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In Situ Digestibility and In Vitro Ruminal Fermentation of Foliage from Native Trees of the Chaco Region: Effects of Tree Species and Tannins

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Abstract

Ruminant production in the Chaco region relies on pastures and native forest foliage, whose nutritional value is poorly characterized and may be influenced by tannins. This study evaluated the in situ digestibility and in vitro ruminal fermentation of foliage from *Prosopis affinis* (PA), *Prosopis nigra* (PN), *Acacia polyphylla* (AP), *Phyllostylon rhamnoides* (PR), and *Tabebuia nodosa* (TN), incubated with or without polyethylene glycol (PEG) to assess the effects of tannin on gas production and nitrogen (N) compounds degradability. Foliage contained ≥17% crude protein (CP) and ≥40% fiber-bound N. Tannin concentration was >4% dry matter (DM) in PN and PA and <1% DM in PR, AP, and TN. In situ digestibility was ≤51% in all species except PR (73%; $p < 0.05$). Gas production was higher in PA, PR, and TN ($p < 0.05$), with no PEG effect. Methane production was not affected by tree species or PEG ($p \leq 0.277$). Both species and PEG affected the effective N compounds degradability (END), with PEG increasing it in PN and AP ($p < 0.05$). Although foliage is high in CP, its digestibility is low; N is largely fiber-bound, and tannins may further limit END, factors to consider when including them in ruminant diets.

Keywords: in vitro gas production; methane; nitrogen compounds degradability; nitrogen fractions; polyethylene glycol

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1. Introduction

Ruminants represent the major domestic herbivores and play a key role in global food security [1] because they can transform large quantities of non-edible biomass into high-nutritive food products (e.g., meat and milk) without competing for land with crop

production [2]. Meat and milk from ruminants account for around 16 and 8% of global protein and energy consumption, respectively [1].

In South America, the Chaco region is a woodland ecosystem that supports more than 5 million beef cattle [3,4], among other ruminants [5], underscoring its significant contribution to global food security. Cattle production in this region is predominantly based on grassland systems, where natural grasslands and exotic grasses coexist with tropical forests [3]. However, the availability and quality of grasses fluctuate significantly due to variable precipitation during the warm season (October to March), with the lowest forage quality and availability typically occurring during the winter [3,4,6]. In this context, the consumption of forest foliage by cattle has been recognized as a dietary supplementation strategy to improve crude protein (CP) supply and overall diet digestibility [7]. Despite this, knowledge about the nutritional value of forest foliage in the Chaco region remains limited. A recent review highlighted that foliage from tropical trees and shrubs may contain substantial levels of CP [8]. However, the binding of CP to fiber fractions can reduce its availability to ruminants. Moreover, foliage from tropical tree and shrub species can contain secondary metabolites, such as tannins, which may further limit nutrient utilization in ruminants.

Tannins are polyphenolic compounds capable of forming complexes mainly with proteins, but also with carbohydrates, thereby reducing their ruminal degradability [9,10]. They have also been recognized for their positive modulation of ruminal fermentation, including the inhibition of methanogenesis, making them potential key metabolites for reducing enteric methane emissions in ruminants [9,11,12]. In foliage from shrubs and trees of tropical regions, tannin concentrations are highly variable depending on the species [8]. This variability is particularly relevant in regions such as the Chaco, where a high diversity of plant species exists under heterogeneous climatic conditions. As a result, limited information is available regarding the tannin concentrations in foliage from native tree species and their potential effects on ruminal fermentation. Furthermore, a knowledge gap exists regarding the protein fractionation of this foliage and the effect of tannins on the potential utilization of its protein. Filling these knowledge gaps is important to better understand how native tree foliage could contribute to ruminant nutrition and diet formulation in regional livestock systems.

Consequently, the objective of this study was to evaluate the in situ digestibility of foliage from five native tree species of the Chaco region, and to assess how tree species and tannin content affect their in vitro ruminal fermentation and N compounds degradation parameters.

2. Materials and Methods

2.1. Study Area and Sampling

The foliage samples were collected from the Fortín Delgado area, Presidente Hayes Department, Paraguay (24°25′48″ S, 59°15′44″ W). This area lies at the transition between the subtropical Humid and Dry Chaco ecoregions, with an annual total rainfall of 951.6 mm and an average temperature of 22.4 °C [13]. The samples were taken from a previous observational study, in which the relative frequency of selected foliage species was recorded from 09:00 to 12:00 h, over a 7-day period in November 2022 (unpublished data). Observations were conducted on a herd of 80 goats browsing within a 20-hectare area consisting of native forest and associated forage species such as *Panicum maximum*, *Digitaria decumbens*, and *Cynodon nlemfuensis*. In that study, 18 trees and shrub species were identified, and the five most frequently browsed species by the goat herd were selected for this study: leaves and twigs of *Prosopis affinis* (PA); leaves, twigs, and fruits of *Prosopis nigra* (PN); leaves and twigs of *Acacia polyphylla* (AP); leaves and fruits of *Phyllostylon rhamnoides* (PR); and leaves of

Tabebuia nodosa (TN). This selection allowed us to focus the analysis on a manageable number of samples while capturing representative variation in foliage characteristics, including tannin content and potential effects on ruminal fermentation. All parts of the plants actually selected by the goats during browsing, including leaves, twigs, and fruits, were collected and, for convenience, considered collectively as "foliage" to simplify the presentation and discussion of the results. Although this approach may limit direct comparability with studies analyzing leaves only, it reflects the plant parts actually selected by the animals and provides a realistic representation for the exploratory nutritional characterization of these species.

Foliage from each tree species was randomly collected from five individual trees and then mixed into a composite sample. The aim of this sampling protocol was not to obtain a representative sample of the entire study area or capture individual-tree foliage variability, but to perform an exploratory analysis of the foliage actually selected by the goat herd.

2.2. Chemical Analysis

The composite samples of foliage were dried in a forced-air oven at 55 °C for 72 h and then weighed and ground to pass a 2 mm screen. Total DM content was determined by oven drying at 110 °C for 24 h. Ash was determined by combustion at 600 °C for 3 h and OM by mass difference. Total N was assayed by the Kjeldahl method [14] and CP was calculated by multiplying N content by 6.25. The neutral (NDF) and acid (ADF) detergent fiber analyses included ash and were based on the procedures of Mertens (2002) [15] and AOAC (1997) [14], respectively, except that the samples were weighed in polyester filter bags (16 µm porosity; SEFAR® PET 1500, Heiden, Switzerland) and treated with neutral or acid detergent in an autoclave at 110 °C for 40 min [16]. For sulphuric acid lignin (ADL) analysis, the bags containing residual ADF were treated with H_2SO_4 12 M for 3 h [14]. Analyses of neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) were performed according to Licitra et al. [17]. Ether extract (EE) concentration was determined according to the Soxhlet method using diethyl ether as the solvent [18]. The content of non-fiber carbohydrates (NFCs, %) was calculated according to Hall [19] as 100 − [(NDF − NDIN × 6.25) + CP + EE + ash]. Total tannin was analyzed following the procedures of Makkar [20]. The chemical composition and N fractions of the evaluated foliage are presented in Table 1.

Table 1. Chemical composition and nitrogen (N) fractions of foliage from five tree species native to the Chaco region.

Item	*Prosopis affinis*	*Prosopis nigra*	*Acacia polyphylla*	*Phyllostylon rhamnoides*	*Tabebuia nodosa*
Dry matter (DM, % as feed)	41.9	48.1	52.5	31.6	41.4
Chemical composition (% DM)					
Organic matter	91.2	90.7	94.4	79.4	90.4
Crude protein	18.8	17.4	21.0	27.7	18.8
Neutral detergent fiber	55.1	55.9	56.9	48.4	56.6
Acid detergent fiber	35.3	32.4	34.6	21.1	34.1
Acid detergent lignin	17.6	19.3	20.5	5.3	8.3
Ether extract	2.36	2.43	2.64	2.97	3.39
Ash	8.8	9.3	5.6	20.6	9.6
Non-fiber carbohydrates	16.2	16.2	15.4	2.7	13.4
Total tannins	0.85	4.58	7.48	0.41	0.99
N fractions (% total N)					
Soluble N	30.3	19.6	37.6	29.3	35.7
Neutral detergent insoluble N	39.5	40.0	46.4	54.9	46.9
Acid detergent insoluble N	11.4	28.5	27.8	15.6	20.2

2.3. In Situ Digestibility Assay

The in situ digestibility assay performed in three consecutive runs which were considered as replicates. The samples of foliage grounded at 2 mm were weighed (1.0 g) in polyester filter bags (5 × 5 cm, 40 µm porosity; SEFAR® PET 1500, Heiden, Switzerland) and incubated in the rumen of a Brangus cannulated cow for 48 h. The cow (4 years old and 470 kg of body weight (BW)) was grazing on native grassland (main species: *Sorghastrum setosum*, *Paspalum* sp., *Steinchisma* sp., *Panicum* sp., *Eleocharis* sp., *Rynchospora* sp., and *Cyperus* sp.) and was supplemented at 0.5% of BW with a commercial concentrate composed of corn grain, rice bran, and soybean expeller (12% CP). The cow received this diet for 14 days before the start of the incubation period and was maintained on the same diet throughout all rumen fluid collection periods. Thereafter rumen incubation, the bags were treated with neutral detergent solution in an autoclave at 110 °C for 40 min [16], washed in tap water, oven dried at 110 °C for 24 h, and ashed at 600 °C for 3 h. The OMD was calculated as the following: (incubated OM (g) − residual OM (g))/incubated OM. Digestible organic matter content (DOM) was calculated as the product of sample OM content and OMD. The digestible energy (DE, Mcal/kg DM) was calculated as DOM (g/kg) × 4.409)/1000, and the metabolizable energy (ME, Mcal/kg DM) was calculated as DE × 0.82 [21].

2.4. In Vitro Gas Production Assay

Samples of foliage grounded to 1 mm were weighed (0.5 g) into 100 mL glass bottles. To evaluate the effect of tannins on gas production parameters, each foliage sample was incubated with 1 or 0 g of PEG (molecular weight = 6000 g/mol), a tannin-binding agent [22]. Subsequently, 40 mL of a buffer solution [23] was added, and then kept refrigerated at 4 °C for 12 h to allow for substrate hydration. After that, the bottles were put in a water bath at 39 °C and 10 mL of ruminal inoculum was added. The inoculum was collected from the rumen of a cannulated Brangus steer (12 years old and 650 kg BW) grazing on a Tifton (*Cynodon dactylon*) pasture and supplemented at 0.5% of BW with a commercial concentrate composed of corn grain, rice bran, and soybean expeller (12% CP). All procedures were performed under continuous carbon dioxide injection. Three in vitro runs were conducted, and each run was considered as a replicate. In each run, samples with or without PEG were incubated in duplicate, along with four additional blank bottles with or without PEG, totaling 24 bottles per run. The bottles were fitted with a needle and a three-way stopcock. For the determination of total gas production, the stopcock was connected to an apparatus that directed the gas into a 25 mL graduated column [24]. The total gas volume (mL) was quantified by liquid displacement under gas pressure and recorded at 12, 24, 36, 48, and 72 h of incubation. The cumulative gas production was expressed in mL per g of incubated OM, and the fractional rate of gas production was estimated using the unicompartmental model proposed by Schofield et al. [25]:

$$V = Vf * (1 + \exp(2 - 4 * S * (t - L))) - 1$$

where Vf = final volume of gas (mL/g OM) at time t; S = fractional rate of gas production (/h); and L = colonization time of the bacteria on the substrate (in hours).

After gas was recorded in each bottle, the three-way stopcock was closed to retain the gas in the graduated column. A hose was then connected to the three-way stopcock and to a syringe equipped with a stopcock, which were simultaneously opened to transfer the gas from the pipette to the syringe. Gas samples were collected using sterile 20 mL Luer Slip syringes (Descarpack, São Paulo, Brazil) with 21G × 1¼″ hypodermic needles (0.80 mm OD) and were immediately transferred into evacuated vials (15.5 mm × 101 mm × 12 mL, Labco Exetainer®, Labco Limited, Lampeter, UK) for compositional analysis. Aliquots were subjected to three successive dilutions in N (1:19 *v*/*v*), resulting in a final dilution factor

of 8000. Specifically, 1 mL of fermentation gas was diluted in 19 mL of N (first dilution), followed by two further 1:19 dilutions. Concentrations of methane were determined by gas chromatography (GC-2014, Greenhouse model, Shimadzu, Kyoto, Japan).

2.5. In Vitro N Degradability Assay

For the in vitro N degradability assay, the foliage samples were previously soaked in distilled water to remove the water-soluble fraction of DM. Briefly, a 10 g sample of each foliage (grounded at 2 mm) was weighed into a 10 × 10 cm polyester bag (40 µ porosity; SEFAR® PET 1500, Heiden, Switzerland) and incubated in distilled water at room temperature for 3 h. Following this, the samples were washed with distilled water and dried at 55 °C for 48 h. The water-insoluble fraction of the samples was analyzed for DM, OM, N, and tannins using the same procedures described above. Soluble N was calculated as the difference between sample N and water-insoluble N. Potentially degradable N was calculated as the difference between total N minus soluble N and ADIN.

Three in vitro runs were conducted for each foliage, and each run was considered as a replicate. For this purpose, water-insoluble residues were weighed (0.5 g) into 100 mL glass bottles. To evaluate the effect of tannins on N compounds degradation; each foliage sample was incubated with 1 or 0 g of PEG (molecular weight = 8000 g/mol). Subsequently, 40 mL of a modified buffer solution [23], containing a reduced concentration of ammonium bicarbonate (i.e., from 1.407 to 0.293 g NH_4HCO_3/L of buffer solution) to minimize its contribution to the ammonia-N released during incubation, was added, and then kept refrigerated at 4 °C for 12 h to allow for substrate hydration. After that, the bottles were put in a water bath at 39 °C and 10 mL of ruminal inoculum was added. The inoculum was collected before the morning feeding (≈9 am) from the rumen of the same cow used for the in situ digestibility assay. All procedures were performed under continuous carbon dioxide injection. In each run, samples with or without PEG were incubated in triplicates, and four additional blank bottles without samples were also incubated. Thus, 34 bottles were incubated in each run.

Gas production was monitored at 0, 6, 12, 18, 24, 36, 48, and 72 h using a graduated column system [24] to ensure that fermentation occurred. After each gas record, a 0.5 mL aliquot of the fluid was collected from each bottle using a syringe, and this fraction was mixed with 4.5 mL of acid solution (2% H_2SO_4, *v*/*v*), then frozen at −20 °C until further analysis. The samples of fluid were thawed at room temperature, centrifuged at 3000 rpm for 20 min, and analyzed for ammonia–N through the phenol-hypochlorite method [26]. The total amount of ammonia-N in the bottle was calculated by multiplying the ammonia-N concentration by the volume of the incubation medium. The ammonia–N release at each time was calculated in proportion of the incubated potentially degradable N, by discounting the NIDA fraction. The in vitro fractional rate of ammonia–N release (in vitro kd) was determined directly as the slope obtained by linear regression of natural logarithms of undegraded N and time [27]. The soluble N (SN) fraction was obtained as the difference between the content of N in the original sample minus the content of N in the water-insoluble fraction. The potentially degradable N (PDN) fraction was obtained as the difference between the content of N in the original sample minus the content of SN and ADIN. The END of feedstuffs incubated in vitro was calculated applying the following equation [28]:

$$END = SN + (PDN \times kd)/(kd + kp)$$

where SN, PDN, and kd are the same as defined above and kp is the fractional rate of N rumen outflow, fixed at 2, 4, and 6%/h.

2.6. Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test through the NORMAL option in PROC UNIVARIATE of SAS software (version 9.1, SAS Institute Inc., Cary, NC, USA). The data from the in situ digestibility assay (i.e., OMD and contents of DOM and ME) were analyzed using PROC GLM in SAS, with species as a fixed effect. The data obtained from the in vitro assays (i.e., gas production, S, kd, and END) were analyzed using the same model, with the inclusion or not of PEG in the incubation medium as a fixed effect. Means were compared by Student's *t*-test, which performs unadjusted pairwise *t*-tests. Differences were declared significant at $p \leq 0.05$.

3. Results

Chemical composition and N fractions of the plant material are shown in Table 1. In general, all species presented high levels of CP (≥17% DM) and NDF (≥48% DM). Foliage from PR presented high levels of ash (20% DM), whereas PA, PN, and AP showed the highest levels of ADL (≥17% DM). Tannins were detected in all species, with high concentrations in PN and PA (>4% DM), whereas the other foliage species showed low concentrations (<1% DM). Despite the high CP content, most of the N fraction was associated with fiber (≥40% of total N) in all species, with a variable proportion bound to ADF (11–29% of total N), which is considered indigestible.

3.1. In Situ Digestibility

The in situ OMD, DOM, and ME of the evaluated foliage are presented in Table 2. The foliage of PR had the highest OMD, DOM, and ME ($p \leq 0.05$), followed by PA and TN, which did not differ from each other, while AP and PN showed the lowest values ($p \leq 0.05$).

Table 2. In situ organic matter digestibility (OMD), digestible organic matter (DOM) and metabolizable energy (ME) content of foliage from five tree species native to the Chaco region.

Item	*Prosopis affinis*	*Prosopis nigra*	*Acacia polyphylla*	*Phyllostylon rhamnoides*	*Tabebuia nodosa*	SEM [1]	*p*-Value
OMD (%)	51.3 ^{b}	38.8 ^{c}	37.9 ^{c}	72.7 ^{a}	48.7 ^{bc}	3.95	<0.01
DOM (g/kg DM)	468 ^{b}	352 ^{c}	358 ^{c}	577 ^{a}	440 ^{bc}	33.4	<0.01
ME (Mcal/kg DM)	1.69 ^{b}	1.27 ^{c}	1.30 ^{c}	2.09 ^{a}	1.59 ^{bc}	0.121	<0.01

[1] SEM = standard error of the mean. Means in the same row with different superscripts (a, b, c) differ significantly ($p < 0.05$). DM = dry matter.

3.2. In Vitro Gas Production Parameters

The effect of PEG on the in vitro total GP and S of the evaluated foliage is presented in Table 3 and Figure 1. Both total GP and S were affected by tree species ($p < 0.001$), whereas PEG addition had no effect ($p = 0.123$), and no Sp × PEG interaction was detected ($p \geq 0.693$). Total GP was higher ($p < 0.05$) in PA, PR, and TN compared to PN and AP. Similarly, S was, in general, higher ($p < 0.05$) in PR and TN than in PN, AP, and PA. No effect of tree species, PEG addition, or their interaction was found for methane production ($p \geq 0.270$).

Table 3. Effect of polyethylene glycol (PEG) on in vitro gas production (GP), fractional rate of gas production (S), and methane production of foliage from five tree species (Sp) native to the Chaco region.

Item	*Prosopis affinis*		*Prosopis nigra*		*Acacia polyphylla*		*Phyllostylon rhamnoides*		*Tabebuia nodosa*		SEM [1]	*p*-Value	
PEG [2]	−	+	−	+	−	+	−	+	−	+		Sp	PEG
Total GP (mL/g OM)	88.8 ^{a}	85.9 ^{a}	63.0 ^{bc}	65.9 ^{b}	47.5 ^{c}	61.0 ^{bc}	89.0 ^{a}	96.8 ^{a}	89.7 ^{a}	98.5 ^{a}	5.89	<0.01	0.123
S (%/h)	2.07 ^{d}	2.10 ^{cd}	2.24 ^{cd}	2.25 ^{cd}	2.18 ^{cd}	2.13 ^{cd}	3.11 ^{a}	2.98 ^{a}	2.77 ^{ab}	2.50 ^{bc}	0.141	<0.01	0.351
Methane (g/kg OM)	9.86	15.55	9.36	12.75	4.79	5.66	13.82	12.08	9.99	11.59	4.254	0.277	0.381

[1] SEM = standard error of the mean. Means in the same row with different superscripts (a, b, c, d) differ significantly ($p < 0.05$). No significant Sp × PEG interaction was detected ($p \geq 0.693$). [2] (+) indicates the incubation medium with PEG and (−) indicates the incubation medium without PEG. OM = organic matter.

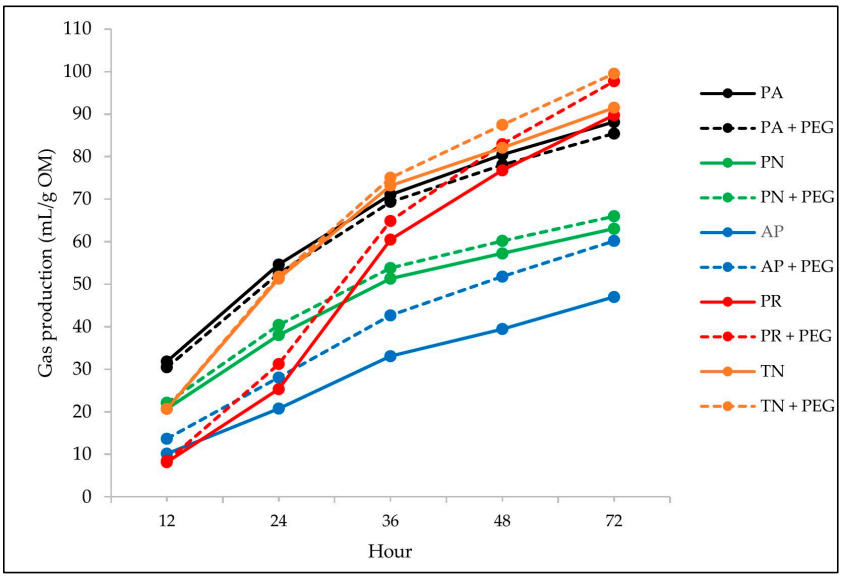


Figure 1. In vitro gas production of foliage from five tree species native to the Chaco region, incubated with or without polyethylene glycol (PEG). PA = *Prosopis affinis*; PN = *Prosopis nigra*; AP = *Acacia polyphylla*; PR = *Phyllostylon rhamnoides*; TN = *Tabebuia nodosa*. OM = organic matter.

3.3. In Vitro N Degradation Parameters

The effect of PEG on the in vitro kd and the END of the evaluated foliage is presented in Table 4. Both tree species and PEG addition affected ($p < 0.01$) the kd and END, while their interaction tended to be significant for kd ($p = 0.052$) and for END estimated at a rumen passage rate of 6%/h ($p = 0.092$). The addition of PEG to the incubation medium increased the kd and, at rumen passage rates of 2, 4, and 6%/h, consistently increased END in PN and AP ($p < 0.05$), whereas no effect was observed in PA, PR, or TN.

Table 4. Effect of polyethylene glycol (PEG) on the in vitro fractional rate of ammonia–nitrogen release (kd) and the effective nitrogen compounds degradability (END) in foliage from five tree species (Sp) native to the Chaco region, assessed at ruminal passage rates of 2, 4, and 6%/h.

Item	*Prosopis affinis*		*Prosopis nigra*		*Acacia polyphylla*		*Phyllostylon rhamnoides*		*Tabebuia nodosa*		SEM [1]	*p*-Value		
PEG [2]	−	+	−	+	−	+	−	+	−	+		Sp	PEG	Sp × PEG
kd (%/h)	1.90 ^{c}	2.49 ^{bc}	1.34 ^{c}	3.55 ^{b}	2.17 ^{bc}	5.34 ^{a}	0.89 ^{c}	1.25 ^{c}	2.09 ^{bc}	2.24 ^{bc}	0.557	<0.01	<0.01	0.052
END (%)														
2	58.6 ^{ab}	62.2 ^{a}	39.7 ^{d}	52.2 ^{bc}	53.5 ^{b}	62.3 ^{a}	46.0 ^{cd}	50.2 ^{bc}	58.2 ^{ab}	58.9 ^{ab}	2.46	<0.01	<0.01	0.167
4	49.1 ^{bc}	52.4 ^{ab}	32.3 ^{e}	43.6 ^{cd}	48.6 ^{bc}	56.9 ^{a}	39.2 ^{d}	42.3 ^{d}	50.8 ^{ab}	51.5 ^{ab}	2.08	<0.01	<0.01	0.110

Table 4. *Cont.*

Item	*Prosopis affinis*		*Prosopis nigra*		*Acacia polyphylla*		*Phyllostylon rhamnoides*		*Tabebuia nodosa*		SEM [1]	*p*-Value		
PEG [2]	−	+	−	+	−	+	−	+	−	+		Sp	PEG	Sp × PEG
6	44.3 [b]	47.2 [b]	28.8 [d]	38.6 [c]	46.1 [b]	53.5 [a]	36.4 [c]	38.8 [c]	47.1 [b]	47.7 [b]	1.77	<0.01	<0.01	0.092

[1] SEM = standard error of the mean. Means in the same row with different superscripts (a, b, c, d, e) differ significantly ($p < 0.05$). [2] (+) indicates the incubation medium with PEG and (−) indicates the incubation medium without PEG.

4. Discussion

To our knowledge, the specific foliage studied here have not been previously evaluated as feed for ruminants in terms of their nutritional composition, in vitro digestibility, N degradability, or the potential effects of tannins on nutrient utilization. In general, the chemical composition of the evaluated foliage falls within the range reported by Castro-Montoya and Dickhoefer (2020) [8] for foliage from tropical tree species. The evaluated foliage presented high CP content (17–28%). However, despite the high CP content, a considerable portion of N (40–55%) was bound to NDF, and a variable fraction of N (11–29%) was bound to ADF (i.e., ADIN), which is associated with lignin, making it unavailable for rumen microbial degradation and post-ruminal digestion, thus effectively limiting the protein availability to the animal [17].

Despite its high ash content, PR foliage showed the highest OMD and DOM concentration, providing energy levels comparable to those of alfalfa hay (≈2.0 Mcal ME/kg DM; [29]), a widely used temperate legume forage. In addition, the higher OMD was consistent with the lower ADL and tannin concentrations found in PR compared to the other evaluated foliage. In fact, a negative correlation between in vitro digestibility and ADL concentration has been reported in legume foliage, as well as in tropical trees and shrubs [8]. Furthermore, PR was among the forages with the highest in vitro gas production, fractional rate of gas production, and methane production, and these parameters were not affected by PEG addition, suggesting that tannins do not interfere with fermentation, likely due to their low concentration in this foliage (i.e., 0.4% DM).

Although PR shows the most promising nutritional attributes in terms of digestibility and CP content, 55% and 16% of its N was bound to NDF and ADF, respectively. The fraction of fiber-bound N, discounting ADIN, which is considered potentially degradable in the rumen but at a low kd [30], accounted for 39% of total N in this foliage. This large pool of slowly degradable N compounds may help to explain why this forage also exhibited the lowest kd among the species evaluated. Consequently, the END for PR was among the lowest observed in this study, despite its high digestibility. Although END parameters are typically estimated using the in situ bag technique [28], in our study they were estimated in vitro. Even so, regardless of the technique, the END value of PR estimated at a passage rate of 4%/h was considerably lower than that reported for alfalfa hay (i.e., 82%; [29]) and lower than that of fresh *Leucaena leucocephala* (i.e., 51%; [31]), a tropical shrub–tree legume. The addition of PEG to the incubation medium did not affect any N compounds degradation parameters, suggesting that tannins did not interfere with protein degradation in this foliage.

The foliage of PA and TN presented similar nutritional attributes, although PA was high in ADL. Both forages showed low concentration of DOM; however, they exhibited gas production values similar to PR. In addition, in vitro gas parameters were not affected by the addition of PEG to the incubation medium, indicating no effect of tannins, which were found at low concentrations in both PA and TN (i.e., <1% DM). Also, PA had high CP levels, and although 40% of its N was bound to NDF, it showed the lowest ADIN value among the forages evaluated, which was below the average ADIN levels reported for tree

foliage [8]. Furthermore, despite the fiber-bound N, the END in this foliage was among the highest of those evaluated, presenting similar values that those reported for *Leucaena leucocephala* (i.e., 51%; [31]), a widely distributed tropical shrub–tree legume.

Despite the high CP levels found in PN and AP, these foliage showed high concentrations of ADL, which may explain the low OMD observed. In fact, they had the lowest DOM values among the forages evaluated, resulting in low energy concentrations, similar to those reported for low-quality roughages such as rice straw (i.e., 1.4 Mcal ME/kg DM; [32]). Additionally, these forages presented high tannin concentrations, close to or above the threshold (i.e., 5% DM), which is generally assumed to depress the intake [33] and in vivo digestibility [34]. However, despite the highest tannin concentrations, no effects were observed on gas production parameters (i.e., no PEG effect). In addition, both the PN and AP foliage exhibited low END values, and the addition of PEG to the incubation medium significantly increased kd and END, indicating a marked negative effect of tannins on the ruminal N compounds degradability of these forages. This response is likely related to the strong affinity of tannins for proteins, whereas tannins do not necessarily affect the fermentation pathways that determine gas or methane production [35,36].

In general, although the utilization of CP from the evaluated foliage is limited due to the high proportion of fiber-bound and indigestible N, they could still represent a valuable source of protein, particularly during the dry season or winter months, when the CP content of native or cultivated grasses is low in the Chaco region [37].

5. Conclusions

The five native tree foliage evaluated showed high CP content, but a large proportion of N was bound to fiber, limiting its availability both in the rumen and post-ruminally. Overall, while some of these forages may have nutritional potential as protein sources for ruminants, their END is restricted by both tannin content and fiber association. These factors should be taken into account when considering their inclusion in ruminant diets. Further research should be conducted to determine the nutritional potential of foliage from a wider range of species, with the most promising ones being evaluated in ruminant diets through in vivo experiments.

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