Abanico Veterinario. January-December 2021; 11:1-13. http://dx.doi.org/10.21929/abavet2021.24
Original Article. Received: 21/01/2021. Accepted: 28/04/2021. Published: 15/05/2021. Code: e2021-01.

Fermentative quality and methane production in corn stubble silage with fermented and unfermented nopal cactus

Calidad fermentativa y producción de metano en ensilados de rastrojo de maíz adicionados con nopal fermentado y sin fermentar

Araiza-Rosales Elia[1] ID, González-Arreola Adolfo[2] ID, Pámanes-Carrasco Gerardo[1] ID, Murillo-Ortiz Manuel[2] ID, Jiménez-Ocampo Rafael[3] ID, Herrera-Torres Esperanza*[4] ID

[1]CONACYT-Universidad Juárez del Estado de Durango, Durango, México. C.P. 34000. [2]Facultad de Medicina Veterinaria y Zootecnia, Universidad Juárez del Estado de Durango. Durango, México. [3]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias-Durango. Km 4.5 Carretera Durango-Mezquital. Durango, México. [4]Instituto Tecnológico del Valle del Guadiana-Tecnológico Nacional de México. Durango, México. e_araiza2002@hotmail.com, junior.glz@hotmail.com, gerardo.pamanes@gmail.com, manuelmurilo906@gmail.com, rafax77@hotmail.com, hetoes99@yahoo.com.mx

ABSTRACT

The objetive of this research was to evaluate the nutritive and fermentative quality and methane emissions in corn stubble silages with nopal (*Opuntia ficus-indica*), for which three experimental treatments were evaluated, T1: corn fodder; T2: 75 % corn stubble + 25 % nopal; and T3: 75 % corn stubble + 25 % fermented nopal. Twenty-one microsilos (7 per treatment) were prepared in plastic containers and left to ferment for 30 days. At the end of fermentation, the chemical composition, fermentation parameters, gas production and methane (CH_4) were evaluated. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were different among treatments ($p<0.05$); CP content increased 44 % with the addition of fermented cactus (T3). Ammonia nitrogen, lactic acid and volatile fatty acids values were different among treatments ($p<0.05$). Maximum gas production (Gmax) and CH_4 concentration decreased 32 % and 49 % at T3, respectively. The addition of cactus and fermented cactus to corn stubble silage increases protein content. In addition, it reduces ruminal methane synthesis *in vitro*.

Keywords: fermentation, corn stubble, greenhouse gases, silage.

RESUMEN

El objetivo de esta investigación fue evaluar la calidad nutritiva, fermentativa y la emisión de metano en ensilajes de rastrojo de maíz con nopal (*Opuntia ficus-indica*), para lo cual se evaluaron tres tratamientos experimentales, T1: forraje de maíz; T2: 75 % rastrojo de maíz + 25 % nopal y T3: 75 % rastrojo de maíz + 25 % nopal fermentado. Se elaboraron 21 micro-silos (7 por tratamiento) en recipientes de plástico y se dejaron fermentar por 30 días. Al término de la fermentación se evaluó la composición química, los parámetros de fermentación, producción de gas y metano (CH_4). Los contenidos de materia seca (MS), proteína cruda (PC), fibra detergente neutro (FDN) y fibra detergente ácida (FDA) fueron diferentes entre los tratamientos ($p<0.05$); el contenido de PC incrementó 44 % al adicionar nopal fermentado (T3). Los valores de nitrógeno amoniacal, ácido láctico y ácidos grasos volátiles fueron diferentes entre tratamientos ($p<0.05$). La máxima producción de gas (Gmax) y la concentración de CH_4 disminuyeron 32 % y 49 % en T3, respectivamente. La adición de nopal y nopal fermentado a ensilados con rastrojo de maíz incrementa el contenido de proteína. Además, reduce la síntesis de metano ruminal *in vitro*.

Palabras clave: fermentación, rastrojo de maíz, gases efecto invernadero, ensilaje.

INTRODUCTION

In northern Mexico, extreme temperatures and prolonged drought have caused a decrease in forage production. Thus, small producers have found it necessary to use forage resources that represent a low nutrient supply for ruminants to counteract the dry season (López-Inzunza *et al.,* 2017). Thus, corn (*Zea mays*) stubble has been used as a forage source in arid and semiarid areas in the north of the country as a shearing in the production of grains for human consumption (SAGARPA, 2009). Under these production conditions, nopal cactus emerges as an alternative in livestock feed (Flores-Hernández *et al*., 2017). Nopal cactus (*Opuntia* spp.) is an important plant resource in northern Mexico; it is considered a natural water store and it is very efficient in water consumption (Orona-Castillo *et al*., 2008). Additionally, nopal provides digestible energy, water and vitamins to the animal during the dry season (Dubeux *et al*., 2018).

In addition, compared to other annual forages, nopal uses less water for its production and growth (Flores-Hernández *et al.* 2019). However, its low protein content (4 % DM) limits its use as a sole forage source. Due to the above, it is recommended to apply different biotechnological processes that help increase its protein content; for example, solid-state fermentation (SSF) (Herrera *et al*., 2017). The SSF process increases the protein content of the substrate by increasing the unicellular protein in the cell wall of the microorganisms. The most commonly used microorganisms are *Saccharomyces cerevisiae* yeasts and some *Kluyveromyces* species ((Van Markis *et al*., 2006). On the other hand, the ensiling process can serve to decrease the problems of cattle feeding and face the shortage of forage in the dry season (Castro *et al*., 2016). This process inhibits the growth of pathogenic microorganisms by decreasing the pH, due to the presence of lactic acid bacteria (LAB), which allows preserving the freshness and nutritional characteristics of forages for later use (Mokoboki *et al*., 2016). Due to the above, the objective of this work was to evaluate the nutritional quality and *in vitro* gas production of corn stubble silages with the addition of cactus and fermented cactus.

MATERIAL AND METHODS

Study area

The study was carried out at the Faculty of Veterinary Medicine and Animal Husbandry of the University of Juárez, Durango State. Forage nopal variety AV6 was randomly harvested from a cultivated nopal plantation, located next to the Faculty and within Durango municipality, Mexico.

Solid state fermentation (SSF) and preparation of microsilos

The nopal cactus stalks were cut into pieces of approximately 1 cm^2 using a stainless steel knife and placed in 19 l plastic containers, where they were inoculated with *Sacharomyces cerevisiae* (1% m/m). The fermentation process was carried out for 48 h at 25 ºC. Treatments consisted of including cactus and fermented cactus to corn fodder, as shown in Table 1. Experimental microsilos were prepared with chopped corn forage without grain, and in mature state with a particle size of 2 to 4 cm (Variety: Hybrid 21/20) (T1, n=7), corn stover with fresh nopal (T2, n=7). Besides prepared with corn stubble with fermented nopal (T3, n=7) in plastic containers (30 cm diameter × 50 cm high), hermetically sealed for 30 d. After this time, the microsilos were opened for later analysis.

Table 1. Ingredients proportions in experimental treatments

(%)	T1	T2	T3
Ingredients			
Corn stubble	100	75	75
Unfermented nopal	--	25	--
Fermented nopal	--	--	25

Fermentative variables

Once the microsilos 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 acid and ammonia nitrogen (NH_3-N) contents, using the procedures proposed by Galyean (2010).

Chemical analysis

Samples from each experimental microsilos were dried in a forced air oven at 55 °C for 72 h; subsequently, the particle size was reduced to 1 mm in a Wileymil mill (Arthur H Thomas, Philadelphia, PA, USA), to determine the dry matter (DM) contents (method 934.01). Crude protein (CP) concentrations were determined by the micro-Kjeldhal technique (method 920.87), using the conversion factor (6.25) (AOAC, 2010). The concentration of NDF, ADF were obtained according to the procedures proposed by Van Soest (1991) and the gas production parameters according to the technique described by Menke and Steingass (1988).

Gas *in vitro* production

Approximately 1 g of sample from each experimental microsilo was placed in glass modules in a pressure transducer equipment, ANKOM brand. In addition, it was incubated in triplicate with a 2:1 solution of ruminal buffer-liquid solution, according to the procedure described by Murillo-Ortiz *et al.* (2018). Incubations were carried out from 0 to 96 h and recording pressure values at the same time. The gas production kinetics was estimated using the Gompertz function (Murillo-Ortiz *et al*., 2018), according to the following 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}) and A= lag phase (h). From the 24 h incubation, the valve was opened to release gas for 2 s from each module. The gas released from each module was connected to a portable CH_4 and CO_2 analyzer through a tube to measure the concentration of these gases according to the procedures established by the manufacturer (GEM^{TM}5000, LANDTEC, USA).

In vitro **fermentation parameters**

To evaluate fermentation parameters, 1 g of sample was placed in nylon bags (ANKOM, F500 nylon bags; ANKOM, 2018), pre-weighed and placed inside ANKOM modules and incubated in triplicate with buffer solution: ruminal fluid, in a 2:1 ratio according to Murillo-Ortiz *et al.* (2018). After 24 h of continuous fermentation, the modules were opened and immediately pH was measured (Hanna instruments, model HI 83142). The bags were removed from the modules and were washed with distilled water and dried at 65°C for 48 h. *In vitro* digestibility of dry matter (IVDDM) was calculated based on the difference in dry matter content of the substrate before and after incubation. Additionally and approximately 1.0 ml of the filtrate was centrifuged at 3,000×g for 5 min; then, 500 μl of the supernatant liquid was acidified with 150 μl of 25 % metaphosphoric acid to evaluate volatile fatty acids. Also approximately 1.0 ml of the filtrate was placed in tubes and was acidified with 30 μl of 50 % v/v sulfuric acid to determine N-NH_3 (Galyean, 2010).

Statistical analysis

The data obtained were analyzed with a completely randomized design, using the GLM procedures of SAS (2010). Means were compared with Tukey's multiple range test and declared significant differences when P≤0.05.

RESULTS AND DISCUSSION

Chemical composition.

Dry matter (DM) content decreased with the inclusion of corn stubble (P<0.05, Table 2). Silages containing stubble and nopal (t2 and t3) presented adequate DM contents, since according to Pineda-Cordero (2016) silages containing between 30 and 35 % DM are considered of good quality. In addition, the moisture present in silages determines the type of fermentation that took place during the ensiling process. Other factors that determine fermentation quality are the soluble carbohydrates present and the buffering capacity of the forage used (Bernal *et al.,* 2002).

Crude protein (CP) concentration was different among treatments (P<0.05, Table 2). The addition of cactus and fermented cactus increased 43.54 % and 79 % the CP concentration in silages, respectively, compared to t1. The increases at t2 and t3 are due to the initial protein content in the corn stubble before ensiling (mature green forage contained 4.9 %, while the stubble contained 5.2 % CP; results not shown) and to the proliferation of cellular protein from the *Saccharomyces cereviseae* yeast used to ferment the nopal. However, Alhanafi *et al.* (2019) recorded a lower CP content in silages of *Opuntia ficus*, *indica* added with Atriplex (6.41 %); compared to t2 of this study.

Table 2. Chemical composition of corn stubble silages added with nopal cactus

(%)	T1	T2	T3	SEM
Dry Matter	42.0±0.29 [a]	35.9±0.48[b]	35.5±0.26[b]	0.25
Crude protein	6.2±0.55[c]	8.9±0.10[b]	11.1±0.05[a]	0.05
Neutral detergent fiber	53.2±2.31[b]	63.1±0.08[a]	58.8±0.12[ab]	1.09
Acid detergent fiber	23.6±0.06[c]	37.6±0.05[a]	35.3±0.73[b]	0.34
Digestibility of dry matter	61.8±2.44	58.4±1.98	64.1±2.20	1.81

[a,b] Different letters in the same row indicate differences (P<0.05). SEM=standard error of the mean, n=3.

The concentration of neutral detergent fiber (NDF) was lower in T1 (P<0.05, Table 2); an increase of 18% was observed in T2. This increase is due to the presence of corn stubble, which has a high amount of fiber; however, part of the hemicellulose is hydrolyzed during the ensiling process. At this stage, pentoses are released and they are fermented into lactic acid and acetic acid (McDonald *et al*., 2002).

Likewise, acid detergent fiber (ADF) content showed the same behavior; ADF increased 59 and 49 % at T2 and T3, respectively (P<0.05, Table 2). In the same way, the increases in ADF were attributed to the presence of corn stubble at T2 and T3. Nevertheless, NDF and ADF contents were within the range of good quality forages.

Fermentation parameters of silage process

The pH values were higher in t2 and t3, with respect to t1 (P<0.05, Table 3); however, all were within the ideal values, which is indicative of a good fermentation and conservation process. It is worth mentioning that the speed with which a silage achieves a pH <4.0 guarantees silage stability and reduces nutrient loss by secondary fermentations, or by bacterial and fungal contamination (Ha Vu *et al*., 2019).

The concentration of ammonia nitrogen in the silages increased with the cactus and fermented cactus inclusion (P<0.05, Table 3). This increase recorded in t2 and t3 silages could have been caused by an increase in protein degrading microorganisms (Berumen *et al*., 2015; Ruangyote and Metha, 2018), or also the use of corn stover reduces the amount of soluble carbohydrates and therefore increases protein degradation (Herremans *et al*., 2019). However, to classify a silage as good quality, the maximum ammonia nitrogen concentration should be 7-20 % of total nitrogen (Sánchez and García, 2017); therefore, the experimental silages obtained values that are within this range and therefore indicate that an adequate fermentation process was carried out.

Table 3. Fermentation parameters of corn stubble silage with cactus addition

	T1	T2	T3	SEM
pH	4.3±0.05[b]	4.7±0.04[a]	4.7±0.01[a]	0.03
$N\text{-}NH_3$ (g/kg DM)	1.9±0.09[c]	5.5±0.09[b]	6.5±0.20[a]	0.03
Lactic Acid (g/kg DM)	24.3±3.45[b]	73.9±1.91[a]	76.7±4.18[a]	2.71
Acetic acid (% DM)	0.7±0.26[c]	0.9±0.00[b]	1.1±0.02[a]	0.01
Propionic acid (% DM)	3.5±0.01[c]	4.2±0.02[b]	4.3±0.01[a]	0.01
Butyric acid (% DM)	0.01±0.002[b]	0.03±0.004[a]	0.04±0.001[a]	0.002

[a,b] Different letters in the same row indicate differences (P<0.05). SEM=standard error of the mean. $N\text{-}NH_3$ =ammonia nitrogen, n=3.

The concentration of acetic acid was different between treatments (P<0.05, Table 3). Volatile fatty acids are the product of fermentations induced by the presence of coliform bacteria that transform lactic acid into acetic and butyric acid, and are present in manure and soil. According to Kung *et al.* (2018), acetic acid concentrations are between 0.5 and 2.0%, when DM content is 45 to 55 %; therefore, the values obtained in this work are within the range for good quality silages (0.5-1.1 %). Propionic acid values were different among treatments (P<0.05, Table 4). The results obtained in this study are equal to those reported by González *et al.*, (2019) in corn silage with cactus and fermented cactus (4.0 %). Butyric acid concentrations were higher in silages containing stubble (P<0.05, Table 3); however, these values indicate that there was adequate fermentation. In addition, Da Silva *et al.* (2020) report lower propionic acid values in cactus silages with gliricidia. According to the lactic and butyric acid values obtained in the silages of this study, it can be inferred that the inclusion of cactus and fermented cactus in the stubble promotes an increase in lactic acid and a decrease in butyric acid, which results in silages with good fermentative and nutritional quality.

Ruminal fermentation parameters

$N\text{-}NH_3$ concentration was lower in t1, compared to t2 and t3 (P<0.05, Table 4). The inclusion of cactus and fermented cactus in the stubble silages promoted an increase of 48 % and 27.7 % in $N\text{-}NH_3$, respectively. Changes in this variable indicate that proteolysis is taking place and it increased due to crude protein increase in the silage by the addition of stubble and fermented nopal. However, there are studies that affirm that increases in $N\text{-}NH_3$ concentration are because the protein is not incorporated into microbial protein synthesis, which would reflect an energy loss in the ruminant (Rodríguez *et al.*, 2007). On the contrary, other authors also found increases for $N\text{-}NH_3$ when crude protein content was increased through the addition of urea in pineapple silages (López-Herrera *et al.*, 2014). Additionally, these results agree with those obtained by Pinho *et al.* (2017) in nopal silages (17 mg/dL) at 9 h of incubation. Thus, in this study, the $N\text{-}NH_3$ concentrations recorded in all treatments presented adequate levels higher than 5 mg/dL, which allows guaranteeing microbial protein synthesis (Rodríguez *et al.*, 2007).

Table 4. *In vitro* ruminal fermentation parameters of corn stubble silage added with nopal cactus

	T1	T2	T3	SEM
pH	6.8±0.008	6.9±0.17	6.9±0.03	0.01
$N\text{-}NH_3$ (mg/dL)	11.9±1.08^b	17.7±0.43^a	15.2±0.08^a	0.55
Acetic Acid (%)	53.3±0.89^a	52.1±0.48^a	46.5±0.43^b	0.52
Propionic Acid (%)	27.1±0.72^b	30.6±0.31^a	32.1±0.45^a	0.43
Butyric Acid (%)	14.7±0.05^a	12.6±0.13^b	14.7±0.05^a	0.07

^{a,b} Different letters in the same row indicate differences (P<0.05). SEM=standard error of the mean. $N\text{-}NH_3$ ammoniacal nitrogen; n=3.

On the other hand, acetic acid concentration decreased and propionate concentration increased in corn stubble and fermented cactus silage (P<0.05; Table 4), compared to t1 and t2. In this sense, a decrease in acetic acid concentration at t2 and t3 is closely related to a decrease in the fermentation of structural carbohydrates (NDF and ADF). Thus, since structural carbohydrates have the highest concentration in the nutrients of t2 and t3, it is suggested that adequate fermentation is not taking place (Van Soest, 1994). However, Sánchez *et al.* (2014) also detected a decrease in acetate concentration and an increase in ruminal propionate. Regularly, the production rate of propionate and other AGV is directly related to the consumption of fermentable substrates from the diet, which favors propionate synthesis from microbial fermentation by amylolytic bacteria (Van Soest, 1994).

Maximum gas production (Gmax) was higher at t1 (P<0.05; Table 5). The inclusion of cactus and fermented cactus in silages with corn stubble decreased maximum gas production 31.9 % and 48.7 %, respectively. The values obtained in this study are lower than those reported in leucaena and star grass (234 and 154 ml/g, respectively) by Naranjo *et al.* (2016). Similarly, González *et al.* (2019) recorded higher Gmax values in corn silages with nopal (176 ml). The decrease in gas production observed in this study is attributed to the decrease in dry matter digestibility at t2 and t3, as a result of the addition of corn stubble that NDF and ADF values are higher than those recorded at t1. As previously mentioned, the presence of structural carbohydrates prevents adequate fermentation, which is reflected in gas production.

Table 5. Gas kinetic parameters of corn stubble silage with nopal added

Parameters	T1	T2	T3	SEM
Gmax (ml/g DM)	124.8±8.48[a]	94.6±6.50[b]	84.0±1.75[b]	5.10
A (h)	4.2±0.21[a]	2.7±0.03[b]	4.4±0.04[a]	0.10
K(%/h)	0.08±0.001	0.07±0.007	0.09±0.003	0.003
Methane (ml/g DM)	9.7±0.29[a]	7.3±0.29[b]	6.5±0.16[b]	0.21
CO_2 (ml/g DM)	59.0±4.49[a]	49.8±0.75[ab]	44.7±0.70[b]	2.17
Methane:CO_2 ratio	0.16±0.010 [a]	0.14±0.003 [b]	0.14±0.005 [b]	0.014

[a,b] Different letters in the same row indicate significant difference (P<0.05). SEM=standard error of the mean; Gmax: maximum gas production; k represents the specific gas production rate; A is the latency period before gas production starts (lag phase).

On the other hand, the dormancy period "A" decreased 55 % at t2 (stubble + nopal silage). To explain this result, variables that were not considered in this study, such as soluble carbohydrate content or lignin, must be taken into account, since the faster onset of fermentation depends on these. In this sense, López-Inzunza *et al.* (2017) reported longer dormancy times for silages with higher ADF content, with respect to NDF content in silages with pineapple stubble; these authors showed structural carbohydrate concentrations and dormancy times similar to those reported in this study (72 % NDF and an A of 3.8 h). Moreover, the A values obtained in this study are also similar to those recorded by González *et al.* (2019) in corn forage silages with nopal cactus. Additionally, methane production decreased 32 and 49 % in silages that included nopal and fermented nopal, respectively (P<0.05; Table 5). Similarly, the methane: CO_2 ratio also decreased with the addition of fermented nopal and nopal without fermented, as well as corn stubble. This variable is closely related to ruminal methane synthesis. Higher values in this ratio suggest an increase in ruminal methane synthesis through the CO_2 reduction pathway (Murillo *et al*., 2018). Thus, although a reduction in methane and CO_2 production is closely related to a decrease in the fermentative quality of t2 and t3 silages, this reduction also suggests changes or even an inhibition in methanogenic populations (Tavendale *et al.,* 2005. In addition, the decrease in methane is directly related to an increase in ruminal

CONCLUSIONS

The addition of nopal and fermented nopal to corn stubble silages increases crude protein content by 43 and 79 %, respectively. In addition, the use of nopal and fermented nopal in corn stubble silages reduces ruminal methane synthesis *in vitro*. On the contrary, the presence of corn stubble increases the structural carbohydrate content of the silage, which compromises ruminal fermentation and gas production *in vitro*; however, *in vivo* studies are recommended to confirm these results.

CITED LITERATURE

ALHANAFI F, Kaysi Y, Muna M, Alkhtib A, Wamatu J, Burton E. 2019. Spineless cactus (*Opuntia ficus-indica*) and saltbush (*Atriplex halimus* L.) as feed supplements for fattening Awassi male lambs: effect on digestibility, water consumption, blood metabolites, and growth performance. *Tropical Animal Health and Production.* 51:1637–1644. https://doi.org/10.1007/s11250-019-01858-6

ANKOM. 2018. RF Gas production system operator's manual. ANKOM Technology, USA. https://www.ankom.com/sites/default/files/document-files/RF_Manual.pdf

AOAC. 2010. Official Methods of Analysis. Association of Official Analytical Chemists International. Gaithersburg, Maryland. 18 ed. 3er. revisión. 2590 Pp. ISBN: 9780935584820

BERNAL J, Chaverra H, Arciniegas A, Acevedeo G, Angel M. 2002. Ensilaje, Heno y Henolaje. 1ª edición, Editorial Angel Comunicaciones. Bogotá, Colombia. ISBN: 978-958-44-1174-7

BERUMEN HL, Páez J, Soto NO, Murillo M, Herrera E, Muro A. 2015. Chemical composition, *in vitro* gas production and energy value of prickly pear fermented with and without *Kluyveromyces marxianus. Journal of BioScience and Biotechnology*. 4(3):359-364.
https://www.ingentaconnect.com/content/doaj/13146238/2015/00000004/00000003/art00014

BORSHCHEVSKAYA LN, Gordeeva TL, Kalinina AN, Sineokii SP. 2016. Spectrophotometric determination of lactic acid. *Journal of Analytical Chemistry.* 71:755–758. https://doi.org/10.1134/s1061934816080037

CASTRO-RINCÓN E, Sierra-Alarcón A, Mojica-Rodríguez J, Carulla-Fornaguera J, Lascano-Aguilar C. 2016. Uso múltiple de leguminosas como abono verde, en rotación con maíz, y heno, para producción de leche. *Corpoica Ciencia y Tecnología Agropecuaria*. 17(1):17-29. https://doi.org/10.21930/rcta.vol20_num3_art:1586

DA SILVA-BRITO GM, Santos EM, García G, Silva de Oliveira J, Zanine AM, Alexandre Fernandes-Perazzo A, Sena-Campos F, Vasconcelos de Oliveira AG, Cavalcanti HS. 2020. Mixed silages of cactus pear and gliricidia: chemical composition, fermentation characteristics, microbial population and aerobic stability. *Scientific Reports. Nature research*. 10:6834. https://doi.org/10.1038/s41598-020-63905-9

DUBEUX JCB, Ben Salem H, Nefzaoui AI. 2018. Producción y utilización del nopal forrajero en la nutrición animal. En: Ingles PC, Mondragon J, Nefzaoui A y Sáenz C. (ed.). Ecología del cultivo, manejo y usos del nopal. FAO-ICARDA. Pp. 229. ISBN 978-92-5-130494-5. http://www.fao.org/3/i7628es/I7628ES.pdf

FLORES-HERNÁNDEZ A, Araújo-Filho JT, Gomes da Silva F, Ramírez-Ordoñez S, Murillo-Amador B. 2017. Dietas a base de forraje tradicional y nopal (Opuntia spp.) enriquecido con proteínas para alimentar cabras. *Nova Scientia*. 9(18): 149-166. https://doi.org/10.21640/ns.v9i18.828

FLORES-HERNÁNDEZ A, Macías-Rodríguez FJ, Meza-Herrera C, García-Herrera G, Esquivel-Arriaga O, Ortiz-Salazar J, Hernández-Bautista C. 2019. Semi-solid fermentation of nopal (Opuntia spp) for use as an animal protein supplement. Revista de Geografía Agrícola. 63: 87-100. https://doi.org/10.5154/r.rga.2019.63.04

GALYEAN ML. 2010. Laboratory Procedures for Animal Nutrition Research, 14thedn. Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas. https://www.dpts.ttu.edu/afs/home/mgalyean/lab_man.pdf

GONZÁLEZ AA, Murillo OM, Pámanes CG, Reveles SF, Herrera TE. 2019. Nutritive quality and gas production of corn silage with the addition of fresh and fermented prickly pear cladodes. *Journal of Animal and Plant Sciences*. 40(1): 6544-6553. https://m.elewa.org/Journals/wpcontent/uploads/2019/04/4.Gonzalez.pdf

HA VU V, Li X, Wang M, Liu R, Zhang G, Liu W, Xia B, Sun Q. 2019. Dynamics of fungal community during silage fermentation of elephant grass (*Pennisetum purpureum*) produced in northern Vietnam. *Asian-Australasian Journal of Animal Science*. 32(7):996-1006. https://doi.org/10.5713/ajas.18.0708

HERREMANS S, Decruyenaere V, Beckers Y, Froidmont E. 2019. Aditivos de ensilaje para reducir la degradación de proteínas durante el ensilado y evaluación de la degradabilidad del nitrógeno ruminal *in vitro*. *Grass and forage sciences*. 74(1):86-96. https://doi.org/10.1111/gfs.12396

HERRERA TE, Murillo M, Berumen L, Soto-Cruz NO, Páez-Lerma JB. 2017. Protein enrichment of *Opuntia Ficus-indica* using *Kluyveromyces marxianus* in solid-state fermentation. *Ciencia e Investigación Agraria*. 44:113-120. https://doi.org/10.7764/rcia.v44i2.1767

KUNG L, Shaver RD, Grant, RJ, Schmidt RJ. 2017. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. *Journal of Dairy Science.* 101: 4020-4033. https://doi.org/10.3168/jds.2017-13909

LÓPEZ-HERRERA M, Jones RWC, Rojas-Bourrillón A, Rodríguez-Chacón S. 2014. Valor nutricional del ensilaje de rastrojo de piña con niveles crecientes de urea. *Nutrición Animal Tropical*. 8(1): 1-20. https://revistas.ucr.ac.cr/index.php/nutrianimal/article/view/14989

LÓPEZ-INZUNZA HJ, Chongo-García BB, O-León OL, Guerra-Liera JE, Luna-López M, Castro-Camacho SJ, López-Juárez LA. 2017. Digestibilidad in situ de rastrojo de maíz tratado con enzimas fibrolíticas. *Revista Ciencia y Agricultura*. 14(1): 31-37. http://www.doi.org/10.19053/01228420.v14.n1.2017.6085

MCDONALD P, Edwards RA, Greenhalgh JF. 2002. Animal Nutrition. 6th Edition. Longman, London and New York. Pp. 543. ISBN-10:9781408204238

MENKE KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development.* 28:7–55. https://www.scienceopen.com/document?vid=e1859372-e696-424a-85fb-d305b0b594bc

MOKOBOKI K, Sebola N, Matlabe G. 2016. Efecto de los niveles de melaza y las condiciones de crecimiento sobre el valor nutritivo y la calidad de fermentación de ensilaje de cladodios de Opuntia. *Journal of Animal Plant Science*. 28: 4488-4495. https://doi.org/10.4141/cjas2013-188

MURILLO OM, Herrera TE, Corral LA, Pámanes CG. 2018. Effect of inclusion of graded level of water hyacinth on *in vitro* gas production kinetics and chemical composition of alfalfa hay based beef cattle diets. Indian *Journal of Animal and Research*. 52(8): 1298-1303. https://doi.org/10.18805/ijar.11417

NARANJO JF, Ceballos OA, Gaviria X, Tarazona AM, Correa A, Chará JD, Murgueitio E, Barahona R. 2016. Estudio de la cinética fermentativa in vitro de mezclas de forrajes que incluyen *Leucaena leucocephala* proveniente de sistemas silvopastoriles intensivos (SSPi) en Colombia. *Revista CES Medicina Veterinaria y Zootecnia*. 11(2): 6-17. https://doi.org/10.21615/cesmvz.11.2.1

ORONA-CASTILLO I, Flores-Hernández A, Rivera GM. 2008. Manual para el establecimiento y manejo del nopal verdura bajo riego por goteo en la Comarca Lagunera. CENID RASPAINIFAP, Gómez Palacio, Durango, México. Pp.18. http://biblioteca2.uaaan.mx/cgi-bin/koha/opac-detail.pl?biblionumber=4441

PINEDA-CORDERO L, Chacón-Hernández P, Boschini-Figueroa C. 2016. Evaluación de la calidad del ensilado de pasto estrella africana (*Cynodon nlemfluensis)* mezclado con tres diferentes aditivos. *Agronomía Costarricense*. 40(1) 1:11-27. http://www.redalyc.org/articulo.oa?id=43646210001

PINHO RM, Santos EM, Oliveira, JS, Loureiro, AHR, Macêdo, AJS, Alves JP, Santos AP, Santos VS. 2017. Effect of spineless-cactus mucilage on the in vitro rumen fermentation of cellulose, starch, and protein. *Revista Brasileira Saúde e Produção Animal.* 18(4):505-517. https://doi.org/10.1590/s1519-99402017000400002

RODRÍGUEZ R, Sosa A, Rodríguez Y. 2007. Microbial protein synthesis in rumen and its importance to ruminants. *Cuban Journal of Agricultural Science*. 41(4): 287-294. https://www.researchgate.net/profile/Rafael-Rodriguez-10/publication/276901672_Microbial_protein_synthesis_in_rumen_and_its_importance_to_ruminants/links/555b266908aeaaff3bfbcad0/Microbial-protein-synthesis-in-rumen-and-its-importance-to-ruminants.pdf

SAGARPA. 2009. Aprovechamiento de esquilmos y subproductos en la alimentación de ganado. [Consulta: 2 de Marzo de 2021]. Disponible en: https://www.academia.edu/19017564/Aprovechamiento_de_esquilmos

SÁNCHEZ-DUARTE, Garcia A. 2017. Ammonia-N concentration in alfalfa silage and its effects on dairy cow performance: A meta-analysis. *Revista Colombiana de Ciencias Pecuarias*. 30(3):175-184. https://doi.org/10.17533/udea.rccp.v30n3a01

SANCHEZ PH, Tracey LN, Browne-Silva J, Lodge-Ivey SL. 2014. Propionibacterium acidipropionici P169 and glucogenic precursors improve rumen fermentation of low-quality forage in beef cattle. *Journal of Animal Science*. 92:1738-1746. https://doi.org/10.2527/jas.2013-7148

SAS Institute. 2010. Statistical Analysis Software SAS/STAT®. version 9.0.2, Cary, N.C., USA: SAS Institute Inc., ISBN: 978-1-60764-599-3. http://www.sas.com/en_us/software/analytics/stat.html#

TAVENDALE MH, Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S. 2005. Methane production from in vitro rumen incubations with *Lotus pedunculatus* and

Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. *Animal Feed Science and Technology*.123-124(1): 403-419. https://doi.org/10.1016/j.anifeedsci.2005.04.037

VAN MARKIS AJA, Abbot DA Bellissimi E. 2006. Alcoholic fermentation of carbon sources in biomass hy-drolysates by *Saccharomyces cerevisiae:* current status. *Antonie Van Leewenhoek*. 90: 391-418. https://doi.org/10.1007/s10482-006-9085-7

VAN SOEST PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition: carbohydrate methodology, metabolism and nutritional implications in dairy cattle. *Journal of Dairy Science*. 74:35-83. https://doi.org/10.3168/jds.s0022-0302(91)78551-2

VAN SOEST PJ. 1994. Nutritional ecology of the ruminant. 2nd Edition, Cornell University Press, Ithaca, USA. Pp. 476. ISBN: 080142772X.