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Original

Nutritional characterization and gas production of vegetative species with potential as feedstuffs for ruminants feeding

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ABSTRACT

Objective. To evaluate the chemical composition, phenolic compounds content, and in vitro methane and gas production kinetics of seven vegetable species as potential feedstuffs for ruminants feeding. **Materials and methods.** Seven species were evaluated: gray oak (GO), red oak (RO), prickly poppies (PP), mesquite (MES), wattle tree (WT), white mulberry (WM) and stevia (STE). The analyses of the samples were: ether extract (EE), ash, crude protein (CP), non-structural carbohydrates (NSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, acid detergent lignin (ADL), condensed tannins (CT), total phenolic compounds (TPC), in vitro dry matter true digestibility (IVDMD); as well as under in vitro ruminal conditions, gas production (GP), methane and carbon dioxide CO_2 production, N-ammonia, and volatile fatty acids (VFA). **Results.** The results show that WT, MES and WM foliage presented the highest content in CP, the highest digestibility's (IVDMD) were observed in PP, WM and STE. Otherwise, the lowest methane productions were generated by MES, RO and WM. **Conclusions.** According to the results in the chemical composition, PP, WM and STE presented the best nutritional quality since they showed the highest protein contents and an adequate digestibility. These results suggest that the use of PP would not affect the nutritional characteristics offered by good quality forage. In addition, the other species may be used as additives or supplements for feeding ruminants because of their higher protein and CT contents.

Keywords: Methane; chemical composition; phenolic compounds; ruminants (*Source: CAB*).

RESUMEN

Objetivo. Evaluar la composición química, contenido de compuestos fenólicos, cinética de producción de gas y emisiones de metano (CH_4) *in vitro* de siete especies vegetales con potencial alimenticio para alimentación de rumiantes. **Materiales y métodos.** Siete especies fueron evaluadas: encino gris

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(EG), encino rojo (ER), chicalote (CHIC), mezquite (MEZ), huizache (HUI), morera (MOR) y la estevia (STE). Los análisis de las muestras fueron: extracto etéreo (EE), cenizas (Cen), proteína cruda (PC), carbohidratos no estructurales (CNE), fibra en detergente neutro (FDN), fibra en detergente ácido (FDA), hemicelulosa, celulosa, lignina detergente ácida (LDA), taninos condensados (TC) y fenoles totales (FT), digestibilidad *in vitro* de la materia seca (DIVMS); así como las condiciones ruminales *in vitro*, producción de gas (PG), producción de metano y dióxido de carbono (CO_2), nitrógeno amoniacal (N-NH_3) y ácidos grasos volátiles (AGV´s). **Resultados.** Los resultados muestran que HUI, MEZ y MOR presentaron un mayor contenido de PC, las mayores digestibilidades (DIVMS) se observaron en CHIC, HUI y STE. De lo contrario, las producciones de metano más bajas fueron generadas por MEZ, ER Y HUI. **Conclusiones.** De acuerdo con los resultados en la composición química, CHIC, MOR y STE presentaron la mejor calidad nutricional ya que mostraron los más altos contenidos de proteína y una digestibilidad adecuada. Estos resultados sugieren que el uso de CHIC no afectaría las características nutricionales que ofrece un forraje de buena calidad. Además, las otras especies pueden usarse como aditivos o suplementos para alimentar a los rumiantes debido a su mayor contenido de proteína y taninos condensados.

Palabras clave: Metano; composición química; compuestos fenólicos; rumiantes (*Fuente: USDA*).

INTRODUCTION

Livestock production is an economic activity which impacts negatively the climate change. Methane and carbon dioxide are the main gases responsible of the greenhouse effect and are synthetized in the ruminants as a product of the ruminal fermentation. In another way, the foliage obtained from trees is a very important forage source for ruminants feeding in zones where very low or not forage fountain are available (1).

Moreover, the great importance lies in the fact that these forage components do not compete with the human feeding; these new sources provide essential nutrients for livestock along the year. According to the latter, mostly all the plants produce diverse biological compounds which are classified as primary and secondary metabolites; primary metabolites are essential for growth and development of plants, whereas the secondary metabolites are mainly produced as defense mechanisms against the presence of predators (2). Thus, the presence of condensed and hydrolysable tannins, as well as saponins, are capable of forming complexes with proteins decreasing the digestibility. Moreover, positive correlations are observed among these compounds and the synthesis of ruminal methane (3). In this way, there is a great number of vegetable species with a great capability to reduce the ruminal methane synthesis; these species offer a high quality of nutrients which may enhance their use as additives or supplements in ruminants feeding in extensive and intensive systems (4). Thus, some species of plants like prickly poppies (*Argemone* Mexicana L.), grey (*Quercus grisea* L.) and red (*Quercus eduardi* Trel.) oak, as well as shrubs like wattles tree (*Acacia tortuosa* Standl), mesquite (*Prosopis laevigata* Humb. & Bonpl. ex Willd.), and the stevia plant (*Stevia rebaudiana* Bertoni) contain phenolic compounds (PC) as flavonoids, anthocyanins, tannins, phenolic acids and nutraceutic properties that are positively correlated with an inhibitory capacity of ruminal protozoa (5,6,7,8). Furthermore, the long-chain polyunsaturated fatty acids contained in the white mulberry (*Morus alba* L.) may contribute with a reduction in the methane synthesis carried out in the rumen (9). Additionally, researchers worldwide are encouraging the use of silvopastoral systems as a sustainable livestock production method since reductions in methane production are presented and remarkable improvement of arable lands are observed. Regarding to these, regions like Africa, India, and South America are feeding livestock with foliage produced in shrubs and trees (10). In addition, some of these vegetable species are being produced in the northern Mexico. Consequently, there is an imperative need of studying forestry species which are not commonly used in livestock production as alternative forage sources and to evaluate the effect of these in the ruminal methane production. Thus, regarding to the latter, this study aimed to determine the chemical composition, phenolic compounds content, and in vitro methane and gas production kinetics of some vegetable species as potential feedstuffs in ruminants feeding.

MATERIAL AND METHODS

Vegetable species. The samples of vegetable species were recollected in the Northern Mexico in April of 2019. Proposed species and recollection areas are presented in table 1.

Three trees were sampled for each vegetative specie and only leaves were recollected randomly from the medium part of each tree, avoiding to take outer leaves or in active growth. Recollected samples were mixed as a pool for each specie.

Table 1. Proposed vegetative species and recollection area proposed in the current study.

Specie	Scientific name	Recollection area	Geographic location
Gray oak (GO)	*Quercus grisea* L.	Durango, Mexico	23° 91′ N y 104° 71′ W
Prickly popies (PP)	*Argemone mexicana* L.	Durango, Mexico	24° 27′ N y 104° 07′ W
Red oak (RO)	*Quercus eduardi* Trel.	Durango, Mexico	23° 91′ N 104° 71′ W
Mesquite (MES)	*Prosopis laevigata a* (Humb. & Bonpl. ex Willd.)	Durango, Mexico	24° 06′ N 104° 41′ W
Wattles tree (WT)	*Acacia tortuosa* Standl	Durango, Mexico	24° 06′ N 104° 41′ W
White mulberry (WM)	*Morus alba L.*	Durango, Mexico	23° 95′ N y 104°57′ W
Stevia (STE)	*Stevia rebaudiana* Bertoni	Nayarit, Mexico	21° 48′ N y 105° 12′ W

Chemical composition. All samples of vegetable species were cut and the foliage obtained was dried in a forced-air stove at 55°C during 48h. Afterwards, dried samples were ground in a Wiley mill (Arthur H. Thomas, Philadelphia, PA, EE. UU) to a 1mm diameter for chemical analyses. Analyses of dry matter (DM), ether extract (EE), ash and crude protein (CP) were performed according to standard procedures (11). Cell wall components as neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose and acid detergent lignin (ADL) were determined as proposed by Van Soest et al. (12) using a Fiber Analyzer equipment (Ankom Technologies, USA). Non-structural carbohydrates (NSC) were estimated according to the following equation NSC=[100-(CP+EE+Ash+NDF)]. *In vitro* dry matter true digestibility (IVDMD) was determined through measuring the dry matter disappearance in a 48h incubation at 39ºC with ruminal liquor and buffer solutions in a 2:1 ratio, respectively. Metabolizable energy (ME) was estimated according to the equation proposed by Menke and Steingass (13) as follows:

ME (MCal/Kg DM) = [2.20 + 0.136(GP24h) + 0.0057(CP) +0.0029(EE)2]/4.184

Phenolic compounds assay. Approximately, 1 g of individual samples of all vegetable species were subjected to alcoholic extractions using 90 mL of ethanol solution (70% v/v) during 24h. Afterwards, extracts were filtered and vaccum-evaporated at 40ºC until ethanol was totally removed. Concentrated extracts were then dried at room temperature and weighed; yields were calculated in a dry matter basis. Condensed tannins (CT) were determined according to procedures proposed by Heimler et al. (14) using catechin as standard and absorbance was measured in a spectrophotometer at 500 nm (Genesys 10S, Thermo Scientific, USA). Otherwise, total phenolic compounds (TPC) were determined using the Folin-Ciocalteau method described previously by Singleton and Rossi (15); galic acid was used as standard and absorbance was measured at 760 nm (Genesys 10S, Thermo Scientific, USA).

***In vitro* gas production.** The *in vitro* gas production was carried out according to methods proposed by Theodorou et al (16). Briefly, about 1 g of dried samples were mixed in glass modules with 120 mL of a solution prepared with buffer solution and ruminal liquor in a 1:2 ratio and incubated by triplicate at 39ºC during 96h. Ruminal liquor was obtained from two ruminally cannulated steers and filtered through four layers of cheesecloth for removal of all feed particles. Glass modules were equipped with electronic pressure transducers and changes in the pressure were recorded at 0, 3, 6, 9, 12, 24, 36, 38, 72 and 96h. Gas production kinetics was estimated by fitting obtained data into the Gompertz function according to the following equation (17):

$$GP=Ae^{-Le^{-(k_d t)}}$$

Where GP= produced gas at time "t" (mL/g DM); A= maximum gas production (mL); k_d= constant rate of gas production (h^{-1}); L= latency time before the gas production begins (lag phase, h). Otherwise, to determine the methane and carbon dioxide proportions, the release pressure valve was opened during 2 sec and the gas was conducted into a gas analyzer (GEM5000, LANDTEC, USA) according to procedures proposed by Mills et al (18).

***In vitro* fermentation parameters.** Ruminal liquor was obtained from two ruminally cannulated steers and filtered through four layers of cheesecloth for removal of all feed particles. Approximately, 1 g of each individual vegetable sample was fermented with 120 mL of a solution prepared with buffer solutions and ruminal liquor in a 1:2 ratio and placed into glass modules equipped with rubber-stoppers according to Theodorou et al (16). Samples were incubated by triplicate during 24h. Once the time was elapsed, modules were opened and pH was measured immediately; then, liquid was filtered. Filtered liquid was divided into two 10 mL subsamples. The first subsample was mixed with 0.3 mL of sulfuric acid (50% v/v), whereas the second subsample was mixed with 2.5 mL of metaphosphoric acid (25% w/v) for N-ammonia and volatile fatty acids (VFA) determinations, respectively (19).

Statistical analysis. All data were analyzed by a completely randomized design using the GLM procedure of SAS. Means comparison was performed with the Tukey test and statistical differences were declared at $p<0.05$.

RESULTS

Chemical composition. As presented in Table 2, highly significant differences are observed in CP, ash, EE and NSC among vegetative species ($p<0.001$). Contents of CP ranged from 8 to 17%; wattles tree (WT) foliage presented the highest content, whereas the lowest was observed in stevia (STE). Otherwise, EE contents ranged from 0.5 to 3%; STE presented the lowest EE content and MES and WT the highest. On the other hand, ash content in white mulberry (WM) is the highest (21.4%), whereas prickly poppies (PP) presented the lowest (4.3%). In the same way, grey and red oak (GO and RO, respectively) presented the higher contents of NDF ($p<0.05$); whereas PP and STE showed the lower values ($p<0.05$). In this way, PP and STE presented the higher NSC proportions due to the rapidly degradable carbohydrates contained in the cell wall. In addition, PP showed the lower value of metabolizable energy (ME) ($p<0.05$); whereas the other vegetable species presented similar values ($p>0.05$). Despite the values obtained in the ME, the lower digestibilities (IVDMD) were presented by GO, RO and WT ($p<0.05$).

***In vitro* gas production.** Statistical differences were observed in gas production kinetics parameters in Table 3 ($p<0.001$). Gmax ranged from 18.6 to 99 (mL/g DM) where the highest gas production was observed with prickly poppies (PP). Otherwise, the lowest gas production was observed with wattles tree (WT). Additionally, the vegetative sources which presented the lower values in gas production presented greater adaptation times ($p<0.05$); PP is the exception ($p>0.05$). Accordingly, the exposed earlier agrees with the microorganisms' required time for adaptation and beginning of the gas production. Similarly, the gas production rate increases in the referred vegetative sources: PP, WT, MES and STE. Likewise, GP_{24} showed the same behavior than the presented by parameter A.

On the other hand, methane production among vegetable species presented statistical differences ($p<0.05$). PP presented the highest methane production among species ($p<0.05$). However, PP presented also the highest maximum gas production (A parameter); hence, the methane production may be overestimated. Otherwise, the lower methane productions were presented by MES, RO and WM ($p<0.05$). The latter is consistent with contents of CT in these species, which were reported to be the highest in this study (1.5, 1.1 and 1.0 % for MES, RO, and WM, respectively).

***In vitro* fermentation parameters**. Table 4 presents the ruminal fermentation parameters obtained with the incubation of the vegetable species. As can be observed, there are differences in the N-ammonia among vegetable species ($p<0.05$); MES and WM presented higher concentrations than the other species (13.1 and 12.8 mg/dL, respectively). These species also presented the highest CP contents which suggests that more protein is being degraded and the microbial protein synthesis is being favored instead. Regarding to the volatile fatty acids (VFA), individual proportions of each VFA presented changes among species

($p<0.05$). However, no differences were observed in the total volatile fatty acids (TVFA) ($p>0.05$). In this way, GO and WT presented the lowest concentrations of acetate among the vegetable species. Hence, WT presented the highest propionate concentration among the vegetable species ($p<0.05$). Otherwise, whether a reduction of acetate is observed, then an increase in the propionate would be expected.

Table 2. Chemical composition (g/100g DM) and *in vitro* dry matter digestibility of proposed vegetative species.

Nutrient (%)	Species							SED
	GO[1]	PP	RO	MES	WT	WM	STE	
CP	9.2c	12.7b	9.1c	15.4ab	17.1a	15.0ab	8.0c	0.30
EE	1.4d	1.6c	0.7e	3.0a	3.0a	2.1b	0.5f	0.02
Ash	7.4c	11.7b	4.3d	5.5cd	7.7c	21.4a	6.9cd	0.29
NSC	23.3b	51.4a	25.5b	26.8b	22.0b	14.1c	52.9a	0.72
NDF	58.6a	22.5d	60.4a	49.3b	50.3b	47.4b	31.2c	0.51
ADF	23.7cd	16.9d	27.2bc	39.4a	40.3a	31.1b	23.4bc	0.67
LIG	4.2b	0.9c	5.0b	9.2a	10.4a	3.8b	5.5b	0.26
CEL	19.2bc	16.0cd	22.1b	29.2a	29. 1a	23.1b	12.9d	0.51
HEM	35.2a	5.6f	33.2ab	23.1c	15.5d	31.6b	8.7e	0.30
ME	12.5ab	8.3c	12.5ab	14.6a	10.0bc	13.8ab	14.2a	0.41
CT	0.8d	0.8d	1.1b	1.0bc	1.5a	0.6e	0.9cd	0.02
TPC	4.3c	1.9e	9.2a	2.9d	8.0b	1.7a	9.8a	0.06
IVDMD	45.6d	82.1a	39.0e	52.1c	38.2e	78.0a	73.2b	0.44

[1]Means with different letters in the same row are statistical different (Tukey, $p<0.05$); GO= gray oak; PP= prickly poppies; RO= red oak; MES= mesquite; WT= wattles tree; WM= white mulberry; STE= stevia; SED= standard error of the difference among means; CP= crude protein; EE= ether extract; NSC= non structural carbohydrates; NDF= neutral detergent fiber; ADF= acid detergent fiber; LIG= lignin; CEL= cellulose; HEM= hemicellulose; ME= metabolizable energy (MJ/kg DM); CT= condensed tannins; TPC= total phenolic compounds; IVDMD= *in vitro* dry matter digestibility.

Table 3. *In vitro* methane, carbon dioxide and gas production kinetic parameters of proposed vegetative species.

Parameter	Species							SED
	GO	PP	RO	MES	WT	WM	STE	
A (mL/g DM)	46.9b	99a	37.9c	27.8d	18.6e	42.5bc	27.8d	0.62
L (h)	2.3b	3.6a	2.1b	3.7a	3.7a	2.3b	3.7a	0.07
k_d (%/h)	0.11c	0.19ab	0.12bc	0.22a	0.21a	0.11c	0.22a	0.01
GP_{24} (mL/g DM)	37.9b	87.3a	30.1c	17.1d	28.1c	30.1c	30.1c	0.52
CH_4 (mL/g DM)	3.2b	7.1a	1.4e	0.8f	0.9ef	1.9d	2.5c	0.04
CO_2 (mL/g DM)	29.3b	67.8a	18.1d	11.0e	22.1cd	20.5cd	23.8bc	0.22
CO_2:CH_4 ratio	9.3c	13.4b	9.6c	13.2b	24.1a	10.4c	9.7c	0.28

*Means with different letters in the same row are statistical different (Tukey, $p<0.05$); A= maximum gas production; L= latency time before the gas production begins h; k_d= gas production constant rate (h^{-1}); GP_{24}: gas production after 24h of fermentation; GO= gray oak; PP= prickly poppies; RO= red oak; MES= mesquite; WT= wattles tree; WM= white mulberry; STE= stevia; SED= standard error of the difference among means.

Table 4. *In vitro* ruminal fermentation parameters of evaluated vegetative species.

Parameter	Species							SED
	GO	PP	RO	MES	WT	WM	STE	
N-NH_3(mg/dL)	4.3e	7.9b	6.7bc	13.1a	5.9cd	12.8a	5.2de	0.19
TVFA (mM)	0.05a	0.07a	0.07a	0.07a	0.05a	0.07a	0.06a	0.01
	VFA concentration (mmol/100 mmol TVFA)							
Acetate	68.4bc	70.9ab	74.2a	75.6a	65.3c	75.9a	72.1ab	0.60
Propionate	16.2ab	16.2ab	13.8b	14.3b	18.6a	14.7b	14.8b	0.28
Butyrate	6.2ab	5.8abc	5.3abc	4.2c	6.6a	4.4bc	5.9abc	0.21

*Means with different letters in the same row are statistical different (Tukey, $p<0.05$); GO= gray oak; PP= prickly poppies; RO= red oak; MES= mesquite; WT= wattles tree; WM= white mulberry; STE= stevia; N-NH_3= N-ammonia; SED= standard error of the difference among means; TVFA: total volatile fatty acids.

DISCUSSION

Chemical composition. Previous research showed similar contents of CP. In fact, the CP contents in a medium quality alfalfa hay is considered about 13%; however, there are some bad quality forages which may range between 8-12% of CP which is highly associated with the maturity of the forage source (20). According to Fox et al (21), the fat contained in EE plays a very important role in the ruminants' energy supply. Even though two of the vegetable species proposed in this study showed higher contents of EE, this content must be recalculated when mixing other feedstuffs in the total mixed ration of livestock since EE values over 7% may represent toxicity for the ruminal microorganisms which may compromise the ruminal fermentation (22). High contents of ash in feedstuffs lead to a reduced ruminal fermentation of organic matter; in addition, high ash contents and metabolizable energy are negatively correlated (23). However, ash contents in vegetable species analyzed in this study agree with previous research (24).

***In vitro* gas production.** Almost all the proposed vegetable species produced gas comparable with medium and high-quality forages. Ivan et al (25) reported that the grade of degradation depends on the structure and type of carbohydrates contained in each specie. Regarding to this, PP and STE presented the higher contents of NSC and the lower contents in NDF. In addition, Han & McCormick (26) reported a maximum gas production of about 96.3 (mL/g DM) when incubated solely alfalfa hay. On the other hand, the lignin content in WT is the highest (10.4%) which leads to an increase in lignocellulosic complexes; these complexes affect the gas production through reductions in the digestibility (27). Likewise, WT presented the highest content in CT (1.5%); CT tend to form complexes with proteins which may affect the gas production (3). Similar behavior is observed with mesquite (MES) that presented similar contents than those obtained for WT; CT content in mesquite is lower than the obtained for WT. Presumably, these complexes affected the maximum gas production as explained earlier. As a matter of fact, the lowest IVDMD was observed in WT and MES; this fact supports the theory of affections due to the presence of lignin and CT. Despite the high IVDMD and NSC observed in STE, this specie presented low values of gas production. Sarnataro & Spanghero (28) reported a reduction in the protozoa when administered stevia extracts to rumen incubations. Regarding to this, previous research reported that defaunation of protozoa affects the ruminal fermentation (29).

Tavendale et al (3) affirmed that CT tend to form complexes with the proteins and with certain methanogens which may block the pathway for the synthesis of methane. According to this, whether methane is not synthetized due to the latter, then the propionate synthesis would be favored since the synthesis of both compounds (methane and propionate) are natural sink of free protons (H^+) contained in the rumen. On the other hand, the CO_2:CH_4 ratio presented a different behavior than the showed by the methane and CO_2 production;

WT, PP and MES showed the highest values ($p<0.05$). The CO_2:CH_4 ratio represents the the volume (mL) of CO_2 remaining by each mL of methane produced; the higher the value of this variable then the lower methane production through the CO_2-reduction pathway. Despite the individual productions of CO_2 and methane for each specie, higher values in CO_2:CH_4 ratio suggest a reduction in methane production when compared to CO_2 production. Thus, the latter may indicate a diminution in methane synthesis through the CO_2 reduction pathway. Furthermore, vegetable species which presents lower values of this ratio are highly desired. Otherwise, species like GO, RO, and WM presented lower values of this ratio as a consequence of lower values in gas production which would consider their characteristics as a low quality forage as discussed earlier in this study. Deutschmann et al (30) reported lower values of gas productions at 24h of fermentation with pangola grass (approximately 10 mL/g DM) than the volume obtained in the present study with the proposed vegetative species. These same authors fed bulls with pangola grass as a part of a mixed ration and obtained average daily gains (ADG) about 0.45 kg/day. The latter suggests that the utilization of the vegetable species proposed in this study may infer superior ADG than those reported by Deutschmann et al (30).

***In vitro* fermentation parameters.** The proteins are degraded to peptides, amino acids and NH_3; the latter is the main nitrogen source used for the microorganisms for microbial protein (31). Cheeke (32) reported that the ideal concentration of N-ammonia in the rumen should be ranged from 5-24 mg/dL. Likewise, Lunsin et al (33) supported this theory reporting a range from 5 to 8 mg/dL as the minimum required for an optimal microorganisms' growth which may promote an efficient feedstuff degradation. According to Pond et al (34), values in N-ammonia depends directly of the available energy. Regarding to the this, only GO presented lower N-ammonia concentrations than the one needed for optimal microbial growth. Nevertheless, the forage is just another ingredient of the mixed ration offered to bovines. In addition, Abdulla et al (35) affirmed that differences in N-ammonia are directly correlated to changes in the dynamic and microbial processes as well as the urea recycling rate in the rumen. Previous research reported that a higher rumen degradable protein contents are directly correlated with higher acetate contents (36); obtained data in the present study agree with the authors.

Whether a fermentation is focused in propionate production a reduction in methane and gases production would be expected as a consequence (37). The fermentation of substrate to propionate is a gas producer reaction only due to the neutralization of the acid; therefore, a lower gas production is associated with a propionic fermentation (38). In this way, results obtained for WT are consistent with the latter. Almaraz-Buendia et al (39) presented similar proportions of acetate, propionate and butyrate (65, 21 and 5%, respectively).

In conclusion, this study proposed different vegetable species as possible forage sources. According to the chemical composition, PP, WM and STE presented the best nutritional quality since they showed the highest contents in protein and adequate digestibility. However, PP also presented optimal values in gas production kinetics which are comparable with commonly used forage sources as alfalfa hay. Additionally, higher values in CO_2:CH_4 for PP suggest an improve in fermentation parameters when compared to the other species. These findings suggest that the use of PP may not affect the nutritional characteristics of a ration in ruminants' feeding in spite of its content of CT; the suggested use of PP would be as a forage source in a ration but not as a solely feeding source. Otherwise, the other species may be used as additives or supplements in the ruminants feeding but digestibility of the ration may be affected instead.

Conflict of interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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