XYL and maguey extract in the present study; the activity of these products, mainly in native corns (i.e., corn stover). Cacahuacintle was also evident, as indicated by a trend (P=0.08) in NDF content. Muttoni *et al*. (2013) found that the range of variability of NDF in local maize varieties was 46.8 to 72% and 42-78% in lines derived from different germplasms, ranking in lower concentrations in maize cultivars evaluated in this study. Nsereko *et al*. (2000) added fibrolytic enzymes to fodder before ingestion and altered the structure of the food, making the fodder more susceptible to ruminal hydrolysis. Moreover, Casler (2001) found a reduction in the NDF content and an increase in the proportion of highly digestible soluble components with the addition of enzymes. The inclusion of enzymes, such as CELL, showed the greatest effect with the least content of NDF and ADF in relation to the control treatment, which was in accordance with the improved fiber digestion noted by Al Sersy *et al*. (2015) following the addition of cellulolytic enzymes to the diet of ruminants. Settimi *et al*. (2013) indicate that fibrolytic enzymes improve the initial degradation of structural carbohydrates of plants. The lowest content of ADF was found using maguey extract, which could be due to the high amount of sugars in this additive, which optimized the fermentation of the corn silage. According to Forsberg *et al*. (2000), polysaccharides provide fermentable carbohydrates to stimulate microbial growth.

In vitro *gas production*

The activity of the XYL, CELL and maguey extracts was reflected more in the Cacahuacintle and San Diego native corns with respect to that in the P-1832 and Victoria hybrids, given their remarkable increase in GP, the time of fermentation rate and the delay of incubation (Table 2, Figure 1), which could be because these germplasms, which are associated with a low lignin content and high content of polyphenolic soluble materials, are adapted to the highlands and tend to accumulate anthocyanins in the midribs and stem pith. The maguey extract added sugars including glucose, fructose and sucrose, in addition to releasing polysaccharides in the corn plant, providing

fermentable carbohydrates to stimulate microbial growth (Vallejo *et al*., 2016) and increasing the number of fibrolytic and non-fibrolytic bacteria in the rumen, which utilize polysaccharides (Vallejo *et al*., 2016).

The criteria linked to the GP were more efficient in the native corns, except in terms of the lag time for the hybrid P-1832, which showed the fastest time (0.96 h); according to Franco *et al*. (2015), the later plant material required 176 days to harvest for silage. Van Soest (1994) stated that genotypes with longer vegetative stages and later maturity tend to be less lignified than early maturing genotypes. XYL was the most efficient enzyme in terms of GP, fermentation time, and lag time and had the highest cumulative GP; if the enzyme affected the GP until the end of fermentation, this meant that the enzyme increased the fermentable material. The addition of XYL, CELL and maguey extract resulted in better GP and other variables than the control (Figure 2). Salem *et al*. (2015a) found that the efficacy of enzymes depends upon the substrate specificity and dose of the enzyme; in this study, the dose of 1 ml kg fresh matter added to corn substrate was effective according to the results obtained, with continuous activity of GP up to 96 h. However, Vallejo *et al*. (2016) noted that the dose of 40 mg g^{-1} of xylanase or cellulase as DM applied to corn fodder resulted in greater GP than other enzyme levels, with continuing effects up to 72 h. Moreover, it was noted that the application of two cellulases from *Aspergillus niger* and *Trichoderma longibrachiatum* at doses of 30 U g^{-1} substrate (70% grass hay and 30% concentrate) showed greater disappearance of DM after 6 and 24 h without effects after 48 h of incubation.

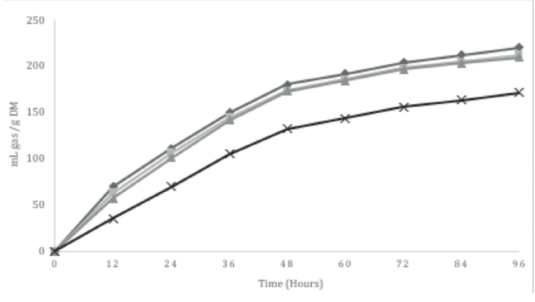

Figure 2. Cumulative gas production profiles (ml gas g^{-1} DM) from *in vitro* fermentation of four corn silages with different exogenous enzyme preparations (ENZ, g g^{-1} DM) in cows. (♦-, Xylanase; -■-, Cellulase; -▲-, Maguey extract; -x-, Control); S.E.M. is for the overall fit, and P' is for the effect of the extract dose.

Ruminal fermentation in vitro

The corn silages from San Diego, P-1832 and Cacahuacintle showed decreased pH, as did that treated with XYL, maguey extract and CELL, compared with that of the control, which could be due to the aggressive fermentation of fresh forage with the addition of sugars of maguey extract and the direct application of exogenous enzymes that favor the formation of a stable substrate, which increases the effectiveness of these enzymes in the rumen (Settimi *et al*., 2013). This was clearly the situation in the present study since the greatest effect on the DMD and OMD was observed for the varieties and additives mentioned. In contrast, the hybrid Victoria had the highest pH, with lower activity of ruminal fermentation for DMD, OMD, ME, SCFA, MCP, and GY_{24h}, similar to the control treatment, which could be due to the difficulty of fermentation of these plants given the thickness of their stems. According to Franco *et al*. (2015), the Victoria cultivar has a thick stem with a diameter of 2.28 cm; moreover, Chalquer-Scott and Fuchigami (1989) demonstrated that low temperature stress can cause cell wall thickening as a physiological response to increased resistance of plants. Boon *et al*. (2008) noted that the stem has the worst digestibility of all maize plant parts, since it is relatively more lignified, and according to Barrière *et al*. (2005), the lignin is the indigestible component of the fiber fraction, playing an important part in strength but decreasing digestibility of the stem cell wall.

The XYL, CELL and maguey extract objectively increased the DMD, OMD, ME, SCFA and MCP during *in vitro* fermentation of corn silage, while

the latter was increased only with XYL and CEL compared with the control treatment; these levels were higher than the results of DMD, OMD and ME reported by Vallejo *et al*. (2016) when working with the addition of CELL and XYL at different doses in corn stover. In this sense, Settimi *et al*. (2013), working with an enzyme complex (cellulase, xylanase and β-glucosidase) applied at different doses, determined that enzymes increased the *in vitro* DM digestibility of corn stover but did not affect digestion after long periods of ruminal incubation; moreover, Casler (2001) found that NDF reduction translates into greater DMD, coinciding with the results of the present study.

Similar increases in OMD, ME and SCFA with the implementation of the three additives were obtained in this study; Elghandour *et al*. (2014) found that increased OMD-related enzymes are correlated with fermented OM; for the case of ME, Zsubori *et al*. (2013) reported that their close correlation is due to the OM content and the degradability of the forage cell wall, agreeing with Elghandour *et al*. (2014), who suggested that increases in these variables were due to supplementation of XYL and CEL in fibrous foods.

The genotypes of yellow grain (San Diego and P-1832) and the treatments XYL and CELL showed the highest MCP, which could be because the grains of yellow corn are sweeter than those of white corn, leading to accelerated fermentation of the former in addition to increases in OMD compared with that of the addition of XYL and CELL. Addition of these enzymes overcame the problem of a slow rate of digestion of low-quality forage due to long retention times in the rumen, so that exogenous fibrolytic enzymes supplemented the enzyme activities of microorganisms in the rumen and allowed better digestion of the substrate during the preliminary stages, which are the most critical for digestion (Settimi *et al*., 2013). The increased production of MCP with the addition of XYL and CELL was evident in the present study. Furthermore, Kung *et al.* (2000) found that the addition of enzymes to the feed can create a stable enzyme-food complex that protects the enzymes from ruminal proteolysis.

The highest GY_{24h} was observed for the native corns San Diego and Cacahuacintle and for the enzymes XYL and CELL, followed by maguey extract, which could be due to increased fiber digestion and altered ruminal fermentation and the contribution of enzymes and sugars present in the corn silage. These results coincide with those of Vallejo *et al*. (2016), who added CELL and XYL at different doses, which together with the release of polysaccharides in the corn forage, caused a higher GY_{24h} compared with that of the control treatment.

The main conclusions are the following. The application of xylanase, cellulase and maguey extract to maize silages increases the gas production and the rate of fermentation; similarly, in *in vitro* ruminal fermentation, the DMD, OMD, ME, SCFA and GY_{24h} were higher in Cacahuacintle and San Diego maize than in other genotypes, and its addition increased the CP content of the silage with respect to that of the control treatment. The silage treated with xylanase showed the lowest pH and had the highest gas production at 96 h of incubation in comparison to that of the silage treated with cellulase and maguey extract, noting with the former treatment a greater impact on DMD and OMD. Cacahuacintle and San Diego, as native corn maize genotypes treated with xylanase, cellulase and maguey extract, showed the highest gas production and better fermentation profiles than the silage of other genotypes. maguey extract can be used as an alternative for the degradation of fiber and fermentation of corn silage.

Resumen

J.R.P. Franco Martínez, A. González Huerta, D.J. Pérez López, R. Serrato Cuevas, Salem A.Z.M., L.E. Robles-Jimenez, y M. Gonzalez-Ronquillo. 2020. Efecto de xilanasa, celulasa y extracto de maguey natural sobre la composición química producción de gas in vitro en rumen de ensilado de maíz. Int. J. Agric. Nat. Resour. El estudio consideró la aplicación de dos enzimas exógenas: xilanasa (XYL) y celulasa (CELL), y extracto de maguey (EM) (aplicando 1 ml por kg de materia fresca) y Control (sin aditivo) en cuatro variedades de ensilaje de maíz (San Diego, Cacahuacintle, P -1832 y Victoria), para investigar su efecto sobre la composición química (CQ), la producción de gas (PG) y la fermentación ruminal *in vitro*. La PG fue medida a las 0, 3, 6, 9, 12, 24, 36, 48, 72 y 96 horas de incubación. La materia seca desaparecida (MSD), la materia orgánica desaparecida (MOD), la energía metabolizable (EM) y los ácidos grasos de cadena corta (AGCC) se determinaron después de 96 h de incubación. Los datos se analizaron mediante un diseño completamente al azar con arreglo factorial 4×4 con tres repeticiones. La CQ mostró un efecto significativo ($P<0.05$) para las variedades, con excepción de la materia orgánica (MO), la inclusión de aditivos incremento la materia seca (MS) y la proteína cruda (PC). Se observó un efecto significativo ($P <0.01$) para las variedades en los parámetros de PG y la fermentación ruminal. La adición de XYL, CEL y EM promueve la degradación de la MS y aumenta la disponibilidad de energía, con una mayor producción de gas *in vitro*.

Palabras Clave: Aditivos, ensilado, enzimas.

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