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Nutritive quality and gas production of corn silage with the addition of fresh and fermented *prickly pear* cladodes

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1 ABSTRACT

This research aimed at evaluating the nutritive quality and *in vitro* gas production of corn silage with the addition of prickly Pear cladodes. Three experimental treatments were evaluated through the elaboration of twenty-one micro-silos based on corn (T1) and with the addition of prickly pear (T2) and fermented prickly pear in the same proportions (T3). Micro-silos were prepared and hermetically sealed in plastic containers (30 cm diameter × 50 cm height) for 30 d. Once the time had elapsed, silages were opened and chemical composition, fermentation parameters, and ruminal gas production kinetics parameters were evaluated. At the end of the silage's evaluation, the dry matter (DM), CP, NDF, AND ADF content were different between the treatments ($p<0.05$). Likewise, nitrogen ammonia concentration, lactic, acetic, propionic and butyric acid were different between treatments ($p<0.05$). Moreover, certain parameters of the ruminal gas production kinetics as Gmax increased 41% when fermented prickly pear was added. These results indicated that these silages may be considered as sustainable and alternative feedstuff in ruminant nutrition. Additionally, this by-product may increase its commercial value by contributing to reduce the ruminal methane production. However, these results should be confirmed in *in vivo* trials.

2 INTRODUCTION

The northern Mexico has extreme temperatures and drought seasons which lead to a diminished forage production. Under these conditions of production, the use of prickly pear cladodes (*Opuntia ficus-indica*) emerges as an alternative in livestock feeding (Herrera *et al.*, 2014). In fact, the cactus provides digestible energy, water and vitamins to the animal during drought seasons (Elizondo *et al.,* 1987). In addition, it is rich in carbohydrates, calcium, and its efficiency in converting water into dry matter and digestible energy is highly desired for producers (Herrera, 2011). In fact, under growing conditions it becomes an alternative for forage production, due to its high efficiency in the use of water compared to other annual crops (Flores and Reveles, 2010). However, its low protein content (4% DM) limits its use as a sole forage

source. Thus, it is highly recommended to apply different biotechnological processes in order to increase its protein content, *i.e.* the solid state fermentation (SSF) Herrera *et al.*, 2017. The SSF process increases the protein content in substrate by increasing the unicellular protein contained in the cell-wall microorganisms. The commonly used microorganisms are yeast cultures as *Saccharomyces cerevisiae* and some species of *Kluyveromyces* (Van Markis *et al.* 2006). Otherwise, the silage process takes place by the acidification and fermentation of soluble carbohydrates into lactic acid and volatile fatty acids by lactic acid-producer microorganisms under anaerobic conditions. This process inhibits the growth of pathogen microorganisms and allows preservation of the nutritional characteristics of forage for later use (Wilkins, 1999). Hence, this research aimed to evaluate the nutritive quality and *in vitro* gas production of corn silage with prickly pear and fermented prickly pear.

3 MATERIALS AND METHODS

3.1 Study area: This study was carried out in the Faculty of Veterinary Medicine and Husbandry of Juarez University of Durango State. Prickly pear cladodes (*Opuntia ficus-indica* variety AV6) were randomly harvested from irrigated crops located nearby the faculty area in Durango, México.

3.2 Solid state fermentation (SSF) and silages preparation: The prickly pear cladodes were cut into small pieces using a sharp knife and were placed into plastic containers where they were inoculated with *Saccharomyces cerevisiae* (1% m/m). The fermentation process was carried out for 48h at room temperature (25ºC).

Silage formulation was determined with the prickly pear addition as a forage fraction substitute (Table 1). Thus, twenty-one experimental micro-silos were prepared by mixing corn forage solely (T1, n=7), forage corn with fresh prickly pear cladodes (T2, n=7) and forage corn with fermented prickly pear cladodes (T3, n=7). After mixing all the ingredients, micro-silos were placed and hermetically sealed in plastic containers (30 cm diameter × 50 cm height) for 30 d. Once the time was elapsed, silages were opened for further analyses.

Table1: Proportion of dietary ingredients of experimental silages

	T1	T2	T3
Ingredient (%)			
Forage corn	100	75	75
Prickly pear	--	25	--
Fermented prickly pear	--	--	25

3.3 Silage fermentation analysis: Once the silages were opened, the following variables were evaluated: pH (Hanna instruments, model HI 83142), lactic acid according to Borshchevskaya *et al.* (2016), as well as volatile fatty acids and ammonia-nitrogen (NH3-N) contents using procedures proposed by Galyean (2010).

3.4 Chemical analyses: Samples of each experimental silage was dried in a forced-air oven at 55 °C for 72 h, ground to 1 mm particles in a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) for the determination of DM (method 934.01; AOAC 1994). The crude protein (CP) content was calculated by determining the total nitrogen (N) content, using the micro-Kjeldhal technique (method 920.87; AOAC 1994) and a fixed conversion factor (6.25). The NDF, ADF concentration was determined following methods proposed by Van Soest (1991) and gas production parameters according to procedures described by Menke and Steingass (1988).

***3.5 In vitro* gas production:** About 1 g of each experimental treatment was placed into ANKOM glass modules equipped with pressure transducers and incubated in triplicate with buffer solutions-ruminal inoculum in a 2:1 ratio according to Theodorou *et al.* (1994). Incubations were performed from 0 to 96 h and pressure was registered every hour in the meantime. *In vitro* gas production kinetics was estimated by fitting data to the Gompertz function (Murillo *et al.*, 2018) according to the follow equation:

$$GP = Gmax * \exp[-A * \exp(-k * t)]$$

Where GP= gas production at time t (ml); Gmax= maximum gas production (ml); k= constant gas production rate (h^{-1}); A= latency time before the gas production begins (h). Once 24h of incubation time was elapsed, pressure release valve was opened during 2 sec in every module individually. The released gas in each module was guided through a tube and connected to a portable gas analyzer for CH_4 and CO_2 measures according to procedures proposed by the manufacturer (GEM^{TM}5000, LANDTEC, USA).

***3.6 In vitro* fermentation parameters:** For the evaluation of the fermentation parameters, approximately 1 g was placed into nylon bags (ANKOM, F500 nylon bags) previously weighed and located into ANKOM modules and incubated in triplicate with buffer solutions-ruminal inoculum in a 2:1 ratio according to Theodorou *et al.* (1994). After 24 h of continuous fermentation, modules were opened and pH was immediately measured (Hanna instruments, model HI 83142). The bags were collected and rinsed with distilled water and dried at 65°C for 48 h. The *in vitro* DM disappearance (IVDMD) was calculated based on the differences in DM content of substrate before and after incubation. Additionally, about 1.0 ml of the filtrate was centrifuged at 3,000×g for 5 min; then, approximately 500 µl of the supernatant liquid was acidified with 150 µl of 25% metaphosphoric acid solution for volatile fatty acids evaluation according to Galyean (2010). For nitrogen-ammonia evaluation, approximately 1 ml of the filtrate was placed into corning tubes and mixed with 30 µl of sulfuric acid (50% v/v) according to Galyean (2010).

3.7 Statistical analysis: The obtained data were analyzed with a completely randomized design using GLM procedures of SAS (2009). Means were analyzed and compared with the Tukey's test declaring significant differences at $p \leq 0.05$.

4 RESULTS AND DISCUSSION

4.1 Chemical composition: Dry matter (DM), CP, NDF, AND ADF content were different among treatments ($p<0.05$; Table 2). The addition of prickly pear decreased above

12% DM content when compared to T1. This reduction may be explained due to an increase in the water content as part of the fermentation process (Kunkle *et al.*, 2006). Otherwise, dry matter values registered in T2 and T3 were within the range proposed as acceptable for good quality silage (NRC, 2001). Jiménez *et al.* (2016) recorded DM values similar to this study (34% y 43.4%) on corn silage. The inclusion of fermented prickly pear (T3) increased the protein content in 11% when compared to T1. These changes are directly attributed to the SSF process of prickly pear and the incorporation of cellular protein of *S. cereviseae*. Likewise, López (2012) registered 6.9% of CP in cactus silage, while Cürek and Őzen (2004) obtained 3.5% of CP, which are lower than the values obtained in this study. The NDF content was lower in T2 when compared to T1 and T3 ($p<0.05$). These results may be explained due to the reduction attributed to the hydrolysis of hemicellulose which occurs during silage fermentation. At this stage, pentoses are released and may be fermented into lactic and acetic acid (McDonald *et al.*, 2002). Higher concentrations were registered by Britos *et al.* (2007) in pasture silage enriched whit buttermilk. However, Mciteka (2008) recorded lower concentration of NDF in cactus pear silage (8.35). Otherwise, the FDA concentration increased 6% in T3 when compared to T1. In spite of the variation in the contents of NDF and ADF among the experimental treatments, these changes did not affect the digestibility ($p>0.05$).

Table 2: Chemical composition of silage elaborated with prickly pear

	T1	T2	T3	SEM
Dry matter (%)	42.0±0.29 [a]	37.2±0.11 [b]	36.2±0.17 [c]	0.14
Crude protein (%)	6.2±0.55 [b]	5.9±0.05 [b]	6.9±0.19 [a]	0.08
Neutral detergent fibre (%)	53.2±2.31 [a]	49.1±1.00 [b]	56.7±0.35 [a]	1.20
Acid detergent fibre (%)	23.6±0.06 [b]	23.7±0.31 [b]	25.3±0.11 [a]	0.16
Dry matter digestibility (%)	61.8±2.44	63.7±1.63	61.1±1.01	1.46

[a,b] Different letters in the same row indicate differences ($p<0.05$).SEM=standard error of mean

4.2 Fermentation parameters of silage process: The pH values were different among treatments ($p<0.05$, Table 3). The pH values were 10.8% lower in T2 with respect to T3. The pH values registered in this research are within the acceptable range (3.5 to 5). These values suggest that fermentation and consequently preservation process was carried out adequately. According to Ben Salem and Abidi (2009), the prickly pear fermentation process is attributed to a higher content in sugars. Additionally, Gusha *et al.* (2013) obtained similar values in pH when fermented prickly pear silage and legumes. On the other hand, nitrogen ammonia concentration (N-NH3) was different between treatments ($p<0.05$, Table 3). The inclusion of cactus pear in T2 increased 14% the N-NH3 concentration. Likewise, Cürek and Őzen (2004) registered similar values to the values reported in this study. However, these results were lower to previous research reported by Mokoboqui *et al.* (2016) in cactus pear silage (49.5 g/kg DM). Apparently, protein contents may go through a deamination when prickly pear is added to the silage due to a reduction in the NDF content. Presumably, microorganisms can be able to improve degradation of proteins when the fibre fractions are reduced by increasing the microorganisms' adhesion to substrate (Berumen *et al.*, 2015).

Table 3: Fermentation parameters of corn silage with prickly pear and fermented prickly pear addition

	T1	T2	T3	SEM
pH	4.3±0.01^{ab}	4.1±0.01^{b}	4.6±0.09^{a}	0.05
N-NH_3 (g/kg DM)	1.4±0.01^{b}	1.6±0.01^{a}	1.1±0.01^{c}	0.03
Lactic acid (g/kg DM)	27.5±1.35^{c}	33.7±0.36^{b}	41.9±0.68^{a}	0.73
Acetic acid (% DM)	0.7±0.26^{b}	0.9±0.00^{a}	0.8±0.02^{b}	0.01
Propionic acid (% DM)	3.5±0.01^{b}	4.0±0.006^{a}	4.0±0.02^{a}	0.01
Butyric acid (% DM)	0.01±0.002^{b}	0.03±0.00^{a}	0.01±0.003^{b}	0.001

^{a,b} Different letters in same row indicate differences ($p<0.05$). SEM= Standard error of the mean. N-NH_3 =ammonia nitrogen

The lactic acid (LA) concentration was different between treatments ($p<0.05$, Table 3). Lactic acid is the most desirable product of the fermentation process. It is mainly produced by bacterial catabolism of carbohydrates. Lactic bacteria offer a high tolerance to low pH values and may comfortably grow with values ranging from 4.0 to 6.8. The reported values in this research are within this range. In spite of Mokoboki *et al.* (2016) and Mciteka (2008) registered 46.5 and 74 g/Kg DM in silages of prickly pear solely respectively, this research offers a mix of forage corn and prickly pear as an alternative feedstuff. The volatile fatty acids (VFA) presented different values among treatments ($p<0.05$). This research showed lower values of acetic acid than those reported by Isnandar *et al.* (2010) when fermented silage with inoculum of lactic bacteria. This result suggests a high lactic acid production at low pH values followed by a steady depletion of fermentation due to clostridia which produces acetic acid and butyric acid (Hafner *et al.,* 2013). Silages with prickly pear and fermented prickly pear produced more propionic acid than corn silage solely. Moreover, the values obtained in this work were lower than those registered by Mciteka (2008), but higher than the results presented by Vendramini *et al.* (2010). Moreover, all treatments presented lower values of butyric acid, which suggest an effective fermentation. Additionally, Cürek and Őzen (2004) obtained higher concentrations of butyric acid. Presumably, the obtained results indicate a fine quality in experimental silages since they offer higher contains in lactic acid and reduced values of butyric acid.

4.3 Ruminal fermentation parameters: No changes in pH were registered among treatments in the *in vitro* ruminal fermentation ($p>0.05$). However, N-NH3 concentrations were different among treatments ($p<0.05$, Table 4). NH_3-N concentrations in the ruminal fermentation were quite similar to those reported by Satter and Slyter (1974) as the optimal level for microbial growth and fibre digestion in the rumen. The higher values in ammonia presented in T2 may be explained due to higher degradation of the ruminal protein (Ricci, 2014). Moreover, the *in vitro* studies showed a disadvantage since the fermentation is made in hermetically sealed bottles, which does not allow the escape of fermentation products. Therefore, the accumulation of NH3-N in *in vitro* incubations may be overestimated (Pengpeng and Tan, 2013). Likewise, butyric acid concentrations presented changes among treatments ($p<0.05$). I*n vitro* ruminal concentrations of butyric acid were 27.2 % higher in T2 compared with T1. Schulze *et al.* (2017) registered lower concentrations (11.4 mol/100 moles) in heifers fed grass/clover silage. Similarly, Pinho *et al.* (2017) obtained a concentration of 12.09 mol/100 moles in spineless cactus mucilage in *in vitro* ruminal fermentation; while, Abidi *et al.* (2009) found

similar values in cladodes of spineless cactus (*Opuntia ficus-indica*). On the contrary, no changes were observed in acetic and propionic acid ($p>0.05$). These results suggest that the addition of prickly pear does not affect the acetic and propionic acids but the butyric acid production. These changes may be elucidated through a diminution in the fibre fraction which may lead to a superior adhesion of the microorganisms and to a higher deamination of proteins when prickly pear is added in T2 (Rodríguez *et al.,* 2007). The maximum gas production (Gmax) presented differences among experimental treatments ($p<0.05$, Table 4). Thus, Gmax increased 41% when prickly pear was added in T2. As stated earlier in this study, this change could be attributed to a reduction in NDF and ADF which affects the availability of hemicellulose. In addition, Tosto *et al.* (2015) reported 183 ml of gas produced *in vitro* with silages based on *atriplex spp.* mixed with prickly pear, which are similar to those observed in this study. Moreover, the volume of produced gas agrees with Del Razo *et al.* (2015), who registered 256 ml. The gas production is a result of the digestibility of the substrate and this is affected by the structural carbohydrates concentration, simple sugars and proteins (Theodorou *et al.*, 1994). Moreover, the shorter lag time (A) was different between treatments ($p<0.05$, Table 5). Lag period decreased 45% when adding prickly pear and fermented prickly pear. The shorter lag time (Lag) observed may be attributed to the physicochemical characteristics of the prickly pear. The soluble fraction constitutes an energetic substrate of rapid fermentation which makes easier the adhesion of microorganisms, presenting and increase in the fermentation of structural carbohydrates and reducing the Lag period as a consequence. Tosto *et al.* (2015) registered lower values in silages fermented with *atriplex spp.* and prickly pear (l h 13 min). Otherwise, the constant rate of gas production presented changes among treatments ($p<0.05$). This constant decreased when prickly pear was added in T2 and T3. Despite of that reaching the maximum gas production value (Gmax) may take a longer time since these rate values are lower, it does not change the fact that asymptotic value will be superior to the presented in T1.

Table 4. *In vitro* ruminal fermentation parameters of corn silage with cactus pear

	T1	T2	T3	SEM
pH	6.86±0.008	6.87±0.03	6.85±0.01	0.01
N-NH_3 (mg/dL)	11.9±1.08^b	15.2±0.04^a	11.3±0.18^b	0.52
Acetic acid (%)	53.3±0.89	51.3±0.70	51.7±0.26	0.55
Propionic acid (%)	27.1±0.72	27.6±0.43	29.1±0.29	0.42
Butyric acid (%)	14.7±0.05^b	16.1±0.17^a	14.4±0.02^b	0.08

^{a,b} Means within the same row with different uppercase superscripts vary ($p<0.05$). SEM= Standard error of the mean. N-NH_3 =ammonia nitrogen.

Table 5. Ruminal gas production kinetics parameters of the experimental treatments

Parameters	T1	T2	T3	SEM
Gmax (mL)	124.8±8.48^b	176.4±1.28^a	167.3±3.73^a	4.41
A (h)	4.2±0.21^a	2.7±0.22^b	2.9±0.06 ^{b}	0.14
k (%/h)	0.08±0.001^a	0.06±0.004^b	0.05±0.0006^b	0.002
Methane (ml/g DM)	9.7±0.29^b	14.0±0.35^a	10.5±0.0.004^b	0.21
CO_2 (ml/g DM)	59.0±4.49^b	81.6±2.63^a	66.6±2.16^b	2.66
Methane:CO_2	0.16±0.01	0.17±0.001	0.15±0.005	0.006

[a,b] Different letters in same row indicate differences ($p<0.05$). SEM= Standard error of the mean; Gmax: maximum gas production; k: rate of gas production; A: latency period before the gas production begins (Lag phase).

Additionally, and in accordance with the results of Gmax among treatments, methane production was lower in T3 when compared to T1 and T2 ($p<0.05$). The inclusion of fermented prickly pear in silage decreased 33% the methane production when compared to prickly pear (T2). Tavendale *et al.* (2005) explained a methane reduction through the reduction in the fibre digestion, which decreases H_2 production. These same authors stated that methanogenesis could be affected by the inhibition of the growth of methanogens. In addition, reductions in methane production may be affected when presented simultaneously a lower proportion of acetate and a higher proportion of propionate. Moreover, acetate synthesis from pyruvate produces metabolic hydrogen in the rumen, which is the main precursor of methanogenesis; in contrast, propionate formation from pyruvate requires hydrogen (Moss *et al.* 2000). Similar values were registered by Denek *et al.*, (2017) in corn silages. Otherwise, the CO_2 production was different between treatments ($p<0.05$). The information of CO_2 production in silages made of cactus is limited; however, the lower concentration of CO_2 was recorded when fermented prickly pear was added. These results are desirable since they may positive affect the use of energy by reducing the methane natural precursors and the methane production by itself. Furthermore, methane: CO_2 ratio was not affected by the inclusion of prickly pear ($p>0.05$). The latter indicates that increases and decreases in methane and CO_2 are proportionally similar among treatments. However, the methane production in T3 is similar to that observed in T1 which indicates that even when more volume of gas is being produced (Gmax), methane and CO_2 production are not increasing.

5 CONCLUSION

As a result, prickly pear and fermented prickly pear silages can be used as an alternative feedstuff when it is added to forage corn. The addition of fermented prickly pear to corn silages increased the nutritional quality. Otherwise, the addition of fermented prickly pear offers an increase in the volume gas production and an improvement ruminal fermentation process without affecting the methane and CO_2 production. The latter suggests that these silages may be considered as sustainable and alternative feedstuff in ruminants' nutrition. Nevertheless, these results should be supported by *in vivo* feeding studies in the near future.

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