IN VITRO METHANE PRODUCTION AND *IN SITU* DEGRADABILITY OF PRICKLY PEAR PRETREATED WITH YEAST CULTURES †

[PRODUCCION DE METANO *IN VITRO* Y DEGRADABILIDAD *IN SITU* DE PENCAS DE NOPAL FORRAJERO PRETRATADO CON UN CULTIVO DE LEVADURAS]

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SUMMARY

Background. Natural cycles and increases in temperature and droughts worldwide are being caused by the climate change effect. In addition, greenhouse gases (GHG) produced by anthropogenic causes are released to atmosphere. In this way, ruminants contribute with 30% of the total methane produced, whereas in Mexico this value represents over 50%. Since droughts are more frequent, production of forage sources which require more water are decreasing alarmingly. Due to the latter, researchers are looking for alternative forages which may alleviate this issue; *Opuntia ficus-indica* arises as a good forage source. **Objective.** To evaluate the effect of the solid state fermentation (SSF) of prickly pear with yeast cultures on *in vitro* gas production, ruminal fermentation parameters and *in situ* degradability. **Methodology.** Three experimental treatments were evaluated: solely prickly pear without previous SSF with yeast cultures (PP) as a control; PP+SC, previously fermented with *Saccharomyces cerevisiae*; and PP+KM, previously fermented with *Kluyveromyces marxianus ITD00262*. Treatments were fermented with ruminal liquor and dry matter and protein degradabilities, as well as ruminal fermentation parameters and gas production kinetics were measured. **Results.** The results obtained showed that total degradability of DM increased due to yeast pretreatment ($p<0.05$). Crude protein degradability and total VFA decreased with PP+SC; however, PP+SC increased total gas production ($p<0.05$). Otherwise, PP+KM increased propionate production and decreased methane and CO_2 production ($p<0.05$). **Implications.** The information presented in this research is focused in the use of PP pretreated with yeast strains in order to increase the nutritional value by increasing its protein content. In addition, the presence of yeast cultures in feedstuffs contributes to decrease the methane synthesis in rumen. Additionally, the use of PP in arid and semiarid zones may increase the water availability for ruminants. **Conclusion:** The use of PP pretreated with strains of *Kluyveromyces marxianus* into livestock feeding increases the protein availability and contributes to a reduction in the *in vitro* methane synthesis in 23%.

Keywords: *Saccharomyces cerevisiae*; *Kluyveromyces marxianus*; gas production kinetics; solid state fermentation.

RESUMEN

Antecedentes. Los ciclos naturales y los incrementos mundiales en la temperatura y en las sequías son causados por el efecto del cambio climático. Además, los gases de efecto invernadero (GEI) producidos por causas antropogénicas son liberados a la atmósfera. En este sentido, los rumiantes contribuyen con el 30% del total de metano producido, mientras que en México este valor alcanza el 50%. Debido a que las sequías son más frecuentes, la producción de forrajes que requieren demasiada agua para su crecimiento están disminuyendo alarmantemente. Debido a lo anterior, investigadores están buscando alternativas forrajeras que puedan mitigar este problema; *Opuntia ficus-indica* emerge como una buena fuente de forraje. **Objetivo.** Evaluar el efecto de la fermentación en estado sólido (SSF) de pencas de

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nopal con cultivos de levaduras en la producción de gas *in vitro*, parámetros de fermentación ruminal y degradabilidad *in situ*. **Metodología.** Tres tratamientos experimentales fueron evaluados: pencas de nopal sin SSF con cultivos de levaduras (PP) como testigo; pencas de nopal pretratado con cepas de *Saccharomyces cerevisiae* (PP+SC); y pencas de nopal pretratado con cepas de *Kluyveromyces marxianus ITD00262* (PP+KM). Los tratamientos fueron fermentados en líquido ruminal y se midieron las degradabilidades de la materia seca y proteína, así como los parámetros de la fermentación ruminal y la cinética de producción de gas. **Resultados.** Los resultados obtenidos muestran que la degradabilidad total de la MS se incrementó debido al pretratamiento con levaduras ($p<0.05$). La degradabilidad de la proteína cruda y los ácidos grasos volátiles totales disminuyeron con PP+SC; sin embargo, PP+SC incrementó la producción total de gas ($p<0.05$). Por otro lado, PP+KM incrementó la producción de propionato y disminuyó las producciones de metano y CO_2 ($p<0.05$). **Implicaciones.** La información presentada en esta investigación se enfoca en el uso de pencas de nopal pretratado con cepas de levaduras para incrementar su valor nutricional mediante un incremento en el contenido de proteína. Además, la presencia de levaduras en el forraje contribuye a reducir la síntesis de metano ruminal. Adicionalmente, el uso de pencas de nopal en zonas áridas y semiáridas pueden incrementar la disponibilidad de agua para los rumiantes. **Conclusiones:** El uso de pencas de nopal pretratado con cultivos de *Kluyveromyces marxianus* en la alimentación de ganado incrementa la disponibilidad de proteína y contribuye a la reducción de la síntesis de metano ruminal in vitro en un 23%.

Palabras clave: *Saccharomyces cerevisiae*; *Kluyveromyces marxianus*; cinéticas de producción de gas; fermentación en estado sólido.

INTRODUCTION

Nowadays, North America as well as different areas worldwide are experiencing an increase in the drought seasons due to the climate change (CC) effects (Seager and Hoerling, 2014). The CC effect is caused by the accumulation of greenhouse gases (GHG) in the atmosphere; CO_2 and methane are the main responsible gases of this effect. Moreover, the livestock activity contributes to the GHG emissions providing almost 30% of the total methane anthropogenic emissions to atmosphere, whereas in Mexico contributes with 50% (Hernández-Medrano, 2018). Additionally, the increasing worldwide population is demanding for feed including protein from animal sources as beef and milk; thus, there is a substantial augmentation of the feeding costs for cattle breeders who exploit the ruminants' ability to convert roughages into edible products for human feeding and costs for human feeding acquisition are increasing as well (Ben-Salem and Smith, 2008). In this way, small cattle breeders are the main affected population. In addition, droughts originated due to the CC affect the water accessibility for cattle, affecting their growing development.

Otherwise, prickly pear (*Opuntia ficus-indica*) is a natural source which may help in the alleviation of the lack of water access for cattle; the water content in prickly pear is approximately 90%. In addition, prickly pear possesses a high efficiency in the use of water when compared to other annual crops. Moreover, prickly pear presents a higher content in carbohydrates and calcium, besides an elevated effectiveness in converting water into dry matter and digestible energy which is highly desired for cattle breeders (Flores and Reveles, 2010). Nevertheless, its low protein content (approximately 4%, DM) limits the use of prickly pear as a nutrients source in animal feeding. In this way, researchers are developing diverse alternatives as a way of abating its low content of protein by pretreating the cladodes with yeast cultures through a solid-state fermentation (SSF) process. This biotechnology use the proliferation of yeast cultures attached to the cladodes through a fermentative process which increases the protein content in cladodes (Herrera *et al*., 2017). Additionally, previous research encourages the use of yeast isolated from the alcoholic fermentation (*Kluyveromyces marxianus* ITD00262) as a strain that degrades pectins and other structural carbohydrates (Páez *et al*., 2013); nevertheless, this strain has only been used for wine production research. Furthermore, Histrov *et al*. (2010) observed that a direct supplementation with yeast strains of *Saccharomyces cerevisiae* slightly decreased methane production by affecting ruminal fermentation in dairy cows. However, livestock performance and production may be reduced by affecting the ruminal fermentation; the latter will hardly be accepted by cattle breeders. To avoid this, diverse ruminal fermentation parameters should be measured in order to determine if production could be compromised when trying alternative feedstuffs. Therefore, the aim of this research was to evaluate the effect of prickly pear pretreated with yeast cultures on *in vitro* gas production, ruminal fermentation parameters and *in situ* degradability.

MATERIALS AND METHODS

Prickly pear and yeast cultures acquisition

Prickly pear cladodes variety AV6 (*Opuntia ficus-indica*) were randomly harvested in September 2019 from a crop located in Durango, México (Latitude 24°1.2192′ N and Longitude 104°39.4536′ W and an elevation above the sea level of 1884 m). *Saccharomyces cerevisiae* cultures were acquired from a local store, whereas *Kluyveromyces marxianus* ITD00262 cultures were provided by the cultures

reservoir at the Durango's Institute of Technology (Durango, Mexico).

Solid State Fermentation (SSF) as pretreatment for prickly pear cladodes

The harvested prickly pear cladodes were cut into small pieces using a sharp knife and collected as a pool sample for further analyses. Solely fresh prickly pear without SSF with yeast cultures was used as a control treatment (PP). Prickly pear cladodes which were subjected to SSF were placed into 19 L plastic containers and were inoculated (1% w/w) with *Sacharomyces cerevisiae* for PP+SC treatment and with *Kluyveromyces marxianus* ITD00262 for PP+KM treatment. Fermentations for each yeast culture were carried out in triplicate. The fermentation process was carried out at room temperature (25ºC) during 48 h for PP+SC and during 144 h for PP+KM according to Herrera *et al*. (2017).

Chemical analysis

Afterwards the SSF process, experimental treatments were dried in a forced-air oven at 55°C for 72 h and ground to 1 mm particles in a Wiley mill (Arthur H Thomas, Philadelphia, PA, USA) for the determination of DM; the crude protein (CP) content was calculated by determining the total nitrogen (N) content, using the micro-Kjeldhal technique and a fixed conversion factor (6.25) (AOAC, 1994). The NDF and ADF concentration were determined following procedures proposed by Van Soest *et al*. (1991).

***In situ* protein and dry matter degradability**

Approximately 10 g (DM) of experimental treatments were placed into nylon bags and sealed; bags were previously weighed and labeled. Afterwards, samples were introduced into two crossbreed steers provided with rumen cannula and left them there for different times (0, 3, 6, 9, 12, 18, 24, 48, 72 and 96h) in triplicate. Once the time was elapsed, nylon bags were retrieved and washed with tap water until all rumen particles were removed. Dry matter digestibility for each time was determined as the difference between the bags weight before and after the fermentation process. Later, each remaining sample was subjected of protein analysis as proposed by AOAC (1994). Data for dry matter and protein were fitted into the Orskov and McDonald model (Orskov and McDonald, 1979) to describe the kinetics parameters as follows:

$$Deg(t)= A+B(1-exp(-c*(t)))$$

Where "Deg(t)" is the degradability at time t (%), "A" is the intercept of degradation at time zero (%), "B" is the potential degradability which will be reached at time t (%) and "c" is the constant rate of degradability (%/h).

***In vitro* gas production and fermentation parameters**

Approximately 1 g (DM) of each experimental treatment was placed into glass modules equipped with electronic transducers for pressure measuring according to manufacturer's procedures (ANKOM, USA) and incubated in triplicate with buffer solutions and ruminal inoculum in a 2:1 ratio, according to Theodorou *et al*. (1994). Incubations were performed from 0 to 96h registering the pressure measure in the meantime. Obtained data were fitted into the Orskov and McDonald model, according to the following equation adapted for gas production:

$$GP(t)= A+B(1-exp(-c*(t)))$$

Where "GP" is the gas production at time t (mL), A=the intercept of gas production curve at time zero; B= the potential gas which will, in time, be produced; c= the rate constant for the gas production of "B". Likewise, the same number of each experimental treatment was incubated in ANKOM glass modules until 24h of fermentation time (Theodorou *et al*., 1994). Once the time was elapsed, modules pressure release valve was opened and released gas was measured for methane and CO_2 compositions according to procedures proposed by Gónzalez-Arreola *et al*. (2019) using the portable gas analyzer GEM5000 (Landtec, USA). Afterwards, glass modules were fully opened and pH was measured immediately; later, an aliquote of ruminal liquor of each fermentation process was used for further analyses of volatile fatty acids (VFA) and nitrogen-ammonia (N-NH_3) according to Galyean (2010).

Statistical analysis

Obtained data for chemical composition, in situ dry matter and protein degradability as well as in vitro gas production kinetics were analyzed as a completely randomized design using the GLM procedures of SAS (2002). Means comparison was performed with the Tukey's test declaring significant differences at $p<0.05$.

RESULTS

Chemical composition

Chemical composition of experimental treatments is presented in Table 1. Dry matter was different among treatments ($p=0.0325$). Likewise, CP was different among treatments ($p=0.0001$). In fact, CP increased 69 and 130% when fermenting PP+SC and PP+KM in a SSF process, respectively. Additionally, NDF and

ADF increased with SSF process ($p<0.05$). Nevertheless, lignin decreased about 55% in PP+SC.

In situ dry matter and protein degradability

Results in Table 2 show significant differences on *in situ* dry matter digestibility kinetics ($p<0.05$). Soluble fraction (A parameter) increased with PP+SC and PP+KM; on the contrary, potentially degradable fraction (B parameter) decreased. Otherwise, no changes were observed in the constant rate of degradability (c parameter) among treatments ($p=0.3495$). In addition, total degradability increased with PP+SC ($p=0.0108$).

Likewise, *in situ* protein degradability kinetics of the experimental treatments is presented in Table 2. On the contrary to the trends observed in dry matter digestibility the opposite effects are observed in protein degradability. Soluble protein fraction and total degradability were reduced with PP+SC whereas potentially degradable protein fraction increased ($p<0.05$). In addition, the constant rate of the protein degradability decreased as well with PP+SC ($p=0.0169$). Moreover, protein potentially degradable fraction increased when fermenting PP+SC ($p=0.0126$).

In vitro gas production kinetics and ruminal fermentation parameters

Table 3 shows the *in vitro gas* production kinetics and the ruminal fermentation parameters of the experimental samples. As observed, there was a reduction in the intercept of gas production curve at time zero with PP+KM ($p=0.0487$). Likewise, the total gas production (A+B fraction) was different among treatments ($p=0.0432$). However, this reduction did not affect the potentially fraction of the gas production (B fraction) which is similar to that obtained with PP ($p=0.0608$). Moreover, constant rate of gas production was not affected by yeast fermentation among the experimental treatments ($p=0.2328$).

Ruminal fermentative parameters, VFA, methane and CO_2 production after 24 h of fermentation are presented in Table 4. As observed, there were no changes in pH among treatments ($p=0.6799$). Nevertheless, nitrogen ammonia increased with PP+SC and PP+KM ($p=0.0441$). Concentration of acetic, propionic and butyric acids, as well as total volatile fatty acids (TVFA) are similar in PP+KM compared to PP ($p>0.05$). However, a reduction in acetic acid and TVFA is observed in PP+SC when compared to PP ($p=0.0001$). Additionally, propionic and butyric acids increased with PP+SC ($p<0.05$).

On the other hand, methane and CO_2 production decreased 23 and 29%, respectively with PP+KM ($p=0.0001$). Conversely, PP+SC presented similar values to those obtained with PP ($p>0.05$). Otherwise, CO_2:CH_4 ratio indicates that PP+KM remains 8.3 mL by each 1 mL of methane produced; whereas, remained CO_2 in PP and PP+SC are 7.8 and 7.6 per mL of methane produced, respectively.

DISCUSSION

Chemical composition

The increase in the protein fraction in both experimental treatments (PP+SC and PP+KM) is a result of the protein provided by the proliferation of yeast cultures used in the pretreatment of cladodes through the SSF process (Herrera *et al.*, 2017). In addition, SSF contributed to increase the structural carbohydrates. However, only pretreatment with

Table 1. Chemical composition of experimental treatments.

Nutrients (%)	PP	PP+SC	PP+KM	SEM	p
Dry Matter	9.9±0.15^{b}	11.0±0.23^{a}	9.8±0.73^{b}	0.37	0.0325
Crude Protein	5.3±0.16^{c}	9.0±0.28^{b}	12.2±0.07^{a}	0.14	0.0001
NDF	38.2±0.17^{c}	55.5±0.57^{a}	45.4±2.70^{b}	1.44	0.0001
ADF	13.0±0.22^{b}	18.3±0.33^{a}	19.2±1.21^{a}	0.72	0.0002
Lignin	3.8±0.08^{a}	1.7±0.05^{b}	3.7±0.08^{a}	0.06	0.0001

^{abc}Means with different letters within same row are statistically different ($p<0.05$); DM=dry matter; CP=crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; SEM=Standard error of difference between means.

Table 2. ***In situ*** **dry matter and protein degradability kinetics of experimental treatments.**

Parameters	PP	PP+SC	PP+KM	SEM	p
In situ **dry matter degradability**					
A (%)	49.9±0.05[c]	54.6±0.10[b]	57.5±0.24[a]	0.26	0.0001
B (%)	42.1±0.17[a]	39.91±0.17[b]	34.6±0.53[c]	0.58	0.0012
A+B (%)	92.0±0.22[b]	94.6±0.27[a]	92.1±0.29[b]	0.46	0.0108
C (%/h)	0.05±0.003	0.04±0.000	0.05±0.004	0.005	0.3495
In situ **protein degradability**					
a (%)	59.12±2.11[a]	43.75±4.826[b]	68.91±0.70[a]	3.06	0.0033
b (%)	29.98±2.17[ab]	37.75±4.24[a]	19.7±1.39[b]	2.87	0.0126
a+b (%)	89.1±0.3771[a]	81.5±0.64[b]	88.6±0.92[a]	0.68	0.0004
c (%/h)	0.065±0.0102[ab]	0.04±0.012[b]	0.10±0.005[a]	0.009	0.0169

[ab]Means with different letters within same row are statistically different ($p<0.05$); A=the intercept of degradation curve at time zero; B= the potential degradability which will, in time, be degraded; C= constant rate for the degradation of "B"; a=the intercept of protein degradation curve at time zero; b= the potential degradability of protein which will, in time, be degraded; c= constant rate for the protein degradation of "b"; SEM=Standard error of difference between means.

Table 3. ***In vitro*** **gas production kinetics of experimental treatments.**

Parameters	PP	PP+SC	PP+KM	SEM	p
A (mL/g)	61.7±1.26[a]	52.5±4.46[a]	20.8±7.62[b]	8.93	0.0487
B (mL/g)	116.8±1.50	149.1±26.45	128.2±0.75	26.50	0.0608
A+B (mL/g)	178.5±0.23[b]	201.7±21.9[a]	149.1±8.37[b]	23.52	0.0432
c (%/h)	0.13±0.005	0.11±0.022	0.09±0.002	0.02	0.2328

[ab]Means with different letters within same row are statistically different ($p<0.05$); A=the intercept of gas production curve at time zero; DM=dry matter; B= the potential gas which will, in time, be produced; c= the rate constant for the gas production of "B"; SEM=Standard error of difference between means.

K. marxianus increased the lignin in PP+KM. Apparently, *S. cerevisiae* contributes to the degradation of lignin instead of the NDF fraction in prickly pear. In fact, Herrera *et al.* (2014) attributed degradation in the lignin fractions to an increase of lignin-cellulolytic enzymes generated during the fermentation process due to the presence of *S. cerevisiae*.

Table 4. *In vitro* methane and CO_2 production and fermentation parameters of experimental treatments.

Parameters	PP	PP+SC	PP+KM	SEM	p
pH	6.95±0.015	6.96±0.005	6.99±0.005	0.009	0.6799
$N\text{-}NH_3$	4.56±0.556^{b}	6.71±0.574 ^{a}	5.41±0.272^{a,b}	0.487	0.0441
Acetic acid (%)	52.0±0.02 ^{a}	47.5±0.10^{b}	53.7±1.07^{a}	0.61	0.0001
Propionic acid (%)	30.8±0.08^{b}	33.3±0.36^{a}	28.7±0.59^{b}	0.40	0.0110
Butyric acid (%)	12.7±0.11 ^{b}	14.2±0.12^{a}	12.4±0.29^{b}	0.19	0.0182
TVFA (mM)	94.8±1.09^{a,b}	84.3±1.85^{b}	111.3±4.72 ^{a}	2.99	0.0148
A:P ratio	1.6±0.00^{a,b}	1.4±0.01^{b}	1.8±0.07 ^{a}	0.04	0.0001
CH_4 (mL/g)	19.2±0.5383 ^{a}	19.0±0.086 ^{a}	14.82±0.146^{b}	0.32	0.0001
CO_2 (mL/g)	151.3±2.24^{a}	145.6±0.86 ^{a}	123.18±1.10 ^{b}	1.52	0.0001
CO_2:CH_4 ratio	7.8±0.10^{a,b}	7.6±0.01 ^{b}	8.31±0.15^{a}	0.10	0.0009

^{ab}Means with different letters within same row are statistically different ($p<0.05$); GP_{24}= gas production at 24h of fermentation process; TVFA: total volatile fatty acids; A:P ratio= acetate:propionate ratio; SEM= Standard error of difference between means.

In situ dry matter and protein degradability

The presence of yeast cultures promotes the proliferation of cellulolytic bacteria (Khadem *et al*., 2007). Despite of no changes were observed in the constant rate of degradability (C parameter) among experimental treatments, the SSF process used as pretreatment improved degradation of fiber in the ruminal fermentative process. The latter is confirmed by augmentations in the soluble fraction (parameter A) and reductions in the potentially soluble fraction (B parameter). Unlike the effects observed with the degradability of dry matter, the degradability of protein showed the opposite effect. As observed, there is a decrease in the protein soluble fraction when cladodes were pretreated with *S. cerevisiae* in PP+SC. Results in chemical composition of experimental treatments indicate that lignin fraction in PP+SC was lower than the other experimental treatments. This effect allowed an increase in the degradability in PP+SC suggesting a reduction in the lignin-carbohydrate complexes. However, an opposite effect was observed with protein degradability fractions. Apparently, non-degraded lignin in PP+SC formed complexes with protein, avoiding its total degradation. Accordingly, the constant rate of the protein degradability supports this theory by reducing its values due to a presence of lignin-protein complexes which limit the activity of proteolytic bacteria. In this way, Stevanic and Salmen (2008) reported lignin-protein and lignin-pectin interactions within the primary cell wall, suggesting that ruminal microorganisms are more likely to degrade simple carbohydrates instead of complex proteins which demand a higher energy consumption (Van Soest *et al*., 1991).

In vitro gas production kinetics and ruminal fermentation parameters

This study reported reduction in the intercept of the gas production curve at time zero when cladodes were pretreated with *K. marxianus* in PP+KM. This reduction may be attributable to a higher content in the hemicellulose and lignin fractions (ADF) in PP+KM. However, these augmentations showed no effect on the other kinetics variables (constant rate of gas production and maximum gas production). Likewise, no changes in pH were attributable to the presence of yeast cultures in PP+SC and PP+KM. Nevertheless, as proposed by Hristov and Jouany (2005), observed changes in nitrogen ammonia among experimental treatments are attributed to a higher proteolysis which

is directly related to higher contents of protein. These results agree with previous reports by Herrera *et al*. (2017). These authors increased yeast cultures presence in the prickly pear due to a SSF process. Moreover, a reduced nitrogen-ammonia in PP+KM could be attributable to the intake of ammonia as nitrogen source in the biomass production of *K. marxianus* which is not possible with *S. cerevisiae* (Löser *et al*., 2015). In addition, pretreatment with *S. cerevisiae* cultures in PP+SC enhances a better energy utilization due to a reduction in the acetic acid concentration and a higher production of propionic acid. In fact, a reduction in the A:P ratio supports this theory. However, these changes were observed in individual proportions; TVFA was reduced with PP+SC, whereas PP+KM presented a similar production in TVFA than that obtained in control (PP). This effect suggests that PP+SC inhibits VFA production somehow.

On the other hand, reduction in methane synthesis in PP+KM cannot be associated as a consequence of a reduction in the total gas production since PP and PP+KM presented similar values. Additionally, there are previous studies which affirmed that increases in propionate production affects directly methane synthesis in ruminal fermentation since both compounds synthesis are natural sinks for hydrogen ions (Ferraro *et al*., 2009). Nevertheless, this effect is not observed with PP+KM. Otherwise, propionic acid production was lower than the produced with PP+SC when PP+KM was fermented. In fact, *K. marxianus* cells present the ability to use urea and ammonia as nitrogen sources for growth. However, a two-step process in the presence of hydrogen is needed in order to metabolize urea (Löser et al., 2015). Because of the latter, compounds considered as hydrogen sinks in the rumen would be affected. In this way, propionic acid and methane would be reduced since less hydrogen ions are available for their synthesis due to urea metabolization. Moreover, previous research has shown that viability in cells of *S. cerevisiae* is affected by the presence of CO_2; this affection is not observed in *K. marxianus* cells (Isenschmid *et al*., 1995). This variable indicates the volume (mL) of CO_2 remaining by each mL of methane produced; the higher the value of this variable then the lower production of methane through the CO_2-reduction pathway (Murillo *et al*., 2018). Thereby, the latter is highly desired when testing alternative sustainable forages as feedstuffs which may contribute to the CC mitigation through reducing the gases emissions in livestock.

CONCLUSION

The results observed in this study showed that the protein provided by *Kluyveromyces marxianus ITD00262* in a SSF, is higher and more bioavailable. In addition, ruminal fermentative parameters like N-NH_3, VFA and *in vitro* gas production kinetics are similar and comparable with those presented for solely prickly pear (without pretreatment). Otherwise, greenhouse gases emissions like methane and carbon dioxide were lower for PP+KM than for solely prickly pear. Therefore, the use of PP pretreated with strains of *Kluyveromyces marxianus* into livestock feeding increases the protein availability and contributes to a reduction in the *in vitro* methane synthesis in 23%.

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Conflict of interest. The authors declare that there is no conflict of interest in the presented research and data.

Compliance with ethical standards. The present research followed the ethical standards declared by the Committee for the Foment and Animal Protection of Durango State.

Data availability. Data are available with Gerardo Pámanes-Carrasco (gerardo.pamanes@gmail.com) upon reasonable request.

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