Abanico Veterinario. January-December 2022; 12:1-17. http://dx.doi.org/10.21929/abavet2022.22
Original Article. Received: 07/01/2022. Accepted:27/07/2022. Published: 19/08/2022. Code: e2022-1.
https://www.youtube.com/watch?v=dNnxkdLbHUA

In vitro ruminal fermentation of *Muntingia calabura* and *Bauhinia divaricata* foliage alone and in combination with *Pennisetum* sp

Fermentación ruminal *in vitro* de follajes de *Muntingia calabura y Bauhinia divaricata* solos y combinados con *Pennisetum* sp

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ABSTRACT

The objetive of this study was to evaluate the ruminal fermentation characteristics of *Bauhinia divaricata* and *Muntingia calabura* foliages alone and combined with *Pennisetum* sp. Five treatments were evaluated: P = *Pennisetum* sp. (100%); Mc = *M. calabura* (100%); Bd = *B. divaricata* (100%); McP = *M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP = B. *divaricata* (30%)/*Pennisetum* sp. (70%). The treatments with the highest *in vitro* dry matter degradability (DMD) were P, BdP, McP and Bd (> 50%) (P≤0.05). There were no significant differences (P> 0.05) in gas production (GP) among treatments Bd, BdP, McP and P; however, Mc produced 35% less gas (P≤0.05) than the other treatments. Conversely, Mc produced 153% more methane (CH_4) than Bd (P≤0.05), being Bd and BdP the treatments with the lowest CH_4 production. No significant differences (P> 0.05) were observed in volatile fatty acid (VFA) concentration, except for acetic acid between Mc and P with Bd, and isovaleric acid between Mc and Bd (P≤0.05). In conclusion, *B. divaricata* foliage decreased CH_4 production and did not negatively affect other ruminal fermentation variables, thus representing a dietary alternative to reduce the ruminal methanogenesis of cattle in the tropics.

Keywords: gas production, methane, secondary metabolites, forage tree, ruminants.

RESUMEN

El objetivo de este estudio fue evaluar las características de la fermentación ruminal de los follajes de *Bauhinia divaricata* y *Muntingia calabura* solos y combinados con *Pennisetum* sp. Se evaluaron cinco tratamientos: P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd= *B. divaricata* (100%); McP= *M. calabura* (30%)/*Pennisetum* sp. (70%), y BdP= *B. divaricata* (30%)/*Pennisetum* sp. (70%). Los tratamientos con la mayor degradabilidad *in vitro* de la materia seca (DMS) fueron P, BdP, McP y Bd (>50%) (P≤0.05). No hubo diferencias significativas (P>0.05) en la producción de gas (PG) entre los tratamientos Bd, BdP, McP y P; sin embargo, Mc produjo 35% menos gas (P≤0.05) que los demás tratamientos. Contrariamente, Mc produjo 153% más metano (CH_4) que Bd (P≤0.05), siendo Bd y BdP los tratamientos con menor producción de CH_4. No se observaron diferencias significativas (P>0.05) en la concentración de ácido grasos volátiles (AGV), excepto de ácido acético entre Mc y P con Bd, y de ácido isovalérico entre Mc y Bd (P≤0.05). En conclusión, el follaje de *B. divaricata* disminuyó la producción de CH_4 y no afectó negativamente otras variables de fermentación ruminal, por lo que representa una alternativa alimenticia

para reducir la metanogénesis ruminal de ganado en el trópico.
Palabras clave: producción de gas, metano, metabolitos secundarios, arbóreas forrajeras, rumiantes.

INTRODUCTION

It is estimated that by 2050, the world's population will be approximately 9.7 billion people (ONU, 2019). As a result, the production and food consumption of animal origin will increase, which will increase greenhouse gas (GHG) emissions. This will have a negative impact on climate change (Garnett, 2010). Livestock is an anthropogenic activity, which in 2010 generated approximately 8.1 gigatonnes of CO_2-eq worldwide, of which 62% was attributed to cattle. From these emissions, about 44% result from enteric fermentation of the digestive process, giving rise to high amounts of methane (CH_4) (FAO, 2017). CH_4 is a GHG with a global warming potential 28 times higher than carbon dioxide (CO_2) (Roehe *et al*., 2016). It is produced by methanogenic archaea using H_2 and CO_2 derived from rumen fermentation of carbohydrates and it is expelled to the environment through burping. It is estimated that an adult bovine can produce 250 to 500 L of CH_4 each day, which also constitutes an energy loss, since approximately 2 to 12% of the gross energy consumed by the animal is used for the production of this gas (Johnson & Johnson, 1995).

Rumen CH_4 production is strongly influenced by feed composition, so research to reduce it has focused on manipulating the rumen environment by modifying microbial diversity and ruminal fermentation patterns through the diet (Haque, 2018). Among the strategies studied to reduce CH_4 production, the use of secondary metabolites (MSec) such as tannins, saponins and flavonoids present in various plants (Bodas *et al.*, 2012), which have been shown to have antimicrobial activity and to reduce H_2 availability in the rumen (Hook *et al*., 2010).

The effect of various tropical forage trees and shrubs in reducing CH_4 emissions has been investigated, with *Leucaena leucocephala* being one of the most widely used species for this purpose (Delgado *et al*., 2012; Meale *et al*., 2012; Molina *et al*., 2015; Rivera *et al*., 2015). Other tree species such as *Samanea saman*, *Sapindus saponaria*, *Albizia lebbeck*, *Tithonia diversifolia*, *Gliricidia sepium* and *Vetellaria paradoxa* have also been shown to reduce ruminal CH_4 emissions *in vitro* (Delgado *et al*., 2012; Meale *et al.*, 2012). However, in the tropical regions of Mexico there is a great diversity of forage plants that have been less studied despite having nutritional and antimethanogenic potential, such as *Bauhinia divaricata* and *Muntingia calabura*. Therefore, the aim of this study was to evaluate ruminal fermentation and gas production (GP) *in vitro* of *B. divaricata* and *M. calabura* foliage alone and combined with *Pennisetum* sp.

MATERIAL AND METHODS

Location and collection of plant material

The collection of plant material was carried out in February 2018 at Xamantún Ranch belonging to the Technological Institute of Chiná of the National Technological Institute of Mexico, located at 19°43' North latitude and 90°24' West longitude. The site has a warm sub-humid A(W1) climate (García, 2004), with mean annual temperature and precipitation of 26°C and 1,138 mm, respectively, and an elevation of 36 m.

One kg of fresh foliage, consisting of leaves and tender stems, was collected, simulating the browsing of an adult bovine at a maximum height of two metres, from the following 10 tree and shrub plant species: *B. divaricata*, *M. calabura*, *Lysiloma latisiliquum*, *Tithonia diversifolia*, *Sida acuta*, *Guazuma ulmifolia*, *Moringa oleifera*, *Acacia farnesiana*, *Samanea saman* and *Coccoloba cozumelensis*, as well as 5 kg of *Pennisetum* sp. var. maralfalfa. Samples were dried in a forced-air oven at a temperature of 55°C for 72 h. The samples were then ground in a milling machine. Subsequently, they were ground in a Willey mill with a 1 mm sieve and stored at room temperature (24°C) until use.

Presence of secondary metabolites

The qualitative presence of various secondary metabolites was determined from the foliage of the 10 forage tree and shrub species mentioned above. For this purpose, 25 g of the plant material was taken and placed in glass jars. Subsequently, 3 v/v of 96 % ethanol was added as a solvent to extract MSec. Once the ethanolic extracts were obtained, the presence of MSec was determined following the protocol of Valencia del Toro and Garín (Valencia-Del Toro and Garín-Aguilar, 2010). The results are shown in Table 1.

Treatments

Taking into account the MSec profiles obtained, as well as the scarce information on their effects on ruminal fermentation, two plant species were selected for their high content of metabolites inhibiting microbial activity: *M. calabura*, which had a high presence of steroidal saponins and flavonoids of the xanthone and flavone type, and *B. divaricata*, which presented a moderate content of tannins derived from catechol. Based on the above, five treatments were evaluated: P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd=*B. divaricata* (100%); McP=*M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP=*B. divaricata* (30%)/*Pennisetum* sp. (70%).

Table 1. Occurrence of secondary metabolites in the foliage of tropical forage trees and shrubs

PLANT SPECIES	SECONDARY METABOLITES												
	Alkaloids	Saponins		Lactones	Tannins		Quinones	Cardenolides	Laucoanthocyanidins	Flavonoids			
		Steroids	Triterpenoids		Gallic acid	Catechol				Aurones	Chalcones	Xanthones	Flavones
Acacia farnesiana	-	+++	-	+++	+++	-	-	-	+	-	-	+++	+++
Muntingia calabura	-	+++	-	+++	++	-	-	-	+	-	-	+++	+++
Moringa oleifera	-	-	+	+++	+++	-	-	-	++	-	-	+++	+++
Samanea saman	-	+++	-	+++	++	-	-	-	+	-	-	++	++
Lysiloma latisiliquum	-	+++	-	+++	+++	-	-	-	+	-	-	+	+
Tithonia diversifolia	-	+++	-	+++	-	++	-	-	+	+	+	-	-
Sida acuta	-	+++	-	+++	-	-	-	-	++	-	-	+	+
Bauhinia divaricata	-	++	-	+++	-	++	-	-	-	-	-	+	+
Guazuma ulmifolia	-	+	-	+++	-	+++	-	-	-	-	-	+	+
Coccoloba cozumelensis	-	++	-	+	-	+++	-	-	-	-	-	-	-

(+++) High; (++) Moderate; (+) Low; (-) No presence

Chemical analysis

Dry and ground samples from the five treatments were analysed in triplicate for dry matter (DM), ash, crude protein (CP) and ethereal extract (EE) content according to AOAC (2006); while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the technique of Van Soest *et al*. (1991). The results are presented in Table 2.

Table 2. Chemical composition of te foliages of *Muntingia calabura* and *Bauhinia divaricata* alone or combined with *Pennisetum* sp

Treatment	DM[1]	ASH[2]	NDF[2]	ADF[2]	CP[1,2]	EE[1,2]
Mc	95.77	8.31	53.02	52.42	13.56	6.55
Bd	96.85	8.46	49.89	48.12	14.74	1.74
McP	96.45	10.97	69.35	57.83	7.94	6.42
BdP	96.56	10.69	68.02	55.24	8.12	1.78
P	96.94	11.84	76.85	59.02	6.02	1.99

[1]Values expressed in percentage; [2]Values expressed on a dry basis; P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd=*B. divaricata* (100%); McP=*M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP=*B. divaricata* (30%)/*Pennisetum* sp. (70%)

In vitro fermentation

The procedure for the collection of rumen fluid (RF) was carried out in strict accordance with the Mexican Official Standard NOM-062-ZOO-1999 "Technical Specifications for the Production, Care and Use of Laboratory Animals" (NOM-062-ZOO-1999., 1999). The RF used were obtained from four uncastrated male steers, from different crosses of Black Sardinian, Gyr, Brahman and Brown Swiss breeds, one year old and with an average weight of 250 kg. Their feed consisted of an integrated diet (70:30 forage:concentrate) composed of *Echynochloa polystachya* and *Brachiaria brizantha* grass straw, ground maize, dried distillers grains, molasses and mineral premix, which was offered twice a day (8:00 and 16:00 hours). Daily the steers consumed the equivalent of 3% DM of their live weight.

RF extraction was performed in the morning, one hour before the first ration of the day using a manual RF collector (Rumen-Mate with RFE, Drench-Mate®). The collected RF was placed in a thermos flask previously heated to 39°C and immediately transported to the laboratory. Once in the laboratory, the RF was filtered through four layers of sky blanket and it was used to prepare the inoculum, which was prepared according to the methodology of Menke *et al*. (1979). Subsequently, 50 mL of inoculum was added to 120 mL amber glass vials containing 0.5 g of each treatment, capped with a rubber stopper, sealed with an aluminium ring and placed in a water bath at 39°C, shaking manually every 2 hours. A total of three fermentations were carried out, with three replicates each.

In vitro dry matter degradability

In vitro dry matter degradability (DMD, mg/g DM) was determined at 24 and 72 hours. At the end of each time, the vials were removed from the water bath and placed in ice water for 2 hours to stop microbial activity. Subsequently, the contents of each vial were filtered with a vacuum pump; the filtered material was placed in an oven at 70°C for 24 hours until constant weight.

In vitro gas production

Gas production (GP) was recorded at 2, 4, 6, 8, 12, 12, 16, 16, 20, 24, 30, 36, 42, 48, 60 and 72 hours using a manometer (Theodorou *et al*., 1994). After each recording, the pressure in each bottle was equalized to zero. To obtain the maximum volume (Vmax), fermentation rate (S) and lag phase (L), the NLIN procedure of the statistical software SAS (2004) version 9.0 was used, using the model mentioned by Kholif *et al*. (2017).

CH_4 and CO_2 determination

The determination of CH_4 and CO_2 was performed according to Kholif *et al.* (2017), with the following modifications: every 6 h for 24 h, the gas produced in vials was collected with a 60 mL glass syringe. Subsequently this gas was transferred to another vial containing 50 mL of a NaOH (1N) solution, shaken to ensure the incorporation of the gas into this solution, collected again and the gas was recorded with the same glass syringe. The mixing of the gas with the NaOH solution allowed the absorption of CO_2, and the volume of gas collected in the syringe was considered as CH_4.

Determination of VFA

The determination of VFA was carried out by gas chromatography (Erwin *et al.*, 1961). After 72 hours of fermentation, 1.6 mL of liquid was collected from each vial, placed in a microcentrifuge tube with 0.4 mL of 25% metaphosphoric acid and stored at -20°C.

Metabolizable energy and partition factor

The calculation of metabolizable energy was performed following the model proposed by Menke *et al.* (1979). The partition factor (ratio of DMD (mg/g) to GP (ml/g DM), was calculated after 24 hours of incubation using the model mentioned by Kholif *et al*. (2017).

Experimental design and statistical analysis

A completely randomized block design was used using the following statistical model:

$$Yij = \mu + Ti + Fj + Eij$$

Where: Yij = is each observation of i-th treatment (Ti) of j-th fermentation (Fj); μ = arithmetic mean; Eij = experimental error.

The results were analysed with the GLM procedure of the statistical programme SAS (2004) version 9.0 and the comparison of means was performed by Tukey's test (P≤0.05).

RESULTS AND DISCUSSION

Presence of secondary metabolites and chemical analysis

The forage species evaluated had the presence of several MSec, but none showed the presence of alkaloids, quinones or laucoanthocyanidins (Table 1). It was observed that *M. oleifera*, *A. farnesiana* and *L. latisiliquum* species had a high content of gallic acid-derived tannins and *G. ulmifolia* in catechol-derived tannins; while *M. calabura* and *S. saman* species had a moderate content in gallic acid-derived tannins, and *B. divaricata* and *T. diversifolia* species in catechol-derived tannins. Regarding saponins, the species with the highest presence of steroids were *M. calabura*, *L. latisiliquum*, *T. diversifolia*, *S*.

*acut*a, *A. farnesiana* and *S. saman*, while only *M. oleifera* had a low presence of terpenoids. From the flavonoids group, the forage species with a high presence of xanthones and flavones were *M. calabura*, *M. oleifera* and *A. farnesiana*, while *S. saman* had a moderate presence; the rest of the species had a low presence, with the exception of *T. diversifolia*, which was the only species with a low presence of aurones and chalcones.

Secondary metabolites are compounds produced in different pathways of plant secondary metabolism, which are not essential for growth and reproduction. These biomolecules perform various functions, notably environmental stress response, immunity and protection against pathogenic microorganisms, pests (Pang *et al*., 2021) and herbivorous animals (Ugbogu *et al*., 2019). It has been shown that some of these secondary metabolites may have antimicrobial activity on ruminal microorganisms, with decreased ruminal methanogenesis being one of their main effects (Patra *et al*., 2017), which has been widely reported for tannins and saponins (Anantasook *et al*., 2013; Ugbogu *et al*., 2019; Patra *et al*., 2017) and to a lesser extent for flavonoids (Patra *et al*., 2017). Contrary to Delgado *et al.* (2012), who reported moderate or high presence of alkaloids in mixtures of tree foliages with grasses, this metabolite was not detected in the present study. Alkaloids possess antimicrobial activity but have also been shown to be toxic to both animals and humans. In general, animals do not consume high amounts of plants with high alkaloid content due to their bitter taste (Guil-Guerrero *et al*., 2016).

The selection of *M. calabura* and *B. divaricata* was due to their phytochemical characteristics and the scarce information on their effects on ruminal fermentation. The nutritional value of the two tree species evaluated in this study is similar to that reported in other studies (Table 2). According to Gómez-Fuentes-Galindo *et al.* (2017), *B. divaricata* has lower NDF content (46%) and higher CP content (12.8%) than grasses such as *Panicum maxima* or *Paspalum langei* which have on average 70% NDF and 6.5% CP, characteristics that could enhance a higher voluntary intake and a faster passage rate. Other authors have reported that *B. divaricata* has between 12 and 18% CP, between 30 and 40% *in vitro* ruminal digestibility, between 1.5 and 3.8% tannins, and the presence of saponins and alkaloids (Albores-Moreno *et al*., 2018; Cab-Jiménez *et al*., 2018; Gómez-Fuentes-Galindo *et al*., 2017; Sosa-Rubio *et al*., 2004). Regarding *M. calabura*, nutritional information is practically non-existent. Kongvongxay *et al*. (2011) reported that *M. calabura* leaves contain 13% CP, but they point out that little is known about its nutritional value as it is little used for animal feed. Regarding the content of secondary metabolites, its fruits (Pereira *et al.*, 2018) and leaves (Pujaningsih *et al.*, 2018) have been reported to contain bioactive compounds with antioxidant and antimicrobial activity, among which several phenolic compounds, including anthocyanins and flavonoids, stand out.

In vitro dry matter degradability

Table 3 shows the results of the DMD at 24 and 72 h of *M. calabura* and *B. divaricata* foliage alone and combined with *Pennisetum* sp. and shows differences between treatments (P≤0.05). The treatments with the highest DMD at 24 h were BdP, followed by P and Bd; however, at 72 h they were P, followed by BdP and McP. *M. calabura* foliage alone had the lowest DMD at both times, which is in agreement with Puspitaning (2012), who mentions that 20% *M. calabura* in the diet decreases DMD. On the other hand, the DMD of *B. divaricata* was higher (53%) than that reported by other authors (32 and 39%) ((Albores-Moreno *et al.*, 2018; Sosa-Rubio *et al*., 2004).

Table 3. *In vitro* dry matter degradability of *Muntingia calabura* and *Bauhinia divaricata* foliage alone and in combination with *Pennisetum* sp

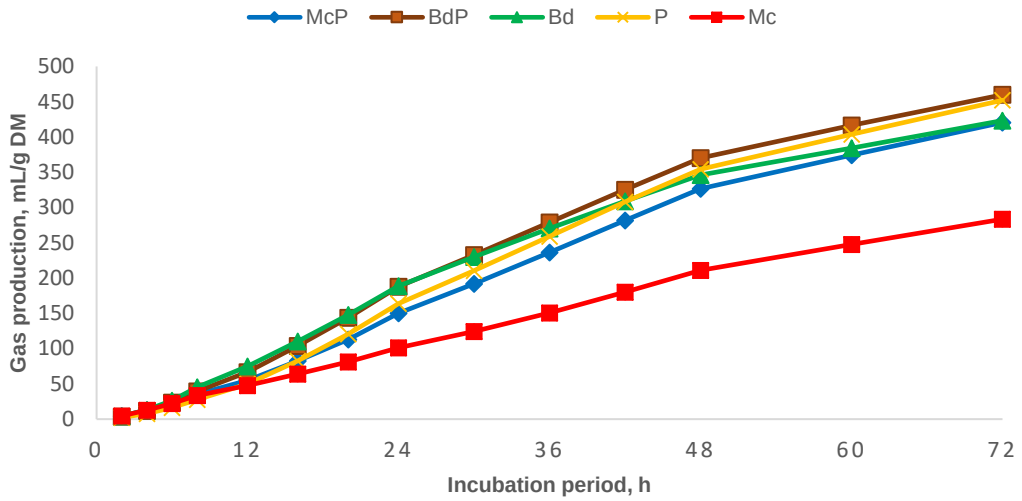
Treatment	DMD (mg/g DM)	
	24h	72h
Mc	231.62^{c}	404.57^{d}
Bd	404.78^{ab}	528.85^{c}
McP	377.86^{b}	570.71^{b}
BdP	461.82^{a}	592.52^{ab}
P	422.86^{ab}	628.33^{a}
S.E.M	18.41	13.31

P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd= *B. divaricata* (100%); McP= *M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP= *B. divaricata* (30%)/*Pennisetum* sp. (70%); S.E.M: Standard error of the mean; $^{a, b, c, d}$, different literals in the same column indicate statistically significant differences (P<0.05)

Kamalak *et al.* (2004) conclude that cell wall content in forages negatively affects ruminal fermentation parameters. Zhang *et al*. (2017) emphasized that the incorporation of foliage of some tropical tree and shrub species in grass-based ruminant diets increases CP content and decreases total structural carbohydrate content in the diet. Such statements were partially observed in this study. Treatments Mc and Bd (corresponding to foliages alone) had ≈33% lower NDF content and ≈57.5% higher CP content, compared to P, which is similar to that reported by Gómez-Fuentes-Galindo *et al.* (2017), who reported NDF and CP contents of 46.5% and 12.8%, respectively, for *B. divaricata*. However, the lower NDF and higher CP content of *M. calabura* and *B. divaricata* did not improve DMD, which contrasts with those reported for other foliages, such as *L. leucocephala*, which increases DMD by 18% when included at 25% in grass-based cattle diets (Molina *et al*., 2015). In the present study, the inclusion of *M. calabura* negatively affected DMD (P≤0.05), as it was 10% lower in the McP treatment than in P.

In vitro gas production

GP is presented in Figure 1. There were no significant differences (P>0.05) during the 72 h of fermentation between treatments Bd, BdP, McP and P, which had an average cumulative GP of 439 mL/g DM. However, there were significant differences (P≤0.05) with Mc, which had a cumulative GP of 283.49 mL/g DM. Albores-Moreno *et al*. (2018) reported a GP of 200.59 mL/g DM at 72h with *B. divaricata* foliage, which is 53% lower than that observed in the present study. On the other hand, the GP of *M. calabura* foliage reported by Silivong *et al*. (2013) was 269 mL/g DM at 24 h, 166% higher than that obtained with Mc. These variations in GP can be attributed to differences in forage composition, generally when they present high concentrations of CP, fiber and polyphenol content (Vargas *et al.*, 2012). In our study, Mc was the treatment with the lowest GP and degradability.



Where: P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd= *B. divaricata* (100%); McP= *M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP= *B. divaricata* (30%)/*Pennisetum* sp. (70%)

Figure 1. Foliage gas production of *Muntingia calabura* and *Bauhinia divaricata* alone and combined with *Pennisetum* sp

In vitro fermentation characteristics and CH_4 and CO_2 production

The results of Vmax, S, L, CH_4 and CO_2 production, ME and PF are shown in Table 4. The Vmax and S of the Mc treatment was significantly lower (P≤0.05) than the rest of the treatments; however, the L of Bd and Mc (the foliage-only treatments) was about 20% lower than the L of the McP, BdP and P treatments. Regarding CH_4 production there were also significant differences (P≤0.05), with Mc being the treatment with the highest CH_4 production, while Bd and BdP had the lowest production. In relation to ME content, it was observed that the highest values were presented by Bd and BdP, being close to 2 Mcal/kg DM, which were significantly different (P≤0.05) to Mc and McP treatments, with 1.5

Mcal/kg DM on average. There were no differences (P>0.05) in PF at 24 h, but there were differences at 72 h (P≤0.05) between treatments with foliage alone, being higher with Mc and lower with Bd.

Table 4. *In vitro* fermentation characteristics and CH_4 and CO_2 production of *Muntingia calabura* and *Bauhinia divaricata* foliages alone and in combination with *Pennisetum* sp

Variable	Treatment					S.E.M
	Mc	Bd	McP	BdP	P	
Vmax, mL/g DM	288.28^{b}	403.71^{a}	414.88^{a}	444.28^{a}	439.47^{a}	10.88
S, h^{-1}	0.019^{c}	0.025^{a}	0.022^{b}	0.02^{ab}	0.024^{ab}	0.00
L, h	8.829^{bc}	7.63^{c}	10.602^{ab}	9.24^{abc}	11.426^{a}	0.32
CO_2, % 24h	85.30^{d}	96.38^{a}	89.88^{bcd}	92.78^{ab}	87.86^{cd}	0.94
CH_4, % 24h	14.70^{a}	3.62^{d}	10.12^{bcd}	7.22^{cd}	12.04^{ab}	0.94
CO_2, mL/g DM 24h	350.22	332.23	339.79	367.23	365.70	11.12
CH_4, mL/g DM 24h	60.33^{a}	16.56^{c}	45.12^{ab}	28.20^{bc}	50.21^{a}	4.04
ME, Mcal/kg DM	1.37^{d}	1.95^{a}	1.61^{c}	1.86^{ab}	1.67^{bc}	0.04
PF 24h*	3.18	2.58	3.05	2.86	3.06	0.09

P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd= *B. divaricata* (100%); McP= *M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP= B. *divaricata* (30%)/*Pennisetum* sp. (70%). Vmax: Maximum volume; PF: Partition factor (mg DM degraded/mL gas produced); L: lag phase; S: fermentation rate; ME: Metabolizable energy; IVDDM: *In vitro* digestibility of dry matter; S.E.M: Standard error of the mean. $^{a, b, c,}$ different literals in the same row indicate statistically significant differences (P<0.05). * mg DM degraded /mL gas produced at 24 hours

The behavior of ruminal fermentation depends on the availability of carbohydrates present in the forages (Zhang *et al*., 2017), so in this study, treatments with higher Vmax of GP could be related to a higher degradation of structural carbohydrates present in the foliage. While the low Vmax in Mc may be due to a low concentration of the soluble fraction of the feed, decreasing the amount of gas produced. In addition, it is likely that the presence of MSec such as saponins and flavonoids may have affected DM fermentation and hence GP. These MSec interact directly on the cytoplasmic membrane function of the rumen microorganisms by inhibiting cell wall synthesis, creating a defaunation effect, thereby decreasing the protozoa and consequently the associated methanogenic archaea and thus decreasing rumen methanogenesis. In the rumen, protozoa are associated with methanogenic archaea and their relationship can generate between 9 and 37% of the total enteric CH_4 emissions produced by ruminants (Rivera *et al.*, 2018; Vargas *et al*., 2012). Meanwhile, Velez-Terranova *et al*. (2014) and Lakhani & Lakhani, 2018 note that plants with high flavonoid content decrease CH_4 production and induce extensive stimulation of microbial metabolism in the rumen, which increases both CP degradability and cell wall constituents by enhancing fermentation by up to 50%. In this regard, Silivong *et al.* 2013 reported that CH_4 production in the *in vitro* fermentation of *M. calabura* was only 3.8%, a

concentration considerably lower than that obtained for *L. leucocephala* (7.8%) or *G. sepium* (15. 6%). It contrasts with what was reported in this study, in which the preliminary chemical analysis showed that the *M. calabura* foliage alone has a high presence of saponins and flavonoids, and a moderate presence of tannins derived from gallic acid; however, in the *in vitro* test, the gas produced with Mc had a higher CH_4 concentration, close to 15%.

Table 5. Concentration of volatile fatty acids from *in vitro* ruminal fermentation of *Muntingia calabura* and *Bauhinia divaricata* foliages alone and in combination with *Pennisetum* sp

VFA	Treatment					S.E.M
	Mc	Bd	McP	BdP	P	
Acetic acid	46.20[a]	38.01[b]	38.76[ab]	39.35[ab]	42.53[a]	0.997
Propionic acid	26.16	26.03	26.87	27.40	28.95	0.487
Butyric acid	19.54	18.84	22.30	21.00	20.01	0.457
Isobutyric acid	3.10	3.64	4.13	4.59	2.63	0.247
Valeric acid	2.32	3.74	3.31	3.08	2.48	0.165
Isovaleric acid	2.68[a]	5.16[b]	4.64[ab]	4.59[ab]	3.40[ab]	0.271

P=*Pennisetum* sp. (100%); Mc=*M. calabura* (100%); Bd= *B. divaricata* (100%); McP= *M. calabura* (30%)/*Pennisetum* sp. (70%), and BdP= *B. divaricata* (30%)/*Pennisetum* sp. (70%); S.E.M: Standard error of the mean. [a, b, c,] different literals in the same row indicate statistically significant differences (P<0.05)

Volatile fatty acids

No significant differences (P>0.05) were observed in VFA concentration, except for acetic acid between Mc and P with Bd, and isovaleric between Mc and Bd (P≤0.05; Table 5). There are no studies evaluating VFA production from ruminal fermentation using *M. calabura* or *B. divaricata* as substrate; however, the modification in ruminal VFA production may be influenced by the presence of MSec in the diet. In this regard, Broudiscou & Lassalas, (2000) conducted a study where they evaluated the effect of dry extracts of *Lavandula officinalis* and *Equisetum arvense*. Ttwo species known for their high flavonoid content, on ruminal fermentation *in vitro* and found that the use of the extract of both species improved the fermentation rate by 50% by increasing the production of acetate and propionate, thus reducing CH_4 production. In the present study, the foliage of *M. calabura* showed a high flavone content, while in the foliage of *B. divaricata* it was low, which may have influenced the higher concentration of acetic acid in the Mc treatment. On the other hand, the higher concentration of isovaleric acid in Bd could have been because of the higher CP content of this foliage (almost 15% in BS), which is produced from the decarboxylation and deanimation of leucine, a branched-chain amino acid (Apajalahti *et al*., 2019).

CONCLUSION

The use of *B. divaricata* foliage with *Pennisetum* sp. decreased CH_4 production and concentration and has a higher ME content, without negatively affecting other rumen fermentation variables. Therefore, the addition of *B. divaricata* in ruminant diets represents an alternative feed that can decrease CH_4 production from rumen fermentation of cattle in tropical regions. However, given the scarcity of information on this subject, it is highly recommended to carry out further studies to confirm this.

Acknowledgements

Authors thank the National Council of Science and Technology (CONACYT) of Mexico for the grant awarded to Adrián Adolfo Sandoval-Pelcastre, as well as the funding for project 417 "Transcriptomic analysis of the rumen microbiota of cattle fed with tropical forages and its correlation with the production of greenhouse gases" of the Call to address national problems 2015, awarded to Dr. Mónica Ramírez-Mella.

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Errata Erratum

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