Laboratory evaluation of meal supplements made of overripe mango flour and parota pod flour

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ABSTRACT

Objective: to evaluate the chemical content [crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF)], and *in vitro* fermentative characteristics [biogas and CH_4 cumulative production, metabolizable energy (ME), dry matter degradation (DMD) and neutral detergent fiber degradation (NDFD)] of three supplements based on overripe mango flour (HMD) and parota pod flour (HVP) through a grazing simulation in the laboratory.

Design/Methodology/Approach: supplements S1=40% HMD+60% HVP, S2=60% HMD+40% HVP, and S3=80% HMD+20% HVP; Treatments to simulate grazing T1=70% cobra grass (PCo)+30% S1, T2=70% PCo+30% S2, and T3=70% PCo+30% S3.

Results: S3 had lower DMD, NDFD and ME ($p \leq 0.05$). T1 had higher CP, DMD, NDFD and ME; and T3 showed lower NDF ($p \leq 0.05$).

Limitations/Implications of the study: this study was implemented in laboratory, so there were some climatological aspects and physiological issues of ruminants that were not considered.

Findings/Conclusions: S1 improved the fermentative characteristics of cobra grass when grazing was lab simulated.

Keywords: alternative supplementation, regional ingredients, *Enterolobium cyclocarpum* (a.k.a; guanacaste), grazing, ruminants, tropics.

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INTRODUCTION

Tropical regions are important for producing grazing cattle, because of the forage biomass produced per hectare. However, grazing ruminant production has low net productivity because tropical grasses provide mainly fibrous carbohydrates. So one strategy to improve productivity is to use supplementation; since grass-based feed does not satisfy the nutrient requirements of animals (protein, energy, and minerals). Due to this, supplementation eliminates deficiencies, stimulates meal consumption, increases digestibility and animal performance (Estrada *et al*., 2019; Souza *et al*., 2021; Cazzuli *et al*., 2023).

Methods of supplementation vary from offering the same amount of supplement for a given period, to offering a certain amount of supplement based on a partcular parameter in ruminant production, such as milk yield (Hills *et al.*, 2015). Supplements can account for up to 70% of production costs. In addition, the lack of technical knowledge, evaluation of forage availability and quality, and basic nutritional requirements, all added encourage producers to use grain-based supplements (Esparza-Jiménez *et al.*, 2021). This is expensive without considering unconventional ingredients which are available in the region that could reduce costs for producers. In that regard, information is required to produce such supplements.

The *in vitro* technique of rumen fluid fermentation offers a fast, low-cost alternative for feed evaluation (Rodríguez *et al.*, 2017; Sobalvarro-Mena *et al.*, 2020). This is complemented with studies on chemical composition. In addition, *in vitro* technique can replace tests *in vivo* and allows for greater control over experimental conditions (Sobalvarro-Mena *et al.*, 2020). However, it is a batch technique, so fermentation products do accumulate. In its implementation, equal amounts of dry matter (DM) or organic matter (OM) from treatments are incubated to be compared and those indicators that are measured are expressed per incubated units of DM or OM (Rodríguez *et al.*, 2017). Thus, the objective was to evaluate the chemical-bromatological content, production and composition of biogas and fermentative characteristics of three supplements based on overripe mango flours and parota pod fluors; as well as to determine these variables also in a grazing simulation implemented in the laboratory.

MATERIALS AND METHODS

Study site

The study was carried out in the Animal Nutrition Laboratory, of the Faculty of Veterinary Medicine and Zootechnics No. 2, under the Autonomous University of Guerrero, located at km 197 of the roadway Acapulco-Pinotepa Nacional, Cuajinicuilapa (Guerrero), Mexico.

Supplements

Pods of the tree *Enterolobium cyclocarpum* (named 'parota' by the locals) was collected in the months of April and May 2020 in lands of the Cuajinicuilapa municipality (Guerrero), Mexico. Four branches were randomly selected from four trees and all physiologically mature pods were harvested; these were placed in paper bags and transferred to the Animal Nutrition laboratory for analysis. The overripe mango flour was processed by collecting overripe mangoes from four trees in the same municipality, they were placed in a 20 L plastic canisters and moved to the laboratory. Mangoes and pods were ground in a mixed mill (M.A.GRO® TR-3500, Mexico) with a 2.54 cm diameter sieve and dehydrated at 55 °C to a constant weight in an oven (Riossa® HCF-41, Mexico). They were then ground in a Thomas-Wiley Mill (Thomas Scientific®, Swedesboro, NJ, USA) with a 1 mm sieve. The supplements made were S1=40% overripe mango flour and 60% parota pod flour; S2=60% overripe mango flour and 40% parota pod flour; S3=80% overripe mango flour and 20% parota pod flour.

Grazing simulation

At the laboratory level, a grazing simulation was done, where a consumption of 70% of cobra grass (*Brachiaria* spp.) 60 d of regrowth and 30% of one of the 3 supplements made was simulated. The treatments in the simulation were: T1=70% grass+30% of S1; T2=70% grass+30% S2; T3=70% grass+30% S3.

Chemical-bromatological analysis

Dry matter (DM), crude protein (CP), ash (A) and organic matter (OM) were determined according to the methodology described by AOAC (2005). In addition, neutral detergent fiber (NDF) and acid detergent fiber (ADF) with the Van Soest *et al.* (1991) methodology, using the ANKOM® Technology Method.

In vitro gas production

The components of the culture medium were 30% clarified rumen fluid [fresh bovine rumen fluid centrifuged 10 min at 12.857 × *g* then sterilized (All American® 1941X, USA) 15 min at 121 °C and 15 psi], 5% 1st mineral solution [6 g K_2HPO_4 (J. T. Baker®) in 1 L of distilled water], 5% 2nd mineral solution [6 g K_2HPO_4 (J. T. Baker®)+6 g $(NH_4)_2SO_4$ (J. T. Baker®)+12 g NaCl (Meyer®)+2.45 g $MgSO_4$ (Meyer®)+1.6 g CaCl-2H_2O (Meyer®) in 1 L of distilled water], 0.1% resarzurine to 0.1% (Sigma-Aldrich®), 0.2% soy peptone (MCDLab®), 0.1% yeast extract (BD Bioxon®), 4% cysteine-sulfide solution [2.5 g L-cysteine (Sigma-Aldrich®) in 15 mL of 2N NaOH (Meyer®)+2.5 g of Na_2S-9H_2O (Meyer®) measured in 100 mL of distilled water], 5% solution to 8% Na_2CO_3 (J. T. Baker®) and 50.6% distilled water. The medium was sterilized for 15 min in an autoclave at 121 °C and 15 psi according to the Cobos & Yokoyama (1995) methodology, modified by Sánchez-Santillán *et al.* (2020).

A serological vial (120 mL) with 0.5 g of sample and 45 mL of culture medium was considered as a biodigester. The vials were kept under anaerobic conditions with CO_2, hermetically sealed with a neoprene cap (20 mm Ø) secured with an aluminum ring.

Table 1. Chemical composition and *in vitro* fermentative characteristics of ingredients.

Variables	Mango flour	Parota pod flour	Cobra grass with 60 d regrowth
Organic matter (g kg^{-1} DM)	972.1	959.5	878.6
Crude protein (g kg^{-1} DM)	55.9	197.2	78.2
Neutral detergent fiber (g kg^{-1} DM)	289.5	389.2	674.3
Acid detergent fiber (g kg^{-1} DM)	156.5	208.4	357.8
Ashes (g kg^{-1} DM)	27.9	40.5	121.4
Biogas production at 72 h (mL g^{-1} DM)	238.68	193.34	136.23
Methane production at 72 h (mL g^{-1} DM)	68.51	60.18	51.19
Dry matter degradation (g kg^{-1} DM)	754.6	712.1	493.2
Neutral detergent fiber degradation (g kg^{-1} DM)	226.9	349.8	326.5
Metabolizable energy (Mcal kg^{-1})	2.80	2.64	1.83

Biodigesters were sterilized at 121 °C and 15 psi for 15 min, and incubated for 24 h at 39 °C to verify sterile condition (Herrera-Pérez *et al.*, 2018). Then, biodigesters were inoculated with 5 mL of total rumen bacteria obtained from the rumen fluid of a Suiz-bu cow; The cow grazed on pangola grass (*Digitaria eriantha*) meadows before taking the rumen fluid sample. Rumen fluid was centrifuged 1.157 × *g* for 3 min to precipitate protozoa and fiber particles (Torres-Salado *et al.*, 2023).

In vitro biogas production was measured by displacing the plunger of a glass syringe (50 mL; BD Yale, Brazil) at 0, 3, 6, 9, 12, 24, 36, 48 and 72 h. The biogas production rate at each incubation time was obtained by estimating the partial production at each time span divided by the incubation hours. Cumulative biogas production was reported at 72 h of incubation (10 independent samples).

In the estimation of methane (CH_4) production, a Taygon® hose (2.38 mm Ø internal and 45 cm in length) was used, at the ends hypodermic needles (20 G × 32mm) were used to couple the biodigester with a trap vial of NaOH solution (2N). The trap vial was placed in reverse in a modified specimen that served to collect the CH_4-displaced NaOH solution produced during incubation by a hypodermic needle placed as an outlet valve (Torres-Salado *et al.*, 2018). CH_4 production was measured at 24, 48 and 72 h. The rate of CH_4 production at each measured time was obtained by estimating the partial production at each time span between the incubation hours. Cumulative CH_4 production was reported at 72 h of incubation (10 independent samples).

At the end of the 72 h of incubation, the biodigesters were used to determine ammonia nitrogen ($N\text{-}NH_3$), dry matter degradation (DMD) and neutral detergent fiber degradation (NDFD). For $N\text{-}NH_3$, 1 mL of the medium contained in the biodigester was taken, then mixed with 0.25 mL of metaphosphoric acid (Meyer®) at 25% (4:1 ratio) then centrifuged for 25 min at 3500 × *g* and the supernatant was restored in 2 mL vials. A volume of 20 μL of this supernatant was mixed with 1 mL of phenol solution [10 mg of $Na_2(NO)Fe(CN)_5$. H_2O (Meyer®) + 10 g of phenol (Meyer®) crystals diluted to the 1 L mark in distilled water] and 1 mL of hypochlorite solution [7.5 g of NaOH (Reasol®) + 21.3 g of Na_2HPO_4 (Meyer®) + 15 mL of hypochlorite (5%; Reasol®) diluted to the 1 L mark in distilled water]. The mixture was incubated for 30 min at 37 °C in a warm water bath. Subsequently, 5 mL of distilled water was added to dilute and stir on a vortex (Genie 2 G-560, USA). Absorbance was measured at 630 nm in a UV/VIS spectrophotometer (Jenway® 6850, USA) calibrated (r^2=0.9994) with a method of ammonia nitrogen concentration according to the technique described by McCullough (1967).

The residual sample from the biodigester was filtered into ANKOM® F57 bags at constant weight. The sample bags were dried at 60 °C for 24 h in a drying oven. The DMD was calculated with the formula:

$$DMD(\%) = (initial\ sample - residual\ sample \mid initial\ sample) * 100$$

(Hernández-Morales *et al.*, 2018). ANKOM® bags were heat-sealed and NDF content determined (Van Soest *et al.*, 1991). The percentage of NDF degradation (% NDFD) was calculated using the formula:

$$NDFD(\%) = (initial\ NDF - residual\ NDF \,/\, initial\ NDF) * 100$$

(Hernández-Morales *et al.*, 2018).

Determination of metabolizable energy (ME) was done with the DMD values; first, the equation described in (NRC, 1982) was used to obtain the digestible energy (DE),

$$DE\left(Mcal\ kg^{-1}\right) = (DMD\,/\,1000) * 4.525$$

Subsequently, the equation described by INRA (1989) was used to estimate the value of ME;

$$ME\left(Mcal\ kg^{-1}\right) = DE * 0.82$$

Statistical analysis

The chemical and bromatological analysis, biogas and CH_4 production rates at different incubation hours, cumulative biogas and CH_4 production, and fermentative characteristics of supplements by a laboratory grazing simulation were analyzed in a completely randomized design. Data were analyzed using the GLM procedure in SAS© (SAS Institute Inc., 2011). Mean differences were compared using Tukey's multiple means test (α, $p \leq 0.05$).

RESULTS AND DISCUSSION

Supplements

The supplements were characterized by a decrease in parota pod flour, which led to ash content (A) and crude protein (CP) decreased as the mango flour content increased. In contrast, organic matter content (OM) increased ($p \leq 0.05$). Neutral detergent fiber content (NDF) showed no differences among supplements ($p > 0.05$); while S3 showed the lowest content of acid detergent fiber (ADF, $p \leq 0.05$) (Table 2). Thus, the nutrient content of the supplements was related to the rate of biogas production and composition (Sobalvarro-Mena *et al.*, 2020).

The rate of biogas production made it possible to indirectly identify potentially fermentable carbohydrates at given times (Amanzougarene & Fondevila, 2020); since, the fermentation process of other nutrients such as proteins and fats are not expressed in the in vitro proportion of gas (Posada & Noguera, 2005; Sobalvarro-Mena *et al.*, 2020). Thus, the rate of biogas production in the different periods, up to 48 h of incubation, did not show differences among supplements ($p > 0.05$), which averaged (mL g^{-1} DM h^{-1}) 12.0, 26.2, 5.0, 5.3, 5.2 y 2.0, at times 0-3, 3-6, 6-9, 9-12, 12-24 y 24-48 h, respectively. In the period 48-72 h, S3 presented the lowest rate ($p \leq 0.05$; Table 2). In the case of CH_4 production rate, the periods were shorter, 24 h, so that supplements evaluated in this study did not present differences at 24, 48 and 72 h of incubation ($p > 0.05$); but they averaged 1.7, 0.6 and 0.3 mL g^{-1} DM h^{-1}, respectively (Table 2).

In cumulative biogas production, S3 had the lowest production ($p \leq 0.05$); in contrast, cumulative CH_4 production at 72 h showed no differences among supplements ($p > 0.05$). Now, the fermentation of carbohydrates from a sample using the *in vitro* gas production technique produces CO_2 and CH_4, since the production of other gases are only traces undetectable with this technique (Amanzougarene & Fondevila, 2020). Therefore, CH_4 represented 21.9%, 22% and 21.5% of the total biogas production produced by S1, S2 and S3, respectively (Table 2). This occurs because, when mainly propionate is fermented, the H_2 ions that form CH_4 are reduced by the proportion of non-structural carbohydrates, thus improving the microbial growth rate (Miranda-Romero *et al.*, 2020).

Rumen fermentation of carbohydrates produces short-chain fatty acids (acetate, propionate, and butyrate), succinate, formate, lactate, ethanol, CH_4, CO_2, and H_2. So, non-fibrous carbohydrates (starch, pectin and glucans) are fermented as part of the soluble components in the first 24 h (Posada & Noguera, 2005; Sobalvarro-Mena *et al.*, 2020; Souza *et al.*, 2021); then, the fermentation of insoluble carbohydrates begins. In both cases there are hydration and colonization by rumen microorganisms. So, biogas production and rate provide information on the degradation and kinetics of fermentation. Thus, the production rates and cumulative production of biogas from supplements can be explained by their chemical and bromatological composition. Also, by the energy content of the foods evaluated (Sobalvarro-Mena *et al.*, 2020), and their interactions and successive changes in microorganisms (Miranda-Romero *et al.*, 2020).

Regarding the rate of production and cumulative production of CH_4 it is assumed that methanogenic archaea use CO_2 and H_2 within their metabolic pathway, generating CH_4 as a product of their fermentation (Torres-Salado *et al.*, 2018). Because of the rate, proportion and amount of CH_4 it can be assumed that the supplements contained mainly non-structural carbohydrates that increased the production of propionate and the H^+ ions that form CH_4, so their production was stoichiometrically reduced (Miranda-Romero *et al.*, 2020).

In fermentative characteristics, S3 had the lowest content of metabolizable energy (ME), and nitrogen ammonia ($N\text{-}NH_3$) in the culture medium at 72 h; As well as the lowest degradation of dry matter (DMD) and neutral detergent fiber degradation (NDFD) ($p \leq 0.05$; Table 2). The *in vitro* degradation of CP is given by the bacteria and protozoa that were used as inoculum, which degraded it into peptides and free amino acids by enzymes, while ammonia was released; this was determined by the $N\text{-}NH_3$ content of the culture medium (Mejía & Mejía, 2007). The $N\text{-}NH_3$ content of S3 can be assumed by supplement composition, since the proportion of parota pod flour was reduced, thereby reducing CP; whereas in S1 and S2 PC content was higher (Bargo *et al.*, 2003) (Table 2).

Neutral detergent fiber (NDF) is an important nutritional factor due to its effect on rumen filling and rumminal digestion. Thus, the supplements in this study can be classified as highly degradable; and the expected increase in degradation occurred when grazing was simulated in laboratory (Baudracco *et al.*, 2010)2010. This is because DMD values were higher than 60%, which indicated that NDF content was lower than 40% (Table 2). In turn, this met a requirement for supplement production, that it contains low concentrations of detergent fibers (Hernández-Morales *et al.*, 2018).

Rojas-García *et al.* (2020) evaluated *in vitro* a supplement made with 50% pumpkin pulp & peel flour and 50% parota pod flour; they reported contents of 166 g kg^{-1} of CP, 409 g kg^{-1} of NDF, 241 g kg^{-1} of ADF (values greater than S1 in this study) and 924 g kg^{-1} of OM (a smaller value than S1 in this study). Regarding the *in vitro* fermentative characteristics, those authors reported values in DMD (776 g kg^{-1}), NDFD (549 g kg^{-1}), N-NH_3 content in the culture medium (9 mg dL^{-1}), cumulative biogas production (149 mL g^{-1}) and CH_4 production (56.2 mL g^{-1}), which are lower than those we consistently obtained with S1 in this study (Table 2).

Grazing simulation

Grazing simulation in the laboratory aimed to establish whether grass fermentation is improved when supplemented. Thus, the content of A, OM and ADF did not present

Table 2. Chemical composition and *in vitro* fermentative characteristics of supplements made of overripe mango flour and parota pod flour.

Variable	S1	S2	S3	SEM
Chemical composition (g kg^{-1} DM)				
Ashes	34.8 a	32.2 b	30.0 c	0.7
Crude protein	138.8 a	111.6 b	79.2 c	9.0
Neutral detergent fiber	332.8	334.9	314.8	4.4
Acid detergent fiber	192.2 a	191.9 a	172.6 b	3.6
Organic matter	965.2 c	967.8 b	970.0 a	0.7
Rate of biogas production (mL g^{-1} DM h^{-1})				
0-3 h	12.36	10.87	12.76	0.45
3-6 h	26.94	25.87	25.92	0.83
6-9 h	4.72	4.13	6.24	0.41
9-12 h	6.53	4.82	4.61	0.37
12-24 h	5.49	4.96	5.09	0.1
24-48 h	2.14	1.96	1.90	0.07
48-72 h	1.18 a	1.17 a	0.83 b	0.06
CH_4 production rate (mL g^{-1} DM h^{-1})				
0-24 h	1.79	1.67	1.63	0.05
24-48 h	0.66	0.53	0.59	0.03
48-72 h	0.27	0.29	0.24	0.01
Biogas production at 72 h (mL g^{-1} DM)	297.1 a	271.7 b	275.2 b	4.18
CH_4 production at 72 h (mL g^{-1} DM)	65.20	59.87	59.24	1.54
DM degradation at 72 h (g kg^{-1} DM)	832.3 a	821.3 a	759.8 b	1.14
NDF degradation at 72 h (g kg^{-1} DM)	592.4 a	572.4 a	505.8 b	1.38
Average ammoniacal nitrogen (mg dL^{-1})	21.26 a	16.15 a	14.52 b	0.97
Metabolizable energy (Mcal kg^{-1})	3.09 a	3.05 a	2.82 b	0.04

a,b: Variables with different letter per row show statistical difference ($p \leq 0.05$). S1=40% overripe mango flour - 60% parota pod flour; S2=60% overripe mango flour - 40% parota pod flour; S3=80% overripe mango flour - 20% parota pod flour; NDF=neutral detergent fiber; CH_4=methane; DM=dry matter; SEM=standard error of the mean.

differences among treatments ($p>0.05$), with an average of 91.1, 908.9 and 308.4 g kg^{-1} DM, respectively. The CP content showed the same trend as in the supplements, since the parota pod flour content decreased. So, T1 was 13.3% higher than T2 and 32.2% higher than T3 ($p\leq0.05$). The lowest NDF content was presented by T3 ($p\leq0.05$); T1 and T3 were both 4.5% higher than T3 (Table 3).

The biogas production rate showed that in the first 3 h of incubation, T1 had the lowest rate ($p\leq0.05$), 44.0% lower than T1 and T2; at 3-6 h, T1 was 30.2% higher than T2 ($p\leq0.05$); at 6-9 h, T3 was 34.4% higher than T1. But, at 9-12 h, 12-24 h and 24-48 h T1 was 103%, 90.8% and 49.4% higher than T3 ($p\leq0.05$). T2 showed no differences with both treatments ($p>0.05$; Table 3) in the same times. The 48-72 h rate showed no difference among treatments ($p>0.05$), and averaged 1.2 mL g^{-1} DM h^{-1}. The CH_4 production rate did not show differences in the measured times ($p>0.05$), but averaged a rate of 1.1,

Table 3. Chemical composition and *in vitro* fermentative characteristics obtained in a grazing simulation in laboratory; cobra grass supplemented with 30% meal supplements made of overripe mango flour and parota pod flour.

Variable	T1	T2	T3	SEM
Chemical composition (g kg^{-1} DM)				
Ashes	90.9	90.2	92.3	0.5
Crude protein	100.6 a	88.8 b	76.1 c	3.8
Neutral detergent fiber	594.4 a	590.9 a	567.0 b	4.7
Acid detergent fiber	308.5	320.7	295.9	4.8
Organic matter	909.1	909.8	907.7	0.5
Rate of biogas production (mL g^{-1} DM h^{-1})				
0-3 h	8.93 b	13.39 a	12.33 a	0.68
3-6 h	9.61 a	7.38 b	7.58 ab	0.4
6-9 h	3.43 b	3.56 ab	4.61 a	0.21
9-12 h	4.67 a	3.55 ab	2.30 b	0.37
12-24 h	2.71 a	1.98 ab	1.42 b	0.21
24-48 h	3.57 a	2.44 ab	2.39 b	0.22
48-72 h	1.38	1.07	1.08	0.08
CH_4 production rate (mL g^{-1} DM h^{-1})				
0-24 h	1.13	1.09	1.10	0.02
24-48 h	0.67	0.73	0.74	0.06
48-72 h	0.41	0.44	0.43	0.03
Biogas production at 72 h (mL g^{-1} DM)	231.16 a	191.93 ab	180.90 b	8.25
CH_4 production at 72 h (mL g^{-1} DM)	53.16	54.33	54.66	1.35
DM degradation at 72 h (g kg^{-1} DM)	705.1 a	640.9 b	637.7 b	11.2
NDF degradation at 72 h (g kg^{-1} DM)	652.9 a	538.3 b	503.9 b	22.9
Average ammoniacal nitrogen (mg dL^{-1})	18.07 a	13.91 b	13.08 b	0.77
Metabolizable energy (Mcal kg^{-1})	2.62 a	2.38 b	2.37 b	0.04

a,b: Variables with the same letter per row are statistically equal ($p>0.05$). T1=70% grass+30% S1; T2=70% grass+30% S2; T3=70% grass+30% S3; NDF=neutral detergent fiber; CH_4=methane; DM=dry matter; SEM=standard error of the mean.

0.7 and 0.4 mL g^{-1} DM h^{-1} in the periods 0-24 h, 24-48 h and 48-72 h incubation, respectively (Table 3).

The cumulative biogas production of T1 was 27.8% higher than T3 ($p \leq 0.05$), with no differences with T2 ($p > 0.05$). In contrast, CH_4 production showed no differences among treatments ($p > 0.05$). However, CH_4 production accounted for 23%, 28.3% and 30.2% of the biogas produced at 72 h. The biogas production rates of the simulation showed that the soluble components were rapidly fermented in the first six hours. This was given by the supplements composition, as it was correlated with carbohydrate content in cells and subsequently with the fibrous components (Sobalvarro-Mena *et al.*, 2020).

In order to explain the behavior of CH_4 in the simulation, two factors are assumed. 1) the fermentation of low-quality forages increases the production of acetate, H^+ ions; therefore, production rates and the accumulated production of CH_4 follow (Miranda-Romero *et al.*, 2020); 2) on the differences in the proportion we assumed that parota flour contained secondary metabolites acting as 'defaunators' that inhibited methanogenic archaea, thus decreasing their production. Thus, as in T1 we had the higher amount of parota pod flour, the proportion of CH_4 was lower compared to the rest of the treatments.

T1 showed 10.6%, 29.6%, 10.3% and 38.1% more DMD, NDFD, ME and N-NH_3 of the medium than T2 and T3 ($p \leq 0.05$). T1 showed a higher content of potentially degradable dry matter; we consider that it will present a better biological performance when offered to the animal. The CP content of S1 was directly related to both degradation levels, and N-NH_3. This follows their importance in the growth and synthesis of rumen microorganisms, which degrade dietary components and release nutrients for absorption (Souza *et al.*, 2021).

Thus, the best fermentative characteristics of S1 in the grazing simulation in laboratory were the result of the interaction of all its nutrients. This shows the benefit of its addition to a grazing rumen diet; DMD, NDFD and ME were improved by 211.9 g kg^{-1}, 326.4 g kg^{-1} and 0.79 Mcal kg^{-1}, respectively.

The trend of increasing N-NH_3 content as the CP content in the supplement increased is similar to that reported by Bargo *et al.* (2003) when they increased soy flour in their supplements for an *in vivo* test. Therefore, this type of *in vitro* studies should be continued; and their limitations considered, because they are useful to understand the potential of the products that can be used afterwards, for *in vivo* grazing trials.

CONCLUSIONS

Supplementation meals in the tropics, using regional products such as overripe mango flour and parota pod flour, represent a viable supplementation alternative for grazing ruminants. The tested supplement with 40% overripe mango flour and 60% parota pod flour improved all the *in vitro* fermentative characteristics of cobra grass in a grazing simulation in laboratory.

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