Fermentation parameters, microbial protein synthesis and gas kinetics of native northern Mexico species *in vitro*

Parámetros de fermentación, síntesis de proteína microbiana y cinética de producción de gas de especies nativas del norte de México evaluadas *in vitro*

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ABSTRACT. Climate change is modifying precipitation patterns in arid and semi-arid rangelands, which may affect forage quality, ruminant nutrition, and the diet composition of grazing cattle. Under these conditions, the identification of alternative forage resources adapted to such environments becomes increasingly relevant. This study evaluated the *in vitro* fermentation characteristics, microbial protein synthesis, and gas production kinetics of native forage species from northern Mexico. An *in vitro* fermentation assay was conducted using *Prosopis juliflora*, *Quercus rugosa*, *Flourensia cernua*, and *Atriplex canescens*, with alfalfa hay (*Medicago sativa*) as the reference forage. Significant differences ($p < 0.05$) were observed among species in total gas production, methane production, fermentable organic matter (FOM), microbial protein production (MP), and gas production kinetics. *Medicago sativa* showed the highest values for total gas production (237 mL g^{-1} DM), FOM, microbial protein synthesis, and maximum gas production, whereas *Quercus rugosa* consistently exhibited the lowest values for gas and methane production (79.9 and 15mL g^{-1} DM), respectively),. In contrast, *Atriplex canescens* recorded 28 mL g^{-1} DM of methane production. Gas production kinetics revealed variability among species, reflecting differences in substrate degradability and fermentation efficiency. Overall, the results indicate that native forage species from northern Mexico exhibit distinct fermentation profiles and could represent alternative feed resources for ruminants. *Q. rugosa* showed potential as a low-methane forage under *in vitro* conditions; however, further *in vivo* studies are required to confirm its nutritional value and applicability under production conditions.
Keywords: Methane emissions, *in vitro* fermentation, gas kinetics, microbial protein, rangeland species.

RESUMEN. El cambio climático modifica la precipitación en pastizales áridos y semiáridos, lo que afecta la calidad de forrajes, la nutrición de rumiantes y la composición de su dieta en pastoreo. En este contexto, la identificación de recursos forrajeros alternativos adaptados a estas condiciones adquiere especial relevancia. El objetivo del estudio fue evaluar las características de fermentación *in vitro*, la síntesis de proteína microbiana y la cinética de producción de gas de especies forrajeras nativas. Se realizó un ensayo de fermentación *in vitro* utilizando *Prosopis juliflora*, *Quercus rugosa*, *Flourensia cernua* y *Atriplex canescens*, con alfalfa henificada (*Medicago sativa*) como forraje de referencia. Se observaron diferencias significativas ($p < 0.05$) entre especies en producción total de gas, producción de metano, materia orgánica fermentable (MOF), producción de proteína microbiana (PPM) y cinética de producción de gas. *Medicago sativa* presentó valores superiores en producción total de gas, MOF, PPM y producción total de gas (237mL g^{-1} MS), mientras que *Quercus rugosa* exhibió consistentemente menor producción de gas y metano (79.9 y 15 mL g^{-1} MS respectivamente). En contraste, *Atriplex canescens* registró 28 mL g^{-1} MS de metano. La cinética de producción de gas evidenció variabilidad entre especies, reflejando diferencias en la degradabilidad del sustrato y la eficiencia de fermentación. Los resultados indican que estas presentan perfiles de fermentación diferenciados y podrían representar recursos forrajeros alternativos para rumiantes. En particular, *Q. rugosa* mostró potencial antimetanogénico bajo condiciones *in vitro*; sin embargo, se requieren estudios *in vivo* para confirmar su valor nutricional y aplicabilidad en sistemas de producción.
Palabras clave: Emisiones de metano, fermentación *in vitro*, cinética de gases, proteína microbiana, especies de pastizal.

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INTRODUCTION

The growing global demand for animal-derived foods is expected to substantially increase livestock production in the coming decades. According to the Food and Agriculture Organization (FAO 2023), meat production is projected to rise by approximately 19% by 2030 compared to the 2015–2017 period, with this increase occurring primarily in developing countries such as Mexico. In this context, livestock production systems face the challenge of ensuring a sustainable supply of high-quality forage resources under increasingly constrained environmental conditions (Jurado-Guerra *et al*. 2021).

In Mexico, livestock production occupies extensive areas of land; however, a gradual reduction in available grazing surfaces has been reported (Jurado-Guerra *et al*. 2021). Official data indicate that the area allocated to cattle production decreased from approximately 108.9 million hectares in 2014 to around 105 million hectares in recent years, with a large proportion of this surface managed under grazing systems based on native and induced pastures (INEGI 2022). In northern Mexico, extensive livestock production in rangelands represents a dominant agricultural activity, highlighting the importance of pasture-based feeding systems (Cisneros-Saguilán *et al.* 2024). Under these conditions, improving the availability and quality of forage resources is essential to sustain productivity. In this regard, the use of native forage species has been proposed as a sustainable strategy to improve rangeland management and livestock nutrition (Boga and Ayasan 2022).

Nevertheless, rangeland degradation associated with overgrazing remains a major constraint, as it reduces vegetation cover, alters hydrological processes, and increases ecosystem vulnerability to drought and desertification (Peprah *et al.* 2022). These changes negatively affect forage quality, limiting nutrient intake and animal performance (Estrada *et al.* 2016). Moreover, low-quality diets can modify ruminal fermentation patterns, increasing methane (CH_4) emissions, which represent both an environmental concern and an energetic loss for the animal (Lileikis *et al.* 2023).

Methane production is an inherent by-product of ruminal fermentation; however, it can be modulated through dietary strategies. Approaches such as the inclusion of high-quality forages, increased concentrate levels, or the use of feed additives have been shown to reduce methanogenesis by inhibiting methanogenic microorganisms or redirecting hydrogen utilization (Bonilla-Cárdenas and Lemus-Flores 2012). However, the use of cereal grains in ruminant diets raises concerns due to competition with human food resources, highlighting the need for alternative feed sources.

In this regard, native trees and shrubs present in rangelands represent a promising alternative. These species can maintain higher crude protein levels than grasses during dry periods, providing a more stable nutrient supply (Estell *et al.* 2016, Fernández-Mayer 2017). Additionally, they contain secondary metabolites capable of modifying ruminal fermentation, potentially improving protein utilization and reducing methane emissions. Condensed tannins have been reported to inhibit certain groups of ruminal microorganisms, particularly methanogenic archaea and protozoa, resulting in lower methane production. This reduction in enteric methane emissions contributes to improved energy efficiency because less dietary energy is lost as methane gas. Furthermore, tannins may alter fermentation pathways by promoting propionate production and reducing acetate formation, which is associated with lower hydrogen availability for methanogenesis (Canul-Solís *et al.* 2020, Ibrahim *et al.* 2022). Despite their potential, the fermentative behavior and nutritional value of many native forage species remain insufficiently characterized.

Therefore, the objective of this study was to evaluate selected native forage species (*Prosopis juliflora*, *Quercus rugosa*, *Flourensia cernua*, and *Atriplex canescens*) in comparison with alfalfa hay (*Medicago sativa*) by assessing the ruminal fermentation characteristics, microbial protein synthesis, and *in vitro* gas production kinetics. We hypothesized that native forage species would exhibit distinct fermentation profiles and differential methane production compared to conventional forage.

MATERIALS AND METHODS

Study area

The study was conducted at the Animal Nutrition Laboratory, Faculty of Veterinary Medicine and Animal Science, Universidad Juárez del Estado de Durango, Mexico. Alfalfa hay (*Medicago sativa*) samples were collected from the Guadiana Valley agricultural region, whereas white oak (*Quercus rugosa* Née) was collected from the mountainous region of Canatlán. Mesquite (*Prosopis juliflora*), hojasén (*Flourensia cernua*), and chamizo (*Atriplex canescens*) were collected from semi-arid rangelands of Cuencamé, corresponding to the natural distribution of each species, between June and August 2023.

All procedures involving rumen-fistulated steers used for rumen fluid collection were carried out in accordance with the guidelines established by the Animal Protection Committee of the State of Durango, Mexico, and the Official Mexican Standard NOM-062-ZOO-1999. Plant material was manually defoliated; leaves were dried in a forced-air oven at 55 °C and ground to a particle size of 1 mm.

Chemical and nutritional composition

All analyses were performed in triplicate. Dry matter (DM), ash content (A), organic Matter (OM), and crude protein (CP) were determined according to AOAC (2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin were analyzed following Goering and Van-Soest (1970). In addition, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) were determined using the ANKOM® method (ANKOM Technology 2008), and condensed tannins (CT) were quantified following Porter *et al.* (1985).

In vitro gas and methane production

Fermentation assays were conducted using 100 mL glass syringes. Two groups of syringes were prepared, each including triplicate samples (0.5 g), two blanks without substrate, and two syringes containing alfalfa as a control. Rumen inoculum was obtained from four adult cattle (Simmental crossbreed; approximately 750 kg body weight) fed irrigated mixed pastures. The inoculum was prepared by mixing rumen fluid with artificial saliva (1:2, v/v) and continuously flushed with CO_2 (Theodorou *et al.* 1994). A volume of 40 mL of inoculum was added to each syringe. Syringes were incubated at 39 °C for 24 h in a water bath. At the end of incubation, total gas production was recorded, methane and carbon dioxide concentrations were determined using the indirect method proposed by Fievez *et al.* (2005) where the gas produced was transferred by means of a two-way valve to a second glass syringe to which 4 mof sodium hydroxide was previously added, subsequently it was shaken and a precipitation reaction occurs, the volume obtained as a result is considered to be suspended methane.

Variable estimation

Fermentable Organic Matter (FOM) was estimated according to Gosselink *et al.* (2004) using the following equation:

$$FOM = 2.815\, GP_{24h} + 0.418\, CP$$

Where FOM is expressed as mg g^{-1} DM, GP_{24h} is the *in vitro* gas production at 24 h (mL g^{-1} DM), and CP is the crude protein content on a dry matter basis.

Microbial protein (MP) was estimated according to Blümmel *et al.* (1997) as follows:

$$MP = DMD_{24h} - (GP_{24h} \times 2.2)$$

Where MP is Microbial Protein (mg g^{-1} FOM), DMD_{24h} is dry matter degradability at 24 h (mg g^{-1} DM), GP_{24h} is gas production at 24 h (mL g^{-1} DM), and 2.2 (mg mL^{-1}) is the stoichiometric factor associated with gas production from short-chain fatty acids.

The Partition Factor (PF) was calculated according to Blümmel *et al.* (1997) as:

$$PF = \frac{DMD_{24h}}{GP_{24h}}$$

Additionally, Dry Matter Degradability at 24 h was estimated as:

$$DMD_{24h} = \frac{GP_{24h} + 11.3}{0.29}$$

Where PF is the partition factor at 24 h, DMD_{24h} is dry matter degradability (mg g^{-1} DM), and GP_{24h} is gas production (mL g^{-1} DM).

Gas production kinetics

In vitro ruminal fermentation was carried out under the conditions previously described, and cumulative gas production was recorded at 0, 3, 6, 9, 12, 24, 48, 72, and 96 h. The Gompertz model was used to estimate gas production kinetics parameters (Lavrencic *et al.* 1997):

$$GP(t) = A \cdot exp[-exp(k_d(Lag - t))]$$

Where GP(t) is cumulative gas production at time t, A is the asymptotic gas production (mL), K_d is the fractional rate of gas production (h^{-1}), and Lag is the lag phase (h).

Statistical analysis

Data were analyzed using a completely randomized design. All variables were subjected to analysis of variance (ANOVA) to evaluate the effect of forage species using GLM procedure of SAS

(2003). When significant differences were detected, means were compared using Tukey's multiple comparison test at a significance level of $p \leq 0.05$.

RESULTS

The results of the evaluation of the nutritional quality of the plants studied are shown in Table 1. Crude Protein (CP) ranged from 8.2% in *Quercus rugosa* to 16.8% in *Medicago sativa*. Neutral Detergent Fiber (NDF) values varied from 31.9% in *Flourensia cernua* to 61.4% in *Q. rugosa*, while Acid Detergent Fiber (ADF) ranged from 19.9% in *Flourensia cernua* to 53.1% in *Q. rugosa*. Lignin content ranged from 7.7% in *Flourensia cernua* to 12.3% in *Prosopis juliflora*. *In vitro* Dry Matter Digestibility (IVDMD) ranged from 20.3% in *Q. rugosa* to 63.0% in *A. canescens*, whereas *in vitro* organic matter digestibility (IVOMD) ranged from 19.1% to 79.0% in the same species. Condensed tannins (CT) ranged from 0.56 to 5.8 mg GAE g^{-1} DM, with the highest value recorded in *Q. rugosa*.

Table 1. Chemical composition of forage species evaluated under *in vitro* conditions.

Variable	Species				
	Medicago sativa	*Prosopis juliflora*	*Quercus rugosa*	*Flourensia cernua*	*Atriplex canescens*
Crude protein (%)	16.8	15.3	8.2	14.6	12.4
NDF (%)	45.0	54.2	61.4	31.9	50.6
ADF (%)	27.6	40.1	53.1	19.9	23.1
Lignin (%)	8.1	12.3	9.2	7.7	12.2
IVDMD (%)	55.8	50.9	20.3	59.0	93.0
IVOMD (%)	50.1	49.0	19.1	89.0	94.0
CT (mg GAE g^{-1} DM)	0.56	1.9	5.8	2.3	2.5

NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; IVDMD = *In vitro* Dry Matter Digestibility; IVOMD = *In vitro* Organic Matter Digestibility; CT = Condensed Tannins.

In vitro gas, methane, and carbon dioxide production after 24 h of incubation are presented in Table 2. Gas production ($GP_{(24h)}$) differed among species ($p < 0.05$), with the highest value observed in *Medicago sativa*, followed by *Atriplex canescens* and *Flourensia cernua*, whereas *Quercus rugosa* showed the lowest value. Methane production (CH_4) also differed among species ($p < 0.05$). *Atriplex canescens* showed the highest methane production, whereas *Q. rugosa* showed the lowest value. *Prosopis juliflora* and *F. cernua* presented intermediate values. The CO_2: CH_4 ratio differed among species ($p < 0.05$), with higher values observed in *M. sativa* and *F. cernua*.

Gas production kinetics parameters are presented in Table 3. Significant differences ($p < 0.05$) were observed among species. The maximum gas production (A) was highest in *M. sativa* and lowest in *Q. rugosa*. The lag phase (L) was higher in *Prosopis juliflora* and *Atriplex canescens*, whereas lower values were observed in *M. sativa* and *Q. rugosa*. The gas production rate ($k_{(d)}$) was highest in *P. juliflora* and lowest in *Q. rugosa*.

Fermentable Organic Matter (FOM), microbial protein (MP), and partition factor (PF) are presented in Table 4. Significant differences ($p < 0.05$) were observed among species. The highest FOM value was recorded in *Medicago sativa*, followed by *Flourensia cernua* and *Atriplex canescens*, whereas

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Quercus rugosa showed the lowest value (225 mg g^{-1} DM) microbial protein (MP) followed a similar pattern, with the highest value in *M. sativa* and the lowest in *Q. rugosa*. The partition factor (PF) differed among species ($p < 0.05$), with higher values observed in *Q. rugosa* and lower values in *M. sativa*.

Table 2. *In vitro* gas, methane and carbon dioxide production from the evaluated species.

Species	GP_{24h} (mL g^{-1} DM)	CH_4 (mL g^{-1} DM)	CO_2 (mL g^{-1} DM)	$CO_2:CH_4$
Medicago sativa	237.7 ± 2.28^{a}	22.0 ± 1.15^{b}	215 ± 3.43^{a}	9.9 ± 0.66^{a}
Prosopis juliflora	138.6 ± 2.42^{c}	19.0 ± 1.00^{bc}	119.6 ± 3.20^{c}	6.3 ± 0.52^{bc}
Quercus rugosa	79.9 ± 1.12^{d}	15.3 ± 0.66^{c}	84.6 ± 0.63^{d}	5.5 ± 0.18^{c}
Flourensia cernua	160.4 ± 4.37^{b}	17.3 ± 0.66^{c}	143.2 ± 3.71^{b}	8.2 ± 0.12^{ab}
Atriplex canescens	165.8 ± 5.00^{b}	28.0 ± 1.15^{a}	137.9 ± 6.00^{b}	4.9 ± 0.42^{cd}
SEM	3.35	0.95	3.79	0.43

Different letters within the same column indicate significant differences (Tukey's; $p < 0.05$). GP_{24h} = Gas production at time t (24h); CH_4 = Methane production at 24h of incubation; CO_2 = Carbon dioxide production at 24h of incubation; $CO_2:CH_4$ = Carbon dioxide:methane ratio. SEM = Standard error of the mean.

Table 3. *In vitro* gas production kinetics for the evaluated species.

Species	A (mL)	L (h^{-1})	K_d (h^{-1})
Medicago sativa	253.6 ± 7.47^{a}	2.4 ± 0.04^{b}	0.17 ± 0.004^{b}
Prosopis juliflora	158.3 ± 2.165^{bc}	4.0 ± 0.046^{a}	0.62 ± 0.023^{a}
Quercus rugosa	121.8 ± 9.24^{c}	2.0 ± 0.132^{b}	0.09 ± 0.014^{c}
Flourensia cernua	177.0 ± 14.19^{b}	2.62 ± 0.154^{b}	0.14 ± 0.013^{bc}
Atriplex canescens	199.3 ± 10.20^{b}	3.9 ± 0.18^{a}	0.17 ± 0.01^{b}
SEM	16.460	0.236	0.258

Different letters within the same column indicate significant differences (Tukey's; $p < 0.05$). A: maximum gas production, L: Lag phase, Kd: gas production rate.

Table 4. Fermentable organic matter, microbial protein and partition factor of evaluated species.

Species	FOM (mg g^{-1} DM)	MP (mg g^{-1} DM)	PF
Medicago sativa	669.3 ± 6.40^{a}	335.7 ± 2.85^{a}	3.6 ± 0.31^{c}
Prosopis juliflora	392.4 ± 6.09^{c}	211.9 ± 3.02^{c}	3.7 ± 0.11^{b}
Quercus rugosa	225.0 ± 3.23^{d}	138.7 ± 1.41^{d}	3.9 ± 0.25^{a}
Flourensia cernua	451.8 ± 2.27^{b}	239.3 ± 5.46^{b}	3.7 ± 0.63^{b}
Atriplex canescens	466.9 ± 1.09^{b}	246.0 ± 6.24^{b}	3.7 ± 0.47^{b}
SEM	9.30	4.19	0.03

Different letters within the same column indicate significant differences (Tukey's; $p < 0.05$). FOM = Fermentable Organic Matter, MP = Microbial Protein; PF = Partition Factor.

DISCUSSION

The differences observed in chemical composition among the evaluated forage species were closely associated with their fermentation behavior, gas production, and microbial protein synthesis. In particular, *Medicago sativa* showed higher crude protein content and intermediate fiber fractions, whereas *Quercus rugosa* combined low crude protein with high neutral detergent fiber and condensed tannin content. These compositional differences are known to influence ruminal fermentation dynamics, as higher fiber concentrations generally reduce the rate and extent of fermentation due to the structural complexity of cell walls (Babayemi 2007).

The patterns observed in gas production are consistent with these compositional characteristics. The higher gas production recorded in *M. sativa* agrees with its greater fermentability, which is typical of leguminous forages with lower structural fiber fractions. Similar results have been reported by Vázquez-Mendoza *et al.* (2023), who observed high *in vitro* gas production values for alfalfa hay. In contrast, the reduced gas production observed in *Q. rugosa* may be associated with its higher fiber content and the presence of condensed tannins. These compounds have been shown to form complexes with nutrients and microbial enzymes, thereby limiting fermentation and reducing gas production (Min *et al.* 2003).

Methane production showed marked differences among species, with *Q. rugosa* presenting the lowest values and *Atriplex canescens* the highest. The reduced methane production observed in *Q. rugosa* is consistent with previous reports indicating that tannin-rich forages can reduce methanogenesis both *in vitro* and *in vivo* (Aboagye and Beauchemin 2019, Berça *et al.* 2023). This effect has been attributed to the capacity of tannins to modify ruminal microbial populations and reduce substrate availability for methanogenic archaea. Additionally, the CO_2:CH_4 ratio observed in some species may reflect shifts in fermentation pathways. According to Murillo-Ortiz *et al.* (2018), higher CO_2:CH_4 ratios suggest that hydrogen is diverted toward alternative metabolic pathways rather than methane formation, which may partially explain the differences observed among species.

The behavior of *A. canescens* is noteworthy, as it showed high digestibility values together with the highest methane production. This can be attributed to the fact that the high digestibility of the forage shrub *Atriplex* occurs mainly during the winter period, when the plant has better nutritional levels compared to rangelands. Furthermore, the leaves and tender stems retain excellent degradability ranging from 65% to 86% (Enríquez *et al*. 2011). Moreover, the greater digestibility does not necessarily result in lower methane emissions, since methane production depends not only on the extent of fermentation but also on the metabolic pathways involved. In highly fermentable substrates, the increased availability of fermentable carbohydrates may enhance total gas production and consequently methane output, depending on hydrogen utilization during ruminal fermentation. The results obtained in this study are consistent with those reported by Araiza-Rosales *et al.* (2022) in different species, similarly, Torres-Fraga *et al*. (2018) registered digestibility values of 60% when species Quercus with 6.0 mg g^{-1} DM.

Gas production kinetics further supported the differences observed among species. The higher asymptotic gas production in *M. sativa* and the lower values in *Q. rugosa* confirm the contrasting fermentability of these forages. The higher gas production rate observed in *Prosopis juliflora* suggests a rapid fermentation once microbial colonization is established, whereas variations in lag phase among species may reflect differences in substrate accessibility and microbial adaptation. These kinetic parameters provide additional insight into fermentation dynamics beyond the 24 h measurements.

The differences observed in fermentable organic matter (FOM) and microbial protein (MP) are consistent with the compositional and fermentative characteristics of the evaluated species. *Medicago sativa* showed the highest FOM and MP values, indicating a greater availability of substrate for microbial growth. Tamminga *et al.* (2007) and Dimas-Villalobos *et al.* (2025) indicated that FOM represents the fraction of organic matter that can be utilized for microbial biomass synthesis, which explains the higher values observed in more fermentable substrates. Similarly, the greater microbial protein production in *M. sativa* is consistent with the concept that microbial growth depends on the availability of fermentable energy and nitrogen in the rumen (Hoover and Stokes 1991). In contrast, *Q. rugosa* showed the lowest FOM and MP values, which may be associated with its high fiber and tannin content. Tannins can reduce protein degradation in the rumen by forming complexes that are less accessible to microbial enzymes, thereby limiting microbial growth (Jenko *et al.* 2018). Despite this, *Q. rugosa* exhibited the highest partition factor (PF), suggesting a greater efficiency of microbial biomass synthesis relative to gas production. According to Makkar (2004), PF values within the range of 2.74 to 4.41 mg FOM per mL of gas indicate efficient fermentation, and all species evaluated in this study fell within this range. For this reason, it is recommended not to consider Q. rugosa as a component of the diet, but as an additive in order to reduce the production of enteric methane gas in livestock production.

The intermediate behavior observed in *Flourensia cernua* and *Atriplex canescens* suggests that native forages can provide fermentation characteristics comparable to conventional forages under certain conditions. Parmar *et al.* (2022) reported that non-conventional forage resources may exhibit similar or even superior fermentation characteristics compared to conventional feeds, highlighting their potential use in ruminant nutrition. In addition, the presence of secondary metabolites in these species may contribute to modulating ruminal fermentation and improving nutrient utilization (Canul-Solís *et al.* 2020, Ibrahim *et al.* 2022).

Overall, the results indicate that native forage species differ substantially in their nutritional and fermentative properties, and their potential use in ruminant feeding systems should be evaluated according to specific production objectives. While *M. sativa* showed the highest fermentative capacity and microbial protein production, *Q. rugosa* exhibited lower methane production, suggesting a potential role in strategies aimed at mitigating enteric methane emissions.

CONCLUSION

The evaluated native forage species showed variable fermentation characteristics under *in vitro* conditions, with some responses comparable to alfalfa hay. *Quercus rugosa* consistently exhibited lower methane production, indicating its potential as a forage resource for mitigating enteric methane emissions. These findings highlight the relevance of native species as alternative feed resources in ruminant production systems, particularly in arid and semi-arid regions. However, further *in vivo* studies are required to determine optimal inclusion levels and to evaluate their effects on animal performance and production efficiency.

DISCLAIMERS

All procedures involving rumen-fistulated steers for rumen fluid collection were carried out in accordance with the guidelines established by the State Committee for the Promotion and Protection of Livestock of Durango, Mexico and complied with the Official Mexican Standard NOM-062-ZOO-1999.

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DECLARATION ON THE USE OF GENERATIVE ARTIFICIAL INTELLIGENCE TOOLS

During the preparation of this manuscript, the authors used the Microsoft Copilot tool to expedite the search for scientific articles, as well as to correct spelling and grammar in English, and to facilitate the review of APA (7th edition) citation guidelines. It should be noted that the review, verification and interpretation of the information provided by Microsoft Copilot were carried out by the authors, who assume full responsibility for the content presented herein.

CONFLICT OF INTEREST

The authors declare no conflicts of interest. The funding source had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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