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CHEMICAL ANALYSIS AND *in situ* DEGRADATION OF DIETS PREPARED WITH AND WITHOUT FRUIT OF *Parmentiera aculeata* (Kunth) L.O. Williams IN SHEEP

Selene **Pérez-Morales**[1], María Magdalena **Crosby-Galván**[1*], Mónica **Ramírez-Mella**[2], José Ricardo **Bárcena-Gama**[1], Omar **Hernández-Mendo**[1], Elsa Margarita **Crosby-Galván**[1]

[1] Colegio de Postgraduados Campus Montecillo. Programa de Ganadería. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264.
[2] CONACyT-Colegio de Postgraduados Campus Campeche. Carretera Haltunchén-Edzná km 17.5, Sihochac, Champotón, Campeche, Mexico. C. P. 24450.
* Author for correspondence: maria@colpos.mx

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ABSTRACT

During the drought period in cattle pastures, the fruits of chote (*Parmentiera aculeata* (Kunth) L.O.) Williams are available, which are satisfactorily consumed by grazing ruminants. However, the nutritional value and ruminal availability of the dry matter of this fruit, to which nutritional properties are attributed, are unknown. The objective was to evaluate the degradation of chote fruit for inclusion in sheep diets using *in situ* degradation and to identify some physiological variables in the rumen. Six rumen fistulated lambs with a live weight of 30 ± 2 kg were adapted for 11 days to the following experimental diets: T 0: 0 % chote fruit; T 15: 15 % chote fruit, and T 30: 30 % chote fruit. *In situ* degradation of chote fruit was determined and experimental diets were analyzed with a 3 × 3 crossover design. The results show that after 3 and 6 h, chote fruit at T 30 presented greater degradation ($p \leq 0.05$). After 24, 48, and 72 h, pH and ammonia nitrogen ($N\text{-}NH_3$) showed no statistical differences ($p > 0.05$). The yields of acetic, propionic, and butyric acids were not different ($p > 0.05$) at T 15 (acetic acid = 77.81 mM L^{-1}; propionic = 14.39 mM L^{-1}; butyric = 0. 67 mM L^{-1}) and T 30 (acetic acid = 77.21 mM L^{-1}; propionic = 18.52 mM L^{-1}; butyric = 8.97 mM L^{-1}), but both were greater than T 0 (acetic = 62.03 mM L^{-1}, propionic = 18.52 mM L^{-1}, butyric = 9.27 mM L^{-1}). In conclusion, the addition of 30 % chote to sheep diets increases degradation without affecting ruminal variables.

Keywords: Bignoniaceae, tropical fruits, cuajilote, pH, volatile fatty acids.

INTRODUCTION

During the dry season, meat and milk production is reduced due to the scarcity of foods such as grains and fodder (Gómez *et al*., 2002), which makes it necessary to look for alternative sources of feed for grazing ruminants. In the tropical zones of Mexico, the chote or cuajilote tree (*Parmentiera aculeata* (Kunth) L.O. Williams), which belongs to the Bignoniaceae family, is produced. The fruit of this tree is used as a complementary food source to enhance the breeding of tropical cattle. The fruits are

available during the dry season (Palma *et al.*, 2019). Its production can begin 2 to 3 years after transplanting, with an annual yield of 1 000 to 2 000 fruits (Morales-Sánchez *et al*., 2015). However, the nutritional value of this fruit and the degradation of dry matter (DM) in the rumen are unknown (García-Castillo *et al.* 2008).

More detailed studies are required to determine the forage capacity of species such as chote and the possibility of its integration into livestock systems. In order to carry out strategic supplementation, it is necessary to determine the influence of this fruit on the rumen fermentation variables of the animals that consume it (Razz *et al*., 2004). For this purpose, it is essential to estimate the ruminal degradation of dry matter (DM), as well as to analyze ruminal fermentation variables such as pH, ammoniacal nitrogen ($N\text{-}NH_3$), and volatile fatty acids (VFAs). This information will support the integration of this fruit as a food source for grazing animals. The objective of this study was to evaluate the *in situ* degradation of chote fruit incorporated in sheep diets on rumen fermentation variables, in order to determine its potential as a supplement for grazing animals.

MATERIALS AND METHODS

Location

The experiment was conducted at the experimental farm and the Animal Nutrition laboratory of the Postgraduate Program of Genetic Resources and Productivity-Livestock, Colegio de Postgraduados Campus Montecillo, located at an altitude of 2240 m in Montecillo, municipality of Texcoco, State of Mexico (19° 27′ 38″ N, 98° 54′ 11″ W).

Animals

Six rumen fistulated Dorper-Pelibuey lambs with a live weight of 30 ± 2 kg and an age of approximately 10 ± 2 months were housed in individual pens and adapted to the experimental diets for 11 days. They were fed 1 kg of the experimental animal diet per day, twice a day (08:00 and 16:00 h) and had unlimited access to water. The cannulas were placed and the lambs were handled in accordance with the regulations for the use and care of animals destined for research at the Colegio de Postgraduados, in accordance with NOM-062-ZOO-1999.

Selection of fruits, distribution, and composition of treatments

Green and ripe fruits were cut and collected from chote bushes in Tehuetlán, Huejutla de Reyes, Hidalgo, at km 198 of the Mexico-Tampico highway, at an altitude of 340 m, 98° 31′ W, 21° 03′ N (INEGI, 1990). In the laboratory, the fruits were cut to facilitate and accelerate drying, placed on aluminum trays, and dehydrated in a forced air oven (RIOSSA® Mod. NCF-62) at 55 °C for 5 d to determine the partial dry matter (DM). For chemical composition analysis of the fruit and diets, grinding was performed using a mill (Thomas Willey Laboratory Mill Mod. 4) with a 1 mm mesh. The feed offered to

the sheep was ground using a mill with a 1 cm screen (Molinos Azteca y Juper S. A. de C. V., Serial No. 025878).

The treatments were as follows: T 0: 0 % chote, T 15: 15 % chote, and T 30: 30 % chote on a dry basis. These diets were formulated according to the nutritional requirements for fattening lambs (NRC, 2007) (Table 1).

Table 1. Experimental diets and their chemical composition (percent dry basis) as lamb feed.

Ingredients	T 0	T 15	T 30
Ground maize (*Zea mays*)	9	1	0
Ground sorghum (*Sorghum bicolor*)	8	1	0
Hay alfalfa (*Medicago sativa*)	30	30	21
Oat straw (*Avena sativa*)	33	33	27
Soybean paste (*Glycine max*)	12	12	14
Chote fruit (*Parmentiera aculeata*)	0	15	30
Molasses	6	6	6
Minerals	2	2	2
Total	100	100	100

T 0: 0 % chote fruit; T 15: 15 % chote fruit; T 30: 30 % chote fruit.

Laboratory analysis

The chemical analysis of the ingredients and the integrated diets are shown (Table 2). Proximal analysis of fruit and diets was performed using the AOAC (2005) methodology, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractionation was performed using the van Soest *et al*. (1991) technique.

Table 2. Chemical analysis of chote (*Parmentiera aculeata* (Kunth) L.O. Williams) fruit and diets offered to lambs.

Sample	DM	H	C	CP	EE	NDF	ADF	HE
				%				
Chote fruit	88.11	11.89	6.37	8.09	0.68	45.95	38.79	7.15
Maize	90.15	9.85	1.15	7.7	3.37	26.63	14.3	12.32
Sorghum	91.18	8.82	1.34	8.1	2.22	37.65	25.05	12.59
Alfalfa	92.28	7.72	11.2	18.8	0.9	57.15	48.75	8.4
Soybean paste	90.29	9.71	6.52	44.8	0.95	52.63	42.45	10.18
Oat straw	95.28	4.72	5.46	5.12	2.01	69.37	45.68	23.69
T0	88.1	11.89	9.4	14.25	1.87	49.11	37.25	11.86
T15	90.1	9.88	10.1	14.26	1.24	54.38	42.65	11.72
T30	89.8	10.22	8.8	14.35	1.37	53.59	43.45	10.13

DM: Dry matter; H: moisture; C: ash; CP: crude protein; EE: ethereal extract; NDF: neutral detergent fiber; ADF: acid detergent fiber; HE: hemicellulose; T 0: 0 % chote fruit; T 15: 15 % chote fruit; T 30: 30 % chote fruit.

Reducing sugars in chote fruit

Sugars were evaluated using liquid chromatography (HPLC) according to Mustafa *et al*. (2003). For this purpose, 2 g of sample were weighed and 60 mL of 80 % ethanol were added. The sample was slowly boiled until 10 mL were obtained. Subsequently, it was volumetrized with water to 17 mL, filtered with acrodisks, and sent to the HPLC for analysis.

In situ degradation of chote fruit and experimental diets in sheep

A 0.5 g sample was deposited in polysilk bags with 5 × 5 cm in size and 60 µm pore size. The bags were heat sealed with Impulse Sealer Type equipment: AIE-2000 (American International Electric) and introduced into the rumen in a cloth net for incubation for 0, 3, 6, 9, 12, 12, 24, 48, and 72 h on day 12 of adaptation. At the end of each incubation period, the bags were removed, washed with running water, dried, weighed, and stored until analysis of DM, NDF, and ADF degradation (Mehrez and Orskov, 1977).

Rumen fluid analysis

The pH of ruminal fluid collected at 3 h postprandial on day 16 of incubation was measured using a portable potentiometer (HANNA Instruments®, pHep Tester®, Pocket pH Tester). Subsequently, the ruminal fluid was acidified with 25 % metaphosphoric acid in a 1:5 mL ruminal fluid ratio and frozen at 4 ° C.

Ammoniacal nitrogen (N-NH_3) was determined using the McCullough (1967) technique. Volumes of 1 mL of phenol, 20 µL of the sample, and 1 mL of sodium hypochlorite were placed in polypropylene tubes and incubated in a water bath at 37 ° C for 30 min before adding 5 mL of distilled water. The standard curve was created with concentrations of 2.5, 5, 10, 15, and 20 mg dL^{-1}. Blanks were prepared with 1 mL phenol, 1 mL sodium hypochlorite, and 5 mL distilled water. The N-NH_3 concentration was measured using a VARIAN Cary 1E spectrophotometer at a wavelength of 630 nm.

The concentration of short-chain volatile fatty acids (VFAs) was analyzed using an HP 6890 gas chromatograph with a flame ionization detector and an Agilent Technologies HP-FFAP 19091F capillary column with dimensions of 30 m length × 0.250 mm inner diameter × 0.25 µ circumference (Ewaschuk *et al*., 2002). The standard used was FAM6C, 1000 µg mL^{-1} H_2O, High Purity Standards. The chromatograph conditions are shown (Table 3).

Statistical analysis

The *in situ* degradation of diets in sheep was analyzed using a 3 × 3 crossover design and the GLM procedure with the SAS® (9.1) statistical package (2011). Comparison of means was performed with Tukey's test ($p \leq 0.05$) (Steel and Torrie, 1992), and the following model was used:

$$Y_{ij} = (t) = \mu + \alpha_i + \beta_j (i) + G (t) + \varepsilon_{ijk} (t)$$

Table 3. Gas chromatograph conditions for analysis of volatile fatty acids.

Detector characteristics:		Injector characteristics:	
Makeup (ON): 14.0 mL min $^{-1}$ Temperature: 240 ° C Injector: Split		Temperature: 230 °C Pressure: 12.0 PSI Split: 10.0	
Total run time: 9.94 min			
Oven temperature ramp:	Speed °C min $^{-1}$	Temperature °C	Time (min)
Ramp 1	65	95	0.15
Ramp 2	15	145	6.0

where $Y_{ijk}(t)$: random variable representing the *j*-th observation of the *i*-th treatment, μ: general mean, α_i: effect of the *i*-th treatment, $\beta_{j(i)}$: average additional effect of the *j*-th level nested in level *I*, *G* (*t*): grand total of treatments, and $\varepsilon_{ijk}(t)$: is the experimental error of unit *ijk*.

RESULTS AND DISCUSSION

In situ ruminal degradation test

In terms of *in situ* DM degradation (Table 4), the chote treatments behave similarly at 3 and 6 h, with T 30 showing the highest degradation (43.77 and 49.88 %), followed by T 15 (40.62 and 48.41 %) ($p \leq 0.05$); T 0 behaves similarly to T 15 with lower values (37.21 and 45.44 %). At 9 h, T 0, and T 15 are equal (51.99 and 54.72 % respectively) ($p > 0.05$); the highest value was with T 30 (59.12 %) with differences with respect to other values ($p \leq 0.05$). At 12 h, T 30 and T 15 presented no differences (62.76 and 61.26 %, respectively) ($p > 0.05$), T 0 presented a lower value than the other treatments (56.26 %) ($p \leq 0.05$). Finally, at the final time periods, no differences were observed between treatments, from 70 to 79 % degradation ($p > 0.05$).

Table 4. *In situ* degradation (%) of dry matter of experimental diets in sheep.

	Time (h)							
Treatments	0	3	6	9	12	24	48	72
T 0	0a	37.21a	45.44a	51.99a	56.26a	72.48a	77.53a	78.93a
T 15	0a	40.62 ab	48.41 ab	54.72a	61.26 b	70.93a	74.37a	79.23a
T 30	0a	43.77 b	49.88 b	59.12 b	62.76 b	71.77a	76.21a	79.04a
SEM	0	0.55	0.79	1.789	1.72	1.79	1.26	1.79

a,b,c: Means with different literal in the column indicate differences (Tukey, $p \leq 0.05$). T 0: 0 % chote fruit; T 15: 15 % chote fruit; T 30: 30 % chote fruit; SEE: standard error of the mean.

The differences in DM degradation observed in this study were due to higher fermentation of soluble carbohydrates (fructose 8.28 %, glucose 5.11 % and sucrose 11.55 % in the chote fruit on a dry basis) present in varying concentrations in the treatments during the first 12 h and found to be more available to ruminal microorganisms (Pulido and Leavep, 2000). After 24 h, fiber fermentation predominates. Moctezuma *et al*. (1993) report digestibilities from 82 to 87.77 %, at 24 h with ripe fruits and the low degradation of this work comes from the combination of green and ripe fruits. In turn, Pinto-Ruíz *et al*. (2010), while evaluating forage trees from three cattle-raising regions of Chiapas, found that for the Frontera region (group in which *P. aculeata* (Kunth) L.O. Williams is found) there is a linear relationship between NDF content and DM degradation of the species studied, increasing fiber and decreasing degradation. In another study with this same fruit at three stages of maturity, incubated for 72 h, a greater disappearance of DM was observed in fruits emerging from the stem of the plant of no more than 8 cm in length, as opposed to green fruits that had developed their maximum size (García-Castillo *et al.* 2008).

Fiber degradation shows no differences ($p > 0.05$) between treatments except at 9 h (Table 5), time at which the mean for T 30 (46.30 %) is higher than T 0 but similar to T 15. This is because increasing the levels of chote increases the levels of sugars,

Table 5. Neutral detergent fiber degradation (%) of experimental diets in sheep.

Treatments	Time (h)							
	0	3	6	9	12	24	48	72
T 0	0	26.84	35.17	40.61a	45.51	59.77	65.66	71.6
T 15	0	26.53	36.61	42.05 ab	49.92	58.68	67.06	70.12
T 30	0	27.27	33.82	46.3 b	47.85	60.6	68.48	72.75
SEM	0	2.66	1.01	2.46	2.05	0.64	2.73	1.51

a,b,c: Means with different literal in the column indicate differences (Tukey, $p \leq 0.05$); T 0: 0 % chote fruit; T 15: 15 % chote fruit; T 30: 30 % chote fruit; SEE: standard error of the mean.

which favor degradation. At 72 h, degradation reached an average of 71.49 %, and no differences ($p > 0.05$) were found between treatments. Ruminal degradation of plant material is known to be related to the proportion of cell wall (Norton *et al*., 1994).

The degradation of DM, NDF, and ADF (Table 6) increased gradually with increasing incubation time and stabilized after 24 h for DM and ADF, and after 48 h for NDF. The low digestibility is attributed to the high content of NDF residue. This degradation behavior is due to the fact that the easily fermented carbohydrates are degraded first, followed by the structural carbohydrates. In general, DM degradation depends on the stage of maturity, showing that soluble DM and degradable DM increased in mature fruits that had yellowish coloration with sweet aroma and soft consistency, followed

Table 6. *In situ* degradation of dry matter, neutral and acid detergent fiber (%) of chote fruit in sheep.

Time (h)	DM	NDF	ADF
0	0	0	0
3	50.11a	12.71a	11.66a
6	55.11 ab	21.77 b	16.70a
9	62.77 bc	26.14 b	18.10a
12	69.76 c	27.23 b	26.03 b
24	79.47 d	65.68 c	61.78 c
48	80.79 d	85.69 d	63.15 c
72	81.18 d	87.14 d	64.31 c
SEM	2.52	2.98	2.13

a,b,c: Means with different literal in the column indicate differences (Tukey, $p \leq 0.05$); DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; SEM: standard error of the mean.

by green chote fruits that correspond to the fruit emerging from the stem of the plant that does not exceed 8 cm in length, and finally green fruits that have developed their maximum size and maintain their light greenish color and hard consistency (García-Castillo *et al.* 2008).

In the research by Pinto-Ruiz *et al.* (2010) only evaluated this fruit at the mature stage, and the results obtained are superior to those reported by other authors. According to van Soest *et al*. (1991), DM degradation of fruit is a variable determined by several factors, such as the amount and type of feed consumed, the fistulated animal serving as animal model, ruminal incubation periods, the characteristics of the nylon bag used, and the physicochemical properties of the fruit evaluated. Compared to other forages, such as introduced grasses (*Brachiaria humidicola* cv) in the tropical region, DM from chote fruit has reduced availability at the rumen level (Rincón *et al*., 2018). However, it is an alternative feed where this forage is abundant.

Ruminal variables estimated *in situ*

The pH did not show differences between treatments ($p > 0.05$) (Table 7). Decreasing the percentage of grains and increasing the percentage of chote fruit in the diets had no effect on ruminal variables. Ammoniacal nitrogen ($N\text{-}NH_3$) showed no differences ($p > 0.05$) between treatments. This indicates that diets are being utilized, despite increasing the chote content and reducing the number of grains such as sorghum and corn.

The production of acetic, propionic, and butyric acids presented differences between treatments ($p \leq 0.05$), with T 30 producing more of these acids than T 0 ($p \leq 0.05$). Differences between treatments were observed in the concentration of isobutyric and isovaleric acid (0.77 mM L^{-1} and 0.73 mM L^{-1}) ($p \leq 0.05$), with T 30 producing the most of these VFAs. In treatments T 0 and T15, isobutyric and isovaleric acids are similar ($p > 0.05$).

Table 7. Ruminal variables of experimental diets in sheep.

Variables	Treatments T 0	T 15	T 30	SEM
pH	6.3a	6.64a	6.4a	0.57
N-NH_3 (mg dL^{-1})	22.39a	17.49a	19.28a	2.14
		VFAs (mM L^{-1})		
Acetic acid	62.03a	77.81 b	77.21 b	5.86
Propionic acid	14.39a	18.52 b	18.52 b	0.77
Isobutyric acid	0.67a	0.64a	0.77 b	0.03
Butyric acid	7.33a	8.97 b	9.27 b	3.66
Isovaleric acid	0.60a	0.55a	0.73 b	0.04
Valeric acid	0.98a	1.10 ab	1.27 b	0.07
A:P	4.31	4.20	4.17	

a,b, c: Means with different literal in the row indicate differences (Tukey, $p \leq 0.05$); T 0: 0 % chote fruit; T 15: 15 % chote fruit; T 30: 30 % chote fruit; SEE: standard error of the mean.

Valeric acid also presented differences between treatments, with T 0 (0.98 mM L^{-1}) being lower than T 30 (1.27 mM L^{-1}) ($p \leq 0.05$). Treatment T 15 (1.10 mM L^{-1}) is similar to treatments T 0 and T 30 ($p > 0.05$). A decrease in ruminal pH from 6.5 to 5.7 reduces N-NH_3 concentrations when ruminal inoculum is obtained from cattle fed an exclusive forage diet, whereas when using inoculum from cattle fed 90 % concentrate, N-NH_3 concentration is affected despite the low pH (Lana *et al*., 1998). These results indicate that protein degradation is influenced by pH and diet type, which can determine the type of microbial population present in the rumen (NRC, 2000; Hristov *et al*., 2001).
The normal concentration of N-NH_3 in the rumen varies from 5 to 25 mg dL^{-1} of rumen fluid (Cheeke, 2004), as reported by Satter and Slyter (1974), where maximum microbial efficiency occurs when ruminal N-NH_3 concentration is between 5 and 8 mg dL^{-1}. In this study, such differences in acetic acid production could be due to the way the forage ration is fed, whether unchopped or in large chunks. Variation depends on forage type, stage of maturity, and soil fertilization (Balch and Rowlan, 1957; Bath and Rook, 1965). Propionic acid varies between 15 and 19 % when the amount of concentrate added to the animal is high (Armstrong and Blaxter, 1957), and its composition is based on grains with high starch content, especially if they have previously been heat-treated (Bath and Rook, 1965). Similarly, these authors state that butyric acid has a wider range of molarity variations, ranging from 8 to 16 %.

CONCLUSIONS

The yield capacity of chote (*Parmentiera aculeata*) fruits, as well as *in situ* dry matter (DM) degradation, neutral detergent fiber (NDF) degradation, and total volatile fatty acids (VFAs), demonstrated that this fruit species is a feeding alternative for ruminants during critical times. The addition of chote to sheep diets up to 30 % does not alter degradation, and rumen variables are adequately maintained.

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REFERENCES

AOAC (Association of Official Agricultural Chemists). 2005. Official methods of the Association of the Agricultural Chemists (18 edition). Washington, DC, USA. https://www.researchgate.net/publication/292783651–AOAC–2005 (Retrieved: November 2022).

Armstrong DG, Blaxter KL. 1957. The heat increment of steam-volatile fatty acids in fasting sheep. British Journal of Nutrition 11 (3): 247–272. https://doi.org/10.1079/BJN19570044

Balch CC, Rowlan SJ. 1957. Volatile fatty acids and lactic acid in the rumen of cows receiving a variety of diets. British. Journal of Nutrition 11 (3): 288–293. https://doi.org/10.1079/BJN19570046

Bath IH, Rook JA. 1965. The evaluation of the cattle foods and diets in terms of the ruminal concentration of volatile fatty acids. II. Roughages and succulents. The Journal of Agricultural Science 64 (1): 67–75. https://doi.org/10.1017/S0021859600010480

Cheeke PR. 2004. Applied animal nutrition: Feeds and feeding (3rd edition). Prentice Hall: Madison, WI, USA. 70 p.

García-Castillo CG, Martínez-Tinajero JJ, Montañez-Valdez OD, Sánchez-Orozco L, Posada-Cruz S, Izaguirre-Flores F, Martínez-Priego G. 2008. Degradación ruminal de la materia seca del fruto cuajilote (*Parmentiera edulis*). Zootecnia Tropical 26 (1): 1–8.

Gómez CH, Tewolde MA, Nahed JT. 2002. Análisis de los sistemas ganaderos de doble propósito en el centro de Chiapas, México. Archivos Latinoamericanos de Producción Animal 10 (3): 175–183.

Ewaschuk J, Zello G, Naylor J, Brocks DR. 2002. Metabolic acidosis: separation methods and biological relevance of organic acids and lactic acid enantiomers. Journal of Chromatography B 781 (1–2): 39–56. https://doi.org/10.1016/s1570-0232(02)00500-7

Hristov A, Ivan M, Rode L, McAllister T. 2001. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. Journal of Animal Science 79 (2): 515–524. https://doi.org/10.2527/2001.792515x

INEGI (Instituto Nacional de Estadística y Geografía). 1990. Hidalgo. Resultados definitivos, datos por localidad (integración territorial), XI censo general de población y vivienda. 13–14. Instituto Nacional de Estadística y Geografía. Ciudad de México, México. https://en.www.inegi.org.mx/contenidos/productos/prod–serv/contenidos/espanol/bvinegi/productos/historicos/1329/702825116705/702825116705–1.pdf (Retrieved: November 2022).

Lana RP, Russell JB, van Amburgh ME. 1998. The role of pH in regulating ruminal methane and ammonia production. Journal Animal Science 76 (8): 2190–2196. https://doi.org/10.2527/1998.7682190X

McCullough H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clinica Chimica Acta 17 (2): 297–304. https://doi.org/10.1016/0009-8981(67)90133-7

Mehrez AZ, Orskov ER. 1977. A study of artificial bag technical for determining the digestibility of feed in the rumen. Journal of Agricultural Science 88 (3): 645–650. https://doi.org/10.1017/S0021859600037321

Moctezuma SA, Lerma D EC, Martínez DR, Salinas ChJ, Yado PR. 1993. Digestibilidad *in vivo* de dietas integrales con distintos niveles de inclusión de fruto de chote (*Parmentiera edulis*) para ovinos. Reunión Nacional de Investigación Pecuaria: Guadalajara, México, pp: 27–30.

Morales-Sánchez V, Osuna-Fernández HR, Brechú-Franco A, Laguna-Hernández G, Vargas-Solís R. 2015. Evaluación del efecto antiurolítico del fruto de *Parmentiera aculeata* en rata Wistar. Botanical Sciences 93 (2): 293–298. https://doi.org/10.17129/botsci.99

Mustafa K, Mustafa E, Mustafa KU, Mehmet AA. 2003. Comparison of different extraction and detection. Methods for sugars using amino-bonded phase HPLC. Journal of Chromatographic Science 41 (6): 331–333. https://doi.org/10.1093/chromsci/41.6.331

Norton BW, Gutteridge RC, Shelton HM. 1994. The nutritive value of tree legumes. *In* Gutteridge C, Shelton Hl. (eds.), Forage Tree Legumes in Tropical Agriculture. CAB: London, UK, pp: 177–192.

NRC (National Research Council). 2000. Nutrient requirements of beef cattle: Seventh revised edition. National Research Council, National Academy of Sciences: Washington, DC, USA. 249 p. https://doi.org/10.17226/9791

NRC (National Research Council). 2007. Nutrient requirements of small ruminants. The National Academies Press, National Research Council, National Academy of Sciences: Washington, DC, USA. 800 p. https://doi.org/10.17226/11654

Palma JM, Zorrilla JM, Nahed J. 2019. Integración de especies arbóreas con residuales agrícolas y agroindustriales en la generación de sistemas ganaderos resilientes. Cuban Journal of Agricultural Science 53 (1): 73–90.

Pinto-Ruíz P, Hernández D, Gómez H, Cobos MA, Quiroga R, Pezo D. 2010. Árboles forrajeros de tres regiones ganaderas de Chiapas, México: Usos y características nutricionales. Universidad y Ciencia 26 (1): 19–31.

Pulido R, Leavep JD. 2000. Degradabilidad ruminal del forraje disponible en la pradera y del aparentemente consumido por vacas lecheras. Pesquisa Agropecuaria Brasileira 35 (5): 1103–1109. https://doi.org/10.1590/s0100-204x2000000500018

Razz R, Clavero T, Vergara J. 2004. Cinética de degradación *in situ* de la *Leucaena leucocephala* y *Panicum maximum*. Revista Científica 14 (5): 424–430.

Rincón A, Flórez H, Ballesteros H, León LM. 2018. Efectos de la fertilización en la productividad de una pastura de *Brachiaria humidicola* cv. Llanero en el piedemonte de los llanos orientales de Colombia. Tropical Grassland 6 (3): 158–168. https://doi.org/10.17138/tgft(6)158-168

SAS (Statical Analysis System). 2011. SAS User´s Guide: Statics, version 9.0. SAS Institute, Inc., Cary, NC.

Satter LD, Slyter LL. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. British Journal of Nutrition 32 (6): 847–849. https://doi.org/10.1017/S000711452100458X

Steel RGD, Torrie JH. 1992. Bioestadística: Principios y procedimientos (2nd edition). McGraw-Hill: Ciudad de México, México. 622 p.

van Soest JP, Robertson J, Lewis B. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74 (10): 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2