Methane production and nutritional content from the diet consumed by grazing cattle

Producción de metano y contenido nutricional de la dieta consumida por ganado bovino en pastoreo

Produção de metano e conteúdo nutricional a partir da dieta consumida por bovinos pastando em pastagem

Elizabeth Yazmin García Piña[1]
Esperanza Herrera Torres[2]
Manuel Murillo Ortíz[3]
Rafael Jiménez Ocampo[4]
Daniel Sierra Franco[3]
Gerardo Antonio Pámanes Carrasco[5]*

Rev. Fac. Agron. (LUZ). 2025, 42(1): e254203
ISSN 2477-9407
DOI: https://doi.org/10.47280/ RevFacAgron(LUZ).v42.n1.III

Animal production

Associate editor: Professor Juan Vergara-López
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela

[1]CONAHCYT. Universidad Juárez del Estado de Durango, Programa Institucional de Doctorado en Ciencias Agropecuarias y Forestales, Río Papaloapan y Boulevard Durango. Col. Valle del Sur. C.P. 34120, Durango, Dgo., Mexico.
[2]Instituto Tecnológico del Valle de Guadiana, México km 45, 34323, Villa Montemorelos, Dgo., Mexico.
[3]Universidad Juárez del Estado de Durango, Facultad de Veterinaria y Zootecnia, Mezquital km 11.5, Durango, Dgo., Mexico.
[4]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias-INIFAP, Campo Experimental Valle del Guadiana, Kilómetro 4.5 Carretera Durango-El Mezquital, Durango, Dgo. C.P. 34170, Mexico.
[5]CONAHCYT. Universidad Juárez del Estado de Durango, Instituto de Silvicultura e Industrias de la Madera, Del Guadiana 501, SAHR, C.P. 34104 Durango, Dgo., Mexico.

Received: 25-10-2024
Accepted: 11-11-2024
Published: 21-12-2024

Keywords:
Gas production kinetics
Nutritional quality
Ruminal fermentation

Abstract

It is a high priority to account for methane emissions from cattle grazing grasslands in order to evaluate the strategies for mitigating GHG emissions in livestock. The aim of this study was to evaluate *in vitro* ruminal methane production and nutritional content of the consumed diet by bovines grazing an open medium grassland in atypical dry and rainy periods in the semi-arid region of the state of Durango, Mexico. Four rumen fistulated bovines were subjected of an *ad libitum* graze under a repeated measure design. Chemical analysis showed that DM, OM, NDF and ADF increased in rainy period ($p<0.05$). Otherwise, CP, EE, phosphorus contains and IVDMD increased in dry period ($p<0.05$). Ruminal fermentation parameters as pH and volatile fatty acids as acetic, propionic and butyric, showed no changes among periods ($p>0.05$). However, ammonia increased in rainy period ($p<0.05$). Moreover, gas production kinetics only showed differences in lag phase ($p<0.05$); whereas, maximum gas production and production constant rate showed no changes among periods ($p>0.05$). Likewise, methane production showed no changes among both periods ($p<0.05$). As conclusion, it is observed that nutritional quality of the consumed diet by bovines grazing and open medium grasslands in dry and rainy periods presents acceptable protein values (8-10 %). In addition, phosphorus contents are higher than the minimum requirements for growing bovines. Furthermore, methane production was not affected by dry and rainy periods.

*Corresponding author:gerardo.pamanes@gmail.com

Resumen

Es prioritario contabilizar las emisiones de metano de ganado en libre pastoreo para evaluar de manera puntual las estrategias en la mitigación de GEI en sistemas pecuarios. El objetivo de este estudio fue evaluar la producción de metano ruminal *in vitro* y contenido nutricional de la dieta consumida por bovinos en pastoreo en un pastizal mediano abierto en épocas atípicas de secas y de lluvias en la región semiárida del estado de Durango, México. Se utilizaron 4 bovinos fistulados de rumen que fueron sujetos a pastorear un pastizal mediano abierto bajo un diseño experimental de mediciones repetidas en el tiempo donde el periodo de la época fue incluido como un efecto de repetición. La MS, MO, FDN y FDA se incrementaron en época de lluvias (p<0,05). Por el contrario, en época seca se incrementó la PC, EE, P y IVDMD (p<0,05). Por su parte, el pH, los ácidos grasos volátiles y la producción de metano no cambiaron entre épocas (p>0,05). No obstante, el N-NH_3 aumentó en época de lluvias. Además, la cinética de producción de gas sólo mostró cambios en la fase de latencia (p<0,05), más no en la producción máxima de gas ni la tasa constante de producción (p>0,05). La calidad nutricional de la dieta consumida por ganado en pastoreo en época seca y lluvias presenta valores de proteína aceptables (8-10 %). Además, los contenidos de fósforo sobrepasan los requerimientos necesarios para bovinos de carne en crecimiento. Por su parte, la producción de metano no se afectó por las épocas evaluadas.

Palabras clave: calidad nutricional, cinética de producción de gas, fermentación ruminal

Resumo

É de alta prioridade contabilizar as emissões de metano provenientes de bovinos em pastagens livres para avaliar as estratégias de mitigação das emissões de gases de efeito estufa (GEE) em sistemas pecuários. O objetivo deste estudo foi avaliar a produção de metano e conteúdo nutricional a partir da dieta consumida por bovinos em uma pastagem aberta de gramíneas médias, em períodos atípicos de seca e chuva na região semiárida do estado de Durango, Mexico. Foram utilizados quatro bovinos fistulados no rúmen, submetidos ao pastejo em uma pastagem aberta de gramíneas médias, em um delineamento experimental de medições repetidas ao longo do tempo, no qual o período da estação foi considerado como um efeito de repetição. Os teores de MS, MO, FDN e FDA aumentaram no período chuvoso (p<0,05). Por outro lado, no período de seca, houve aumento nos teores de PB, EE, P e IVDMD (p<0,05). Os parâmetros de fermentação ruminal como opH, os ácidos graxos voláteis e a produção de metano não apresentaram alterações significativas entre os períodos (p>0,05). Entretanto, o N-NH_3 aumentou durante o período chuvoso. Ademais, a cinética de produção de gases apresentou diferenças apenas na fase de latência (p<0,05), enquanto a produção máxima de gás e a taxa constante de produção não mostraram alterações entre os períodos (p>0,05). A qualidade nutricional da dieta consumida pelo gado em pastagem livre, tanto na estação seca quanto na chuvosa, apresentou teores de proteína aceitáveis (8-10 %). Além disso, os níveis de fósforo estão acima dos requisitos necessários para bovinos de corte em crescimento. Por sua vez, a produção de metano não foi influenciada pelas variações sazonais entre seca e chuva.

Palavras-chave: qualidade nutricional, cinética de produção de gás, fermentação ruminal.

Introduction

Livestock farming in Mexico is one of the main economic activities, developed in an area of 108.9 million ha, in which 32.6 million head of cattle and 12 million head of sheeps and goats, respectively, are found (Enriquez-Quiroz *et al*., 2021). In the state of Durango, rangelands account for 70 % of the forage supply for livestock grazing in these areas, mainly cattle (INEGI, 2023; SIAP, 2023). Extensive livestock farming is a major emitter of anthropogenic methane (CH_4); methane has a calorific value up to 23 times higher than CO_2 (Sánchez *et al*., 2021). Furthermore, ruminal enteric CH_4 production is the main cause of the high carbon footprint of ruminants. Livestock-related CH_4 production alone contributes 14 % of non-CO_2 emissions in this sector (Sandoval-Pelcastre *et al*., 2020). Hence the importance of estimating CH_4 emissions in this production system. In Mexico, there are few studies that determine the production of enteric methane in extensive systems, so they have to be estimated according to the methodology proposed by the IPCC based on general equations, without taking into account the breed, sex, age or physiological stage of the animals, much less the type of pasture where it is produced, and above all the diet selected by the grazing cattle.

There are some results in which it has been found that those animals with lower dry matter intake are due to poor nutritional quality, and therefore, higher methane emissions (Rao *et al*., 2015). Another factor that causes a decrease in nutritional quality and availability of forage is low rainfall (Olivera-Castro *et al*., 2022; Rodríguez *et al*., 2019); this increases the cellulose fraction, which causes ruminants to have increased enteric methane production (Palangi *et al*., 2019; Nampoothiri *et al*., 2018). In addition, 2023 was an atypical year with low rainfall in northern Mexico. Other studies have shown that there are seasonal variations in pasture nutritional quality, which may lead to an increase in ruminal enteric methane production (Reyes-Estrada *et al*., 2014); however, information regarding methane production in extensive systems is very limited due to the complexity of the methodologies.

The objective was to evaluate the rumen methane production and nutritional characteristics of the diet consumed by grazing cattle in a medium open pasture during the dry and rainy season.

Materials and Methods

Description and botanical composition of the study area

The study was conducted in a medium open grassland located at 24°19'22.20 N, -104°45'57 W belonging to the area of Malpais de la Breña, Durango, with a mean temperature of 22.9 °C and an average rainfall of 8.56 mm for the dry season (April - June 2023) and a mean temperature of 24.1 °C and an average rainfall of 59.56 mm for the rainy season (August - October, 2023) (SMN, 2024). The pasture has an area of 194 ha, which is divided into paddocks; the present experiment was carried out in a 33 ha paddock. The predominant species are grasses: navajita grass (*Bouteloa gracilis*), pata de gallo (*Chloris submutica*), liendrilla (*Muhlenbergia rigida*), Rhodes grass (*Chloris gayana*) and shrubs: mesquite (*Prosopis julifora*) and huizache (*Acacia tortuosa*).

Experimental cattle, periods and sample collection

Four rumen fistulated Criollo cattle weighing 682 ± 10 kg (BW) were used under continuous grazing in the pasture described above. Samples of the diet consumed by the cattle were obtained at the beginning and end of the dry season (April to June), as well as at the beginning and end of the rainy season (August to October). The sampling and sample collection periods were 15 d, of which the first 10 days were for saturation with chromium sesquioxide as a marker. From day 11, faecal samples were collected rectally until day 15 (Reyes-Estrada *et al*., 2014). Finally, on day 15, the rumen contents were emptied and immediately afterwards a 10 mL aliquot was taken to evaluate rumen fermentation parameters, such as ammonia nitrogen ($N-NH_3$) and volatile fatty acids (VFA) (Galyean, 2010); in addition, the pH of the rumen fluid per animal was measured with a potentiometer (model HI 83142, Hanna Instruments, Granjas, Mexico). Once this phase was completed, and with the rumen empty, the cattle were left to graze for one hour, and then samples of the diet consumed were taken (Reyes-Estrada *et al*., 2014). Once the samples were obtained, they were dried in a forced air oven at 55 °C for 72 h (Arthur H. Thomas, Philadelphia, PA, USA) for subsequent analysis of nutritional quality and gas and methane production.

Chemical analysis of the consumed diet

Samples of the diet consumed by cattle at each time of the year were dried and ground to determine dry matter (DM), ash, crude protein (CP) and ether extract (EE) contents (AOAC, 2019). Similarly, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin according to standardised methodology (Van Soest *et al*., 1991). *In vitro* dry matter digestibility was determined by obtaining rumen inoculum from two fistulated rumen fistulated Criollo cattle weighing 682 ± 10 kg (BW) and mixed in a 1:2 ratio with buffer solutions. Subsequently, ANKOM F57 bags (Ankom Technology, Macedon, NY, USA) containing 1 g sample of the consumed diet were immersed in jars of the Daysi II incubator ANKOM DAISYII Incubator, NY, USA) for 48 h (ANKOM, 2005).

***In vitro* gas production kinetics**

The number of replicates for each variable was sixteen and approximately, 1 g sample of the diet consumed by cattle was placed in modules equipped with a pressure transducer (ANKOM, RF Gas production system, Macedon, NY, USA) and left to ferment with rumen inoculum for 0, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h (Teodorou *et al*., 1994). The data obtained from the gas production kinetics were fitted with the France model (France *et al*., 2000) according to the following equation proposed by Elghandour *et al*. (2013):

$$A = b \left(1 - e^{-c\,(t-l)}\right)$$

Where: A= gas volume (mL) at incubation time t (h), b= the asymptote of gas production ($mL.g^{-1}$ DM), c= gas production rate (h^{-1}), l= lag phase (h).

Methane and CO_2 production *in vitro*

To evaluate methane (CH_4) and carbon dioxide (CO_2) production, the 8 replicates of the consumed diet were fermented by placing 1 g sample of the consumed diet in modules equipped with pressure transducer (ANKOM, RF Gas production system, Macedon, NY, USA) for 24 h and using a gas analyser (GEM5000, Landetc, MI, USA) the proportion of methane and carbon dioxide in the headspace was measured and multiplied by the gas production at 24 h according to the proposed literature (González-Arreola *et al*. , 2019).

Statistical analysis

Data obtained from proximal chemical analysis as well as ruminal fermentation parameters were analysed with a repeated measures experimental design over time using the MIXED procedure of SAS (2015), where the period of the season was included as an effect of repetition. Results are expressed as least square means. The number of replicates for each variable was sixteen. For the variables of the *in vitro* gas production kinetics parameters, as well as methane and carbon dioxide production, they were analysed under a completely randomised design using the GLM procedure of SAS (2015). The number of replicates for each variable was eight. Significant differences were declared under Tukey's mean comparison test, with a significance value of $p<0.05$.

Results and discussion

Table 1 shows the proximate chemical analysis of the diet consumed by grazing cattle. It is observed that DM and OM increased by 35 and 3 % in the rainy period with respect to the dry period, respectively ($p<0.05$). In the same way, NDF and FDA increased 7 and 11 % during the rainy season with respect to the drought season, respectively ($p<0.05$). On the other hand, CP, EE, phosphorus and IVDMD decreased in the rainy season by 13, 50, 42 and 5 %, respectively ($p<0.05$). These results are caused by the irregular rainfall during 2023, which caused a decrease in the quality of the pasture. According to CONAGUA (2024), only 332.7 mm of rainfall was recorded in Durango, and there are even sources that report less water volume (SMN, 2024). These values are lower than the average for the state of Durango, which is 550 mm per year. The decrease in pasture quality is attributed to water scarcity, which causes an increase in the fibrous fraction of the forage and compromises regrowth. In fact, the phenology of the grassland itself affects the nutritive value, as it decreases as the grassland ages; as the leaves mature, they tend to become smaller or fall off, while the stem increases in thickness, which increases structural carbohydrates. Thus, an increase in the fraction of cellulose and hemicellulose from NDF and FDA is expected. On the other hand, neither lignin nor CNF changed between seasons ($p>0.05$), suggesting that the decrease in IVDMD was not due to increases in lignocellulosic compounds, but to an increase in the fibrous fraction of the diet consumed.

Table 1. Least square means of the chemical composition of the diet consumed by grazing cattle in a medium open pasture during two seasons of the year.

Parameter (% DM)	Dry	Rainy	SEDM
DM	51.824[b]	70.374[a]	1.944
OM	79.416[b]	82.572[a]	0.757
CP	10.603[a]	9.198[b]	0.159
EE	1.304[a]	0.646[b]	0.114
FND	67.510[b]	72.727[a]	0.752
FAD	42.798[b]	47.648[a]	1.818
Lignin	8.488[a]	7.644[a]	0.661
NFC	6.685 [a]	7.140 [a]	0.781
Phosphorus	0.407[a]	0.271[b]	0.020
IVDMD	63.694[a]	60.055[b]	0.759

[ab]Literals in the same row indicate significant differences ($p<0.05$); DM=Dry Matter; OM=Organic Matter, CP=Crude Protein, EE=Ether Extract, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre, NFC= Non-fibre carbohydrates, IVDMD=*In vitro* dry matter digestibility, SEDM= Standard error of the difference between means.

In fact, Dong *et al*. (2021) observed that when the NDF:NFC ratio was increased, IVDMD was reduced. In this study, NFC was not affected, but NDF was increased, so the results are consistent with those reported above. In conjunction, previous studies have found high nutritional values in the rainy season with grazing animals during the dry and rainy season (Reyes-Estrada *et al*., 2014); consequently, the values presented by these authors are comparable with those found in this study (10, 64 and 67 % CP, NDF and IVDMD, respectively). The concentrations shown in the present study exceed the amounts recommended by NRC (2010). This manual indicates that the recommended amounts of phosphorus should be between 0.10 and 0.20 % (DM) of the diet. As has been shown, even in the rainy season, this requirement is met. In fact, in the dry season the minimum amount required is doubled (0.40 % DM). The presence of phosphorus in a ruminant diet is of utmost importance. Phosphorus reductions between seasons may be due to the fact that water-stressed plants in pastures redistribute nutrients to the stem, roots and soil in a protective manner; this effect also causes a mobilisation of minerals to the root (Van Soest *et al*., 1991, Romero *et al*., 2020). A deficiency of phosphorus in the diet can be reflected in reduced appetite in animals (Crespo *et al*., 2015).

Table 2 shows the rumen fermentation parameters of cattle grazing on pasture. As can be seen, pH and volatile fatty acids were equal between seasons (p>0.05). Low forage diets have been reported to stimulate changes in butyrate to acetate synthesis (Espinoza-Velasco, Ramírez-Mella and Sánchez-Villareal, 2018); no such changes were found in the present study because the diet consumed was high in fibre. Likewise, no changes were found in propionate ratio or A:P ratio, parameters indicative of better energy utilisation by cattle. However, according to previous studies, the value found in the A:P ratio is adequate because it is close to 2 (Holguín *et al*., 2020).

Table 2. Least square means of rumen fermentation parameters of the diet consumed by grazing cattle in a medium open pasture during two seasons of the year.

Parameter	Dry	Rainy	SEDM
pH	6.812[a]	6.850[a]	0.044
N-NH_3 (mg.dL^{-1})	4.639[b]	5.309[a]	0.196
Acetate (%)	46.715[a]	46.467[a]	1.785
Propionate (%)	34.821[a]	34.984[a]	1.150
Butirate (%)	13.216[a]	13.287[a]	0.462
Isobutyric (%)	1.536[a]	1.538[a]	0.103
Valeric (%)	2.368[a]	2.372[a]	0.105
Isovaleric (%)	1.327[a]	1.350[a]	0.070
A:P ratio	1.357[a]	1.328[a]	0.073

[ab]Different literals in the same row indicate significant differences (p<0.05), N-NH_3= Ammonia nitrogen, TVFA=total volatile fatty acids, A:P=acetate:propionate ratio; SEDM=standard error of the difference between means.

On the other hand, the pH is in the optimal range for proper fermentation and adequate growth of cellulolytic bacteria (Zhang *et al*., 2017). The N-NH_3 content increased by 14 % in the rainy season compared to the dry season (p<0.05). Ammonia nitrogen is the preferred N source of cellulolytic bacteria. According to previous studies by Hackmann *et al*. (2015), increases in the bacterial population are positively correlated with an increase in the passage rate. Furthermore, ruminants can adapt to high-fibre feeds through changes in their microbial population (Espinoza-Velasco *et al*., 2018). Thus, it is suggested that the higher the amount of ammonia nitrogen, the higher the amount of cellulolytic bacteria. This assertion makes sense when an increase in the fibre fraction (cellulose and hemicellulose) in the diet consumed during the rainy season is observed. Thus, the rumen is prepared for more fibre degradation in the rainy season than in the dry season. However, IVDMD decreased in the rainy season. In addition, some authors indicate that the recommended minimum concentration of ammonia nitrogen in the rumen for optimal microbial protein synthesis is between 5 and 10 mg.dL (Ramos-Juárez *et al*., 2021).

Table 3 shows the data for the parameters of gas production kinetics. Gas production kinetics can be directly correlated with the digestive processes of ruminants by fitting mathematical models (Murillo *et al*., 2020); these models expose parameters that have a justification for the biological processes taking place in the rumen.

Table 3. Kinetics of gas, carbon dioxide and methane production *in vitro* from diet consumed by grazing cattle in a medium open pasture during two seasons of the year.

Parameter	Dry	Rainy	SEDM
c (h^{-1})	0.023[a]	0.025[a]	0.0008
l (h)	0.186[b]	1.063[a]	0.027
b (mL.g^{-1} DM)	242.350[a]	217.500[a]	2.504
CO_2 (mL.g^{-1} DM)	71.144[a]	67.65[a]	6.296
CH_4 (mL.g^{-1} DM)	7.728[a]	8.845[a]	0.434
CO_2:CH_4 ratio	6.95[a]	6.7[a]	0.511

[ab]Different literals in the same row indicate significant differences (p<0.05), c=gas production rate, l=lag phase, b=maximum gas production, CO_2=carbon dioxide production, CH_4=methane production, SEDM=standard error of the difference between means.

As can be seen in the table 3, the adaptation phase (parameter 'l') decreased 82 % during the rainy season compared to the dry season (p<0.05). This decrease in the adaptation time or start of gas production can be attributed to a higher concentration of non-structural or non-fibrous carbohydrates, as shown in the nutritional content in Table 1. Thus, changes in the lag phase of gas production can be attributed to microbial activity (Torres *et al*., 2019); these changes are more consistent with cell wall thickening. On the other hand, the constant rate of gas production and maximum gas production were similar between seasons (p>0.05). This similarity suggests that although the nutritional quality decreased in the rainy season, the rumen microorganisms carried out an integral utilization of the consumed diet; the decrease in the nutritional quality of the pasture is attributed to an atypical water shortage in the rainy season in the year of analysis. In fact, the data obtained on CH_4 and CO_2 production, as well as the CO_2:CH_4 ratio, support this theory, as their values are similar between seasons (p>0.05). On the contrary, increases in the fibre fraction of the diet consumed could have led to an increase in methane production. However, methane production remained similar between seasons, as did CO_2 production. In this regard, Carro *et al*. (2018) associate CH_4 production and forage quality with digestibility, so the results shown here are consistent with the latter. Several studies have shown that forage quality influences ruminal methane synthesis. Thus, forages with lower nutritional quality and mature forages increase the content of structural carbohydrates and reduce the content of more rapidly fermentable carbohydrates, such as starch. These changes in the plant cell wall lead to an increase in methane production (Almaraz-Buendía *et al*., 2019). However, although there was an increase in fibre in the rainy season, the fraction of CNF remained similar between seasons, which may have led to no changes in ruminal methane production and CO_2 consumption for its synthesis.

Conclusions

The results shown indicate that despite an atypical year with high levels of drought, the nutritional content of the diet consumed by grazing cattle showed acceptable levels. In addition, the methane production of the diet consumed between seasons is similar, showing no changes due to the drought that the pasture was subjected to. This study proposes a novel way to evaluate methane production from the diet consumed by grazing cattle.

Literature cited

Almaraz-Buendía, I., García, A. M., Sánchez-Santillán, P., Torres-Salado, N., Herrera-Pérez, J., Bottini-Luzardo, M. B., y Rojas-García, A. R. (2019). Análisis bromatológico y producción de gas *in vitro* de forrajes utilizados en el trópico seco mexicano. *Archivos de zootecnia*, *68*(262): 260-266. https://doi.org/10.21071/az.v68i262.4145

ANKOM. (2023). Daisy II Incubator, Operator´s Manual. https://www.ankom.com/sites/default/files/document-files/D200_D200I_Manual_0.pdf?srsltid=AfmBOorriYFNLhHm4sKPDm_06XJ1KYlMjaZ-xOuKhTn_dloj_HN-s2lE

AOAC. (2019). Official Methods of Analysis. *Association of Official Analytical Chemists International*. 21th ed. Virginia, USA, 1997: 69-83. https://www.researchgate.net/publication/292783651_AOAC_2005

Carro Travieso, M. D., Evan, T. D., and González Cano, J. (2018). Emisiones de metano en los animales rumiantes: influencia de la dieta. *Albéitar*, *220*: 32-35. https://www.portalveterinaria.com/pdfjs/web/viewer.php?file=%2Fupload%2Friviste%2FAlbeitar221_MR.pdf

CONAGUA "Comisión Nacional del Agua" (2023). Precipitación (mm) por Entidad Federativa y Nacional. México 2023: CONAGUA. https://smn.conagua.gob.mx/tools/DATA/Climatolog%C3%ADa/Pron%C3%B3stico%20clim%C3%A1tico/Temperatura%20y%20Lluvia/PREC/2023.pdf

Crespo, G., Rodríguez, I. y Lok, S. (2015). Contribución al estudio de la fertilidad del suelo y su relación con la producción de pastos y forrajes. *Revista Cubana de Ciencia Agrícola 49* (2): 211-219. https://www.redalyc.org/articulo.oa?id=193039698011

Dong, L. F., Yang, X. Z., Gao, Y. H., Li, B. C., Wang, B., and Diao, Q. Y. (2021). Effects of dietary NDF: NFC ratio on growth performance, nutritive digestibility, ruminal fermentation characteristics and methane emissions of Holstein heifers. *Acta Prataculturae Sinica, 30*(2), 156.https://doi.org/10.3390/ani9100725

Elghandour M. M., Salem A. Z. M., Gonzalez-Roquillo M., Bórquez J. L., Gado H. M., Odongo N. E. and Peñuelas G. G. (2013). Effects of exogenous enzymes on *in vitro* gas production kinetics and ruminal fermentation of four fibrous feeds. *Animal Feed Science and Technology, 179*: 46-53. https://doi.org/10.1016/j.anifeedsci.2012.11.010

Enríquez-Quiroz J. F., Esqueda-Esquivel V. A. y Martínez-Mendez D. (2021). Rehabilitación de praderas degradadas en el trópico de México. *Revista Mexicana de Ciencias Pecuarias; 12*(3)*:* 243-260. https://doi.org/10.22319/rmcp.v12s3.5876

Espinoza-Velasco B., Ramírez-Mella M. y Sánchez-Villareal A. (2018). Elucidando la relación entre la microbiota ruminal y la emisión de gases de efecto invernadero mediante la aplicación de la genómica. *Agroproductividad*, *11*(2): 3-8. https://revista-agroproductividad.org/index.php/agroproductividad/article/view/111

France J., Dijkstra J., Dhanoa M. S., López S. and Bannink A. (2000). Estimating the extent of degradation of ruminant feeds from adescription of their gas production profiles observed *in vitro*: Derivation of models and other mathematical considerations. *British Journal of Nutrition, 83*: 143–50. https://doi.org/10.1017/S0007114500000180

Galyean M.L. (2010). Laboratory procedures in animal nutrition research. *Department of Animal and Food Sciences*. Texas Tech. University, Lubbock, TX, USA. https://www.depts.ttu.edu/agriculturalsciences/vetSciences/mgalyean/lab_man.pdf

González-Arreola Adolfo, Murillo-Ortiz Manuel, Pámanes-Carrasco Gerardo A., Reveles-Saucedo Fanny y Herrera-Torres Esperanza. (2019). Pricly pear cladodes. *Journal Animal and Plants Science, 40*(1):6544-6553. https://www.m.elewa.org/JAPS

Hackmann, T.J. and Firkins, J.L. (2015). Maximizing efficiency of rumen microbial protein production. *Frontiers in Microbiology, 6*: 465. https://doi.org/10.3389/fmicb.2015.00465

Holguín V.A., Cuchillo-Hilario M., Mazabel S. y Mora. Delgado J. (2020). Efecto de la mezcla ensilada de *Pennisetum purpureum* y *Tithonia diversifolia* sobre la fermentación ruminal *in vitro* y su emisión de metano en el sistema RUSITEC. *Revista Mexicana de Ciencias Pecuarias, 11*(1): 19-37. https://doi.org/10.22319/rmcp.v11i1.4740

INEGI "Instituto Nacional de Estadística y Geografía". (2023) Aspectos geográficos. INEGI. https://www.inegi.org.mx/contenidos/app/areasgeograficas/resumen/resumen_10.pdf

Murillo, M., Reyes-Jáquez, D., Herrera-Torres, E., and Pámanes-Carrasco, G. A. (2020). Gas Production Technique as a Powerful Tool for the Evaluation of the Nutritional Quality of Feedstuffs in Ruminants' Production. In O. P. Jenkins (Ed.), Nova Science Publishers. *Advances in Animal Science and Zoology, 16*:1-16. https://www.researchgate.net/publication/354077538_Gas_Production_Technique_as_a_Powerful_Tool_for_the_Evaluation_of_the_Nutritional_Quality_of_Feedstuffs_in_Ruminants'_Production

Nampoothiri, V.M.; Mohini, M.; Malla, B.A.; Mondal, G.; Pandita, S. (2018). Animal performance, and enteric methane, manure methane and nitrous oxide emissions from Murrah buffalo calves fed diets with different forage-to-concentrate ratios. *Animal Production and Science, 60:*780. https://doi.org/10.1016/j.aninu.2018.01.009

NRC "National Research Council". (2010). Research at the Intersection of the Physical and Life Sciences. Washington, DC: The National Academies Press. https://.doi:10.17226/12809

Olivera-Castro Y., Mendez de Acevedo M., Arias-Avilés L. L., Pinheiro-Machado Filho L. C. y del Pozo-Rodríguez P. P. (2022). Rendimiento y calidad nutricional del pastizal de la finca Ressacada en Florianópolis-SC, Brasil. *Pastos y Forrajes, 45*(3)*:* 1-7. https://www.redalyc.org/articulo.oa?id=269173684003

Palangi, V. (2019). Effects of Processing Legume Forages with Organic acids on *In vitro* gas production, rumen fermantation and ethan *Production. Animal Science; Ataturk University: Yakutiye, Turkey*, p. 83. https://doi.org.10.12681/jhvms.3

Ramos-Juárez, J. A., Martínez-Urbina, E., Izquierdo-Reyes, F., Aranda-Ibañez, E. M., Vargas-Villamil, L. M., Hernández-Sánchez, D., y Joaquín-Torres, B. M. (2021). Efecto de Suplementos Fermentados con Pollinaza sobre el consumo y degradación del pasto Cuba CT-115. *Revista Fitotecnia Mexicana*, 44(4-A): 773-773.https://doi.org/10.35196/rfm.2021.4-A.773

Rao, I., Peters, M., Castro, A., Schultze-Kra, R., White, D., Fisher, M., *et al*., (2015). Livestock Pluse sustainable intensification of foragebased agricultural systems to improve livelihoods and ecosystems services in the tropics. *Topical Grasslands, 3*(2):59–82. https://hdl.handle.net/10568/68840

Reyes-Estrada O., Murillo-Ortiz M., Herrera Torres E., Gurrola-Reyes N. y Carrete-Carreón F. O. (2014). Cambios estacionales en consumo, composición química y degradabilidad ruminal de la dieta seleccionada por novillos en pastoreo. *Revista cubana de Ciencia Agrícola, 46*(4): 375-380. http://www.scielo.org.mx/scielo.php

Rodríguez Tenorio D., Gutiérrez Luna R., Bañuelos Valenzuela C., Rochín Berumen F. y Ruiz Fernández E. (2019). Hábitos de comportamiento del ganado bovino pastoreando en un pastizal mediano abierto. *Investigación Científica, 12*(2)*:* 1-6. https://revistas.uaz.edu.mx/index.php/investigacioncientifica/article/view/732

Romero Delgado G., Echeverría Rojas M., Trillo Zárate F., Hidalgo Lozano V., Aguirre Terrazas L., Robles Rodríguez R. y Núñez Delgado J. (2020). Efecto del faique (*Acacia macranthak*) sobre el valor nutricional del pasto guinea (*Panicum maximun* Jaq.) en un sistema silvopastoril. *Revista de Investigación Veterinaria de Perú, 31*(1)*:* 1-16. http://dx.doi.org/10.15381/rivep.v31i1.17562

Sánchez Zubieta A., Savian J.V., de Souza Filho G., Osorio Wallau M., Marín Gómez A., Bindelle J., Franciscois Bonnet O.J., César de Faccio P. (2021). Does grazing management provide opportunities to mitigate methane emissions by ruminants in pastoral ecosystems?. *Science of The Total Environment, 1*(754): 142029. https://doi.org/10.1016/j.scitotenv.2020.142029

Sandoval-Pelcastre, A. A., Ramírez-Mella, M., Rodríguez-Ávila, N. L., and Candelaria-Martínez, B. (2020). Árboles y arbustos tropicales con potencial para disminuir la producción de en rumiantes. *Tropical and Subtropical Agroecosystems, 23*(33): 1-16.https://www.researchgate.net/publication/370509194_ARBOLES_Y_ARBUSTOS_TROPICALES_CON_POTENCIAL_PARA_DISMINUIR_LA_PRODUCCION_DE_METANO_EN_RUMIANTESetano

SIAP "Servicio de Información Agroalimentaria y Pesquera" (2023). Población ganadera nacional. SIAP, https://nube.siap.gob.mx/poblacion_ganadera/

SMN "Servició Metereologíaco Nacional". (2024). Resúmenes mensuales de temperaturas y lluvia. Servicio Meteorológico Nacional. Comisión Nacional del Agua. https://smn.conagua.gob.mx/es/climatologia/temperaturas-y-lluvias/resumenes-mensuales-de-temperaturas-y-lluvias

Teodorou M.K, Williams B.A., Dhanoa M.S., McAllan A.B. and France J. (1994). A simple gas production method a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology, 48*:185-197. https://.doi.org/10.1016/0377-8401(94)90171-6

Torres S. N., Sánchez S. P., Rojas G. A. R., Almaraz B. I., Herrera P. J., Reyes V. I., y Mayren M. F. J. (2019). Producción de gas *in vitro* y características fermentativas de consorcios bacterianos celulolíticos ruminales de búfala de agua (*Bubalus bubalis*) y vaca suiz-bu. *Agrociencia, 53*: 145-159. http://ri.uagro.mx/handle/uagro/1341

Van Soest, P.J., J.B. Robertson, B.A Lewis. (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.http://.doi.10.3168/jds.S0022-0302(91)78551-2

Zhang, L., Chung, J., Jiang, Q., Sun, R., Zhang, J., Zhong, Y. and Ren, N. (2017). Characteristics of rumen microorganisms involved in anaerobic degradation of cellulose at various pH values. *RSC Advances, 7*: 40303-40310. https://doi.org/10.1039/C7RA06588D