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Laboratory evaluation of ripe carob, parota and cubato pods

Evaluación a nivel de laboratorio de vainas maduras de algarrobo, parota y cubato

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ABSTRACT

In the tropics, collecting legume pods to feed livestock during the dry season is a common practice; however, they require nutritional characterization. The objective was to determine the chemical composition, gas production, and fermentation kinetics estimators using the *in vitro* gas production technique for pods of rain tree, devil's ear tree, and boat-thorn acacia. Pods of rain tree (*Samanea saman*), boat-thorn acacia (*Acacia cochliacantha*), and devil's ear tree (*Enterolobium cyclocarpum*) were obtained to determine nutrient content, *in vitro* gas production, gas production kinetics, and fermentation fractions. A completely randomized experimental design was used. Mean differences were assessed using Tukey's test. Rain tree pods had lower ash, neutral detergent fiber, nitrogen-free extract, and rapidly fermentable fraction contents; boat-thorn acacia pods had lower organic matter and metabolizable energy contents; devil's ear tree pods showed higher protein content, cumulative gas production during the first 12 h and at 72 h of incubation, slow and total fermentation fractions, maximum gas production potential, and lag time phase ($p < 0.05$). In conclusion, mature devil's ear tree pods exhibited superior chemical-bromatological and *in vitro* fermentative features at the laboratory level, making them a viable alternative for ruminant feeding.

KEY WORDS: Gas production, *in vitro*, fermentation fractions, gas production kinetics, legumes, tropics, bromatology.

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RESUMEN

En el trópico, la recolección de vainas de leguminosas para alimentar al ganado durante el período seco es una práctica común, pero requieren caracterización nutrimental. El objetivo fue determinar el contenido químico, producción de gas y estimadores de la cinética de fermentación con la técnica de producción de gas *in vitro* de las vainas de algarrobo, parota y cubato. Se obtuvieron vainas de algarrobo (*Samanea saman*), cubato (*Acacia cochliacantha*) y parota (*Enterolobium cyclocarpum*) para determinar contenido de nutrientes, producción de gas *in vitro*, cinética de producción de gas y fracciones de fermentación. El diseño experimental fue completamente al azar. La diferencia entre medias fue con la prueba de Tukey. La vaina de algarrobo presentó menor contenido de cenizas, fibra detergente neutro, extracto libre de nitrógeno, fracción rápida de fermentación; la vaina de cubato presentó menor contenido de materia orgánica y energía metabolizable; la vaina de parota mostró mayor contenido de proteína, producción acumulada de gas en las primeras 12 h y a 72 h de incubación, fracciones lenta y totales de fermentación, máximo potencial producción de gas y fase de retardo del tiempo lag ($p < 0.05$). Se concluye, la vaina madura de parota mostró mejores características químico-bromatológicas y fermentativas *in vitro* a nivel laboratorio, por lo que es una alternativa para usarse en la alimentación de rumiantes.

PALABRAS CLAVE: Producción gas, *in vitro*, fracciones fermentativas, cinética de producción gas, leguminosas, trópico, bromatológico.

Introduction

Tropical leguminous trees can enhance the nutritional value of animal feed. They are used as sources of forage, fuelwood, or timber in Africa, Asia, Australia, and the Americas. The use of pods has attracted research interest from institutions investigating their application in these regions. Some species are now widely distributed and utilized outside their native range. The foliage, flowers, and pods of leguminous trees can be used, even though these trees are often planted primarily for shade (Blair *et al.,* 1990).

Forage trees and shrubs play an important role in ruminant feeding as protein suppliers under adverse environmental conditions. They represent an alternative protein source for ruminants in the tropics (Devendra, 1991; Zapata-Campos *et al.,* 2020). The collection of pods to feed livestock during the dry season is a common practice among small-scale farmers in dry tropical areas; however, this practice has not been widely adopted due to the difficulty involved in harvesting pods (Esquivel-Mimenza *et al.,* 2014).

The rain tree (*Samanea saman*) belongs to the Fabaceae family and is native to the dry tropics of the Americas, ranging from Mexico to South America (Cisneros, 2018). It is a deciduous tree 25-30 m tall, with a broad, umbrella-shaped crown whose diameter exceeds the tree's height. The fruit is an elongated, compressed, indehiscent pod measuring 10-22 cm in length, 1.5-2.2 cm in width, and 0.5-1 cm in thickness. Pods are green and fleshy before maturity, turning brown upon ripening. The mesocarp is pulpy and sweet, containing 5-10 seeds per pod. Seeds are ellipsoid, strongly biconvex, 8-11.5 mm long and 5-7.5 mm wide, with a characteristic U-shaped pleurogram (Cisneros, 2018; ForrajesTropicales, 2024). Pods are harvested when mature, between February and June, with an average yield of 200-250 kg of pods per tree per season. These pods contain 16.4 % protein, 35.65 % neutral detergent fiber, 66.3 % dry matter digestibility, and secondary metabolites such as alkaloids, condensed tannins, saponins, nitrogenous compounds, glycosides, resins, and mucilages. Despite these compounds, pods are an important forage supplement for cattle, sheep, goats, and pigs during the dry season (Cisneros, 2018).

The devil's ear tree (*Enterolobium cyclocarpum*) belongs to the Fabaceae family and is native to tropical America, ranging from western and southern Mexico to Venezuela and Brazil. In Mexico, it occurs in states such as Campeche, Chiapas, Colima, Guerrero, and Jalisco, among others, as a component of disturbed vegetation in tropical zones (Rosales, 2018). It is a showy, fast-growing, deciduous forage legume that reaches up to 30 m in height, with a deep and extensive root system (Rosales, 2018; Feedipedia, 2024). The species has been reported in agroforestry systems in Veracruz and Yucatán, as well as scattered for shade and browsing in agricultural areas. The fruit is a woody, circular, indehiscent pod measuring 7-15 cm in diameter, flattened and coiled like an ear. Mature pods are shiny, dark brown, sweet-tasting, and contain 10-15 seeds (Rosales, 2018; Feedipedia, 2024). In Jalisco, it is used for fruit production as a forage source (Rosales, 2018). During the dry season, brown pods can be seen hanging high in the tree canopy (Feedipedia, 2024; Rosales, 2018). The fruits are a nutritional alternative for livestock in regions with pronounced dry seasons, as they can contain up to 30 % protein, 60.45 % nitrogen-free extract, and approximately 69 % digestibility (Rosales, 2018).

The boat-thorn acacia (*Acacia cochliacantha*), synonymous with *A. milleriana* Standl., is classified within the Fabaceae family and is native to tropical America, with a heterogeneous distribution. In Mexico, it ranges from southern Sonora to Chiapas and occurs discontinuously along the Atlantic slope. Its uses include shade trees in pastures, live fences, incorporation into secondary vegetation, posts, firewood, and shade (Pinto *et al.,* 2018). It is a shrub or tree up to 20 m tall with a trunk diameter of 5-10 cm, armed with concave, spoon-shaped stipular spines up to 3.5 cm long, whitish when mature and reddish when young (Pinto *et al.,* 2018; iNaturalistMexico, 2024). The fruit is woody, dark purple-brown, 5-13 cm long, each containing eight seeds, found singly or in clusters at the axils of the spines. Pods contain approximately 12 % protein, 4.3 Mcal/kg DM gross energy, 45 % neutral detergent fiber, and 56 % ruminal degradability (Pinto *et al.,* 2018). The use of pods from these legumes as a forage resource in grasslands could improve the sustainability of livestock production units during periods when herbaceous forage is scarce or of low quality (Zapata-Campos *et al.,* 2020). Studies have evaluated the use of such pods in animal feeding as part of a ration (Albores-Moreno *et al.,* 2017; Carbajal-Márquez *et al.,* 2019; Molina-Botero *et al.,* 2019), but few have examined pods individually (Hernández-Morales *et al.,*

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2018). Therefore, the objective was to determine the chemical composition, gas production, and estimators using the *in vitro* gas production technique for pods of rain tree, devil's ear tree, and boat-thorn acacia.

Materials and Methods

Geographic location of the study

The research was conducted at the Animal Nutrition Laboratory of the Faculty of Veterinary Medicine and Animal Science No. 2 of the Universidad Autónoma de Guerrero, located in the Cuajinicuilapa municipality, Guerrero, Mexico.

Pods

Pods of rain tree (*Samanea saman*), boat-thorn acacia (*Acacia cochliacantha*), and devil's ear tree (*Enterolobium cyclocarpum*) were obtained from four randomly selected branches of each tree (10 trees per evaluated species). All physiologically mature pods were harvested, placed in paper bags, and transported to the laboratory. Pods were dehydrated at 60 °C for 48 h in an oven (RIOSSA HCF-41, Mexico) and then ground using a 1-mm screen in a Thomas-Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA).

Proximate analysis

In samples from each pod type (10 replicates per type), total nitrogen content was determined using the Kjeldahl method (method 976.05), and crude protein (CP) content was estimated by multiplying nitrogen content by 6.25. Ash content was determined by incineration at 600 °C for 3 h (method 942.05). Ether extract (EE) was obtained with ether extraction at 80 °C (method 20.39-1920), according to AOAC official methods (AOAC, 2023). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined following the ANKOM Technology method, with ash correction according to Van Soest *et al.* (1991). Organic matter (OM) was calculated as 100 minus ash percentage, while nitrogen-free extract (NFE) was calculated as 100 minus the sum of CP, NDF, A, and EE contents.

In vitro gas production technique

The culture medium consisted of two-thirds reduced buffer-mineral solution and one-third fresh ruminal fluid. The reduced buffer-mineral solution was prepared as described by Hernández-Morales *et al.* (2018). Fresh ruminal fluid was collected from a fistulated bovine grazing on pangola grass pastures and filtered through cheesecloth to remove large organic matter particles. The bovine was managed according to the internal bioethics and welfare regulations of UAGro, in compliance with official standards (NOM-062-ZOO-1999, 1999).

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Biodigesters (20 replicates per pod type) consisted of 120 mL serum vials containing 0.5 g of constant-weight pod sample, to which 50 mL of culture medium was added under continuous CO_2 flow. Vials were sealed with neoprene stoppers and aluminum crimps with removable centers. Biodigesters were incubated in a water bath at 39 °C for 72 h. Gas production was measured as the displacement of the plunger of a glass syringe at 3, 6, 9, 12, 24, 48, and 72 h of incubation. A blank biodigester (three replicates) without a pod sample was used to adjust gas production values for gas originating from the inoculum.

Gas production data were used to estimate maximum gas production potential (*A,* mL/g DM), gas production rate of potentially degradable material (*b*, mL/h), and lag time phase (*k,* h) using the Gompertz model: Y = $A * \{\exp[-b * \exp(-k * t)]\}$, where *Y* = gas volume at time t (mL/g DM), *A* = total gas production potential at t = ∞ (mL/g DM), *b* = constant gas production rate of potentially degradable material (mL/h), *k* = lag time (h), microbial efficiency constant defined as the intercept of the time axis by the tangent line at the inflection point, and *t* = incubation time (Lavrenčič *et al.,* 1997). Gas volume was also used to determine fermentable fractions: cumulative gas production (GP) from 0 to 9 h (rapid fermentation fraction (RF); RF (mg/g) = GP/0.427), > 9 to 24 h (medium fermentation (MF); MF (mg/g) = GP/0.615), and > 24 h (slow fermentation (SF); SF (mg/g) = GP/0.345). The sum of the three fractions represented the total fermentable fraction (TFF; mg/g) (Miranda-Romero *et al.,* 2015). Organic matter degradability (OMD) was estimated using equation 1 (Menke & Steingas, 1988), while metabolizable energy (ME) content was estimated using equation 2 (Jarrige, 1989), based on digestible energy (DE) estimated by equation 3 (Arruda & Ribeiro, 2009) and gross energy (GE) by equation 4 (McDowell *et al.,* 1974).

OMD (%) = 14.88 + 0.889 Gp + 0.45 CP + 0.0651 A [Ec. 1]

ME (Mcal/kg) = 0.82 * DE [Ec. 2]

DE (Mcal/kg) = 0.6592 * GE [Ec. 3]

GE (Mcal/kg) = 5.72(CP/100) + 9.5(EE/100) + 4.79(NDF/100) + 4.7(NFE/10) [Ec. 4]

Where: OMD = organic matter degradability; Gp = mL of gas produced per 200 mg DM during 24 h of fermentation; CP = crude protein (%); A = ash (%); ME = metabolizable energy; DE = digestible energy; GE = gross energy; EE = ether extract (%); NDF = neutral detergent fiber (%); NFE = nitrogen-free extract (%).

Statistical analysis

Proximate analysis and *in vitro* gas production variables were analyzed using a completely randomized design. Data were analyzed with the GLM procedure of the SAS statistical package (2011). Mean differences were compared using Tukey's test (α = 0.05).

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Results and Discussion

Rain tree pods showed the lowest ash and NDF contents but the highest EE and NFE contents, whereas boat-thorn acacia pods had the lowest OM and ME contents. In contrast, devil's ear tree pods presented the highest CP content, while boat-thorn acacia pods had the highest dry matter (DM) and ADF contents ($p < 0.05$; Table 1). This suggests that, although these are mature plant structures, rain tree pods contain less fiber, which was reflected in their OMD (Table 2). This can be explained by the fact that in mature plant structures, lignification begins; in trees, lignin synthesis intertwines with cellulose to provide structural support to pods, which in turn affects their ruminal degradation (Hoffman *et al.,* 2007).

According to FEDNA (2019), for an ingredient to be considered a protein source, it must contain at least 20 % CP; therefore, rain tree and devil's ear tree pods are classified as protein ingredients. Lower values of NDF (343.7 g/kg DM), Ash (42.9 g/kg DM), and CP (16.8 g/kg DM), as well as higher values of ADF (250.6 g/kg DM), have been reported for mature *S. saman* pods; lower values of ADF (204.0 g/kg DM), Ash (41.3 g/kg DM), and CP (195.0 g/kg DM), along with higher NDF content (283.5 g/kg DM), have been reported for devil's ear tree pods compared to those in the present study; higher values of NDF (573.6 g/kg DM), ADF (455.4 g/kg DM), and Ash (52.2 g/kg DM), and lower CP content (109.1 g/kg DM) have been reported for boat-thorn acacia pods collected in the dry tropics of Guerrero, Mexico (Hernández-Morales *et al.,* 2018). Additionally, *S. saman* pods collected in Mokola, Ibadan, Nigeria, showed higher NDF (530.0 g/kg DM), ADF (420.0 g/kg DM), Ash (50.0 g/kg DM), and CP (245.0 g/kg DM) contents, and lower EE content (150.0 g/kg DM) compared with the same pod species evaluated in this study (Babayemi *et al.,* 2010). Variations in these results are attributed to agronomic conditions affecting the trees, as well as climatic conditions during flowering and pod harvesting (Muñoz-González *et al.,* 2016).

Table 1. Proximate analysis and metabolizable energy of rain tree, boat-thorn acacia, and devil's ear tree mature pods.

Variable	Rain tree	Boat-thorn acacia	Devil's ear tree	SEM
DM (g/Kg DM)	973.0[b]	993.9[a]	944.7[c]	3.9
Ash (g/Kg DM)	30.7[c]	44.1[a]	37.2[b]	1.2
NDF (g/kg DM)	366.8[c]	734.8[a]	457.6[b]	29.8
ADF (g/kg DM)	231.2[b]	526.1[a]	245.7[b]	26.6
CP (g/kg DM)	234.7[b]	170.1[c]	290.0[a]	9.5

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Continuation

Table 1. Proximate analysis and metabolizable energy of rain tree, boat-thorn acacia, and devil's ear tree mature pods.

Variable	Rain tree	Boat-thorn acacia	Devil's ear tree	SEM
EE (g/kg DM)	16.0[a]	4.5[b]	7.6[b]	1.8
NFE (g/kg DM)	351.9[a]	46.5[c]	207.6[b]	23.6
OM (g/kg DM)	969.3[a]	956.0[c]	962.8[b]	1.2
ME (Mcal/kg DM)	2.65[a]	2.57[b]	2.65[a]	0.01

[a,b,c] Values with different letters per row indicate differences (p≤ 0.05).

DM= Dry matter, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, CP= Crude protein, EE= ethereal extract, NFE= nitrogen free extract, OM= Organic matter, ME= metabolizable energy, SEM = standard error of the mean.

Source: Own elaboration.

The estimation of energy content was particularly important given that values for these pods are scarce or even absent in the literature, making it necessary to determine their energy content (Mendoza *et al.,* 2022). Energy estimation can be carried out through the gas production technique (Menke & Steingas, 1988) or proximate analysis (Jarrige, 1989). Accordingly, the present study applied a series of equations that allowed for the estimation of ME content for use in future research requiring this information to formulate rations for ruminants in which any of the pods evaluated here might be included.

Cumulative gas production showed that rain tree pods yielded greater gas volumes at 3, 6, 9, and 12 h ($p < 0.05$) compared with the other pods; however, at 24 and 48 h, both devil's ear tree and rain tree pods produced the highest average gas volumes, with 166 and 198 mL/g DM, respectively. At 72 h, devil's ear tree pods once again produced the highest cumulative gas volume ($p < 0.05$; Figure 1). With the gas production technique, carbohydrate fermentation yields volatile fatty acids, methane, carbon dioxide, and hydrogen as end products. Therefore, greater gas production can be taken to indicate greater availability of carbohydrates (Sobalvarro-Mena *et al.,* 2020) for ruminal microbial fermentation. During the first 24 h, this can be attributed to the high availability of non-structural carbohydrates (Texta *et al.,* 2019), suggesting that rain tree pods had higher non-structural carbohydrate availability, consistent with their NFE content (Table 1).

Beyond 24 h, fermentation of structural carbohydrates occurs (Texta *et al.,* 2019), with devil's ear tree pods showing greater availability of this carbohydrate fraction compared with rain tree pods. Between 24 and 48 h, gas production did not differ between these two pods, with devil's ear tree pods producing 80 mL/g DM and rain tree pods 43 mL/g DM (Figure 1). Furthermore, devil's ear tree pods exhibited higher gas production at 72 h, indicating greater availability of carbohydrates for *in vitro* ruminal fermentation.

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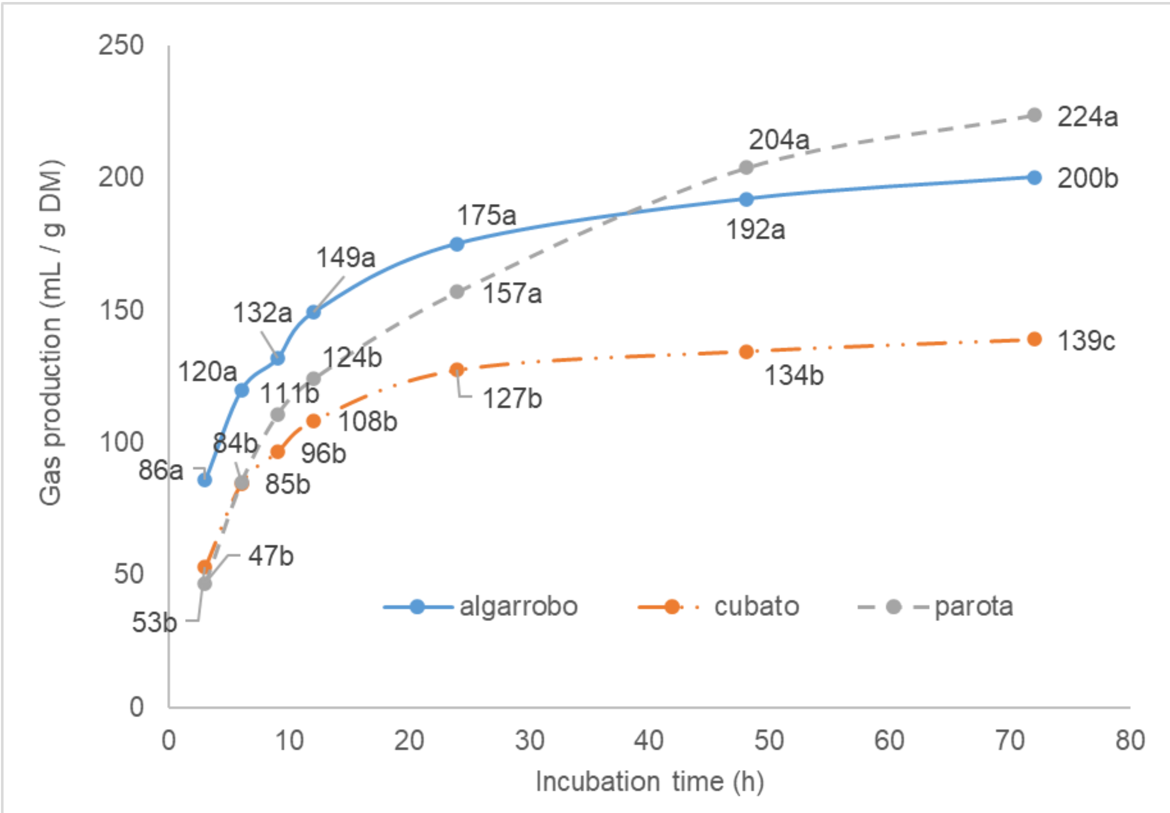

Figure 1. *In vitro* cumulative gas production of rain tree, boat-thorn acacia and devil's ear tree of mature pods.

Source: Own elaboration based on data collected using the *in vitro* gas production technique

Babayemi *et al.* (2010) reported gas production from *S. saman* pods of 10.0, 18.3, 23.3, 28.3, and 63.3 mL/g DM at 3, 6, 9, 12, and 24 h of incubation, respectively, values lower than those obtained for the same pod species in the present study. Hernández *et al.* (2017) reported gas productions of 160 and 240 mL/g DM at 72 h of incubation for rain tree and devil's ear tree pods collected in Cuajinicuilapa, Guerrero, Mexico, respectively; these were lower for rain tree pods and higher for devil's ear tree pods compared to the present study. García-Montes de Oca *et al.* (2011) reported an *in vitro* gas production of 155 mL/g DM for devil's ear tree pods collected in Valle de Bravo, State of Mexico, Mexico, values below those observed here.

As fermentation progresses, the gas produced and the gas accumulation profile provide information on fermentation fractions, feed digestibility, and fermentation kinetics (Getachew et al., 1998). Rain tree pods showed the highest FR, whereas devil's ear tree pods exhibited the highest FL and TFF ($p < 0.05$). Rain tree and devil's ear tree pods showed no differences in the moderately fermentable fraction (FM) ($p > 0.05$). It should be noted that boat-thorn acacia pods had the lowest

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MF, SF, and TFF values ($p < 0.05$; Table 2). The estimation of these fractions can be attributed to soluble sugars and oligosaccharides for RF; storage polysaccharides such as starch, dextrans, and pectin for MF; and cell wall polysaccharides such as cellulose and hemicellulose bound to lignin for SF (Sandoval-González *et al.,* 2017; Miranda-Romero *et al.,* 2020). This confirms that rain tree pods have a higher content of rapidly fermentable nutrients, as indicated by their proportion of NFE (Table 1). In contrast, devil's ear tree pods had higher structural carbohydrate content (Table 1) and showed better *in vitro* fermentation than the other pods evaluated. Comparisons of these fractions under the present study conditions are limited, as the literature mostly reports studies on *Leucaena leucocephala* in association with *Cynodon nlemfuensis* (Sandoval-González *et al*., 2017; Miranda-Romero et al., 2020) or sorghum silage (Ramírez-Díaz et al., 2020), among others, using mathematical models similar to those applied here.

OMD did not differ between rain tree and devil's ear tree pods ($p > 0.05$), averaging 523.3 g/kg OM. Babayemi *et al.* (2010) reported OMD of 404.2 g/kg OM in *S. saman* pods, lower than the present findings. Mendoza *et al.* (2022) noted that OMD can be estimated from dry matter degradation (DMD) using Eq. 5; accordingly, Hernández-Morales *et al.* (2018) obtained OMD values of 607.3 and 607.2 g/kg OM for rain tree and devil's ear tree pods, respectively, which are higher than those reported in the present study.

$$\text{OMD (g/kg OM)} = \text{DMD} * 0.92 \qquad \text{[Eq. 5]}$$

Gas production kinetics indicated that devil's ear tree pods had the highest *A* ($p < 0.05$), consistent with their higher gas volumes in both direct measurement (Figure 1) and mathematical modeling (Table 2). Likewise, devil's ear tree pods had a higher *k* value, suggesting that microorganisms took longer to adhere to the pod particles during the gas production assay. This is consistent with the type of carbohydrates available for fermentation, as the higher content of structural carbohydrates means ruminal bacteria require more time to attach before initiating fermentation (Dunne, 2002; McDonald *et al.,* 2012). In contrast, the pods evaluated in the present study showed no differences in *b* ($p > 0.05$), indicating that the fermentation rate was similar for all pods, but the adhesion time, availability, and composition of structural and non-structural carbohydrates influenced the variables analyzed in this study. Lower *A* and *k* values have been reported for rain tree pods: *A* = 161.7 mL/g DM and *b* = 0.04 (Torres-Salado *et al.,* 2018); *A* = 63.35 mL/g DM and *b* = 0.05 (Babayemi *et al.,* 2010). Similarly, for devil's ear tree pods, *A* values were similar but *b* values were lower (*A* = 223.0 mL/g DM and *b* = 0.04) (Torres-Salado *et al.,* 2018). However, beyond these factors, it should be considered that this is a laboratory technique, and variables such as inoculum source, gas venting system, substrate particle size, inoculum-to-buffer ratio, and concentrate addition to the medium can influence the results obtained in the present study (Yang, 2017).

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Tabla 2. *In vitro* fermentation fractions, fermentation kinetics, and organic matter degradation of rain tree, boat-thorn acacia, and devil's ear tree mature pods.

Variable	Rain tree	Boat-thorn acacia	Devil's ear tree	SEM
RF (mg/g)	308.23[a]	225.91[b]	258.95[b]	9.25
MF (mg/g)	70.54[a]	50.32[b]	75.29[a]	2.14
SF (mg/g)	72.45[b]	33.31[c]	193.41[a]	9.87
TFF (mg/g)	451.22[b]	309.53[c]	527.65[a]	14.04
OMD (g/kg OM)	539.4[a]	454.7[b]	507.2[a]	7.88
A (mL/g DM)	197.47[b]	134.67[c]	225.72[a]	5.76
k (h)	1.07[c]	1.42[b]	1.73[a]	0.06
b (mL/h)	0.11	0.16	0.12	0.01

[a,b,c] Values with different letters per row indicate differences ($p \leq 0.05$).

RF= rapid fraction, MF= medium fraction, SF = slow fraction, TFF= total fermentation fraction, OMD= organic matter degradation, *A*= total biogas production potential; *k*= Lag time; *b*= constant rate of biogas production from potentially degradable material, SEM = standard error of the mean.

Source: Own elaboration.

Conclusions

At the laboratory level, mature devil's ear tree pods exhibited superior nutritional characteristics and *in vitro* performance, making them suitable as a protein-rich ingredient for inclusion in ruminant diet formulations, followed by mature rain tree pods. Both can serve as alternatives to reduce the use of oilseed meals, given their bromatological composition and ruminal fermentation characteristics; however, further characterization of secondary compounds and their effects on dry matter intake by the animal is required.

Author contributions

Work conceptualization, methodology development, data analysis, manuscript writing and preparation: SSP, TRJC; software management, data handling: HPJ; experimental validation: TSN; writing, review, and editing: SSP, TRJC, HPJ, TSN.

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Ethical statements

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Informed consent statement

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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