v. 4, n. 8, 2024

Journal of Agricultural Sciences Research

MESQUITE PIPERIDINE ALKALOID EXTRACT LEVELS IN LAMB DIETS CHANGE RUMINATION RATE AND RUMEN MICROBIAL EFFICIENCY

Karine Pinheiro de Oliveira Ribas
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0002-5895-5008

Mara Lúcia Albuquerque Pereira
Department of Exact and Natural Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0001-9704-7064

Josivânia Rodrigues de Araújo Santos
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil

Leandro Santos e Silva
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0002-0537-7451

Otanael Oliveira dos Santos
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil

George Soares Correia
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0003-4510-077X

Virgínia Patrícia dos Santos Soares
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0003-3690-7889

Edileusa de Jesus dos Santos
Department of Education, Federal Institute of Roraima, Campus Novo Paraíso
Caracaraí, Roraima, Brazil
https://orcid.org/0000-0001-7646-7692

Ana Cláudia Maia Soares
Postgraduate Program and Graduate Course in Animal Science, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0003-0078-9518

César Augusto Ramos Pereira
Department of Rural and Animal Technology, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil

Herymá Giovane de Oliveira Silva
Department of Rural and Animal Technology, Southwestern Bahia State University, Campus Juvino Oliveira
Itapetinga, Bahia, Brazil
https://orcid.org/0000-0003-3944-9633

Abstract: The use of plant secondary compounds to modify rumen microbiome has an imperative effect on animal physiology. This study was realized to evaluate the effects of diets supplemented with enriched *Prosopis juliflora* (mesquite) piperidine alkaloid extract (MPA) levels (0, 2.3, 4.6, and 9.2 mg/kg of diet DM) compared to monensin (MON; 2.1 mg/kg DM) on feeding behavior, intake, digestibility and microbial protein synthesis in lambs. Five lambs with initial body weights of 21.05 ± 0.54 kg were allocated to metabolic cages and randomly distributed in a 5 × 5 Latin square design for 95 days, being 14 days of adaptation and 5 days of sampling in each experimental. The MPA levels showed a quadratic effect with a minimum point of 5 mg/kg for rumination rate of DM (P = 0.016) and NDF (P = 0.006) (g/h), which was higher than that of MON and the control diet. The NFC intake increased (P < 0.001) with MPA levels. The digestibility of DM and OM was higher (P = 0.017) for MON than control diet, not differing from MPA (P = 0.288; 0.260). Microbial protein synthesis (MPS) and efficiency were similar (P = 0.664; 0.668) between MON and MPA. MPA showed a dose-dependent effect on rumen digestion, whereby doses of 2.3 to 4.6 mg/kg decreased rumination chew and MPS, and the dose of 9.2 mg/kg caused the opposite effect of increasing rumination chew and MPS. The major concentration of MPA intensifies rumination with potential to increase efficiency of microbial protein synthesis.

Keywords: feeding behavior, ionophore, microbial efficiency, phytogenic additive, *Prosopis juliflora*, rumination intensity

INTRODUCTION

Feed quality may cause changes in the temporal activity of digestive processes (Nikkhah, 2014). Digestion efficiency is determined, among other factors, by dietary chemical characteristics, food particle size, and available digestion time. Findeisen et al. (2021) stated that for a diet with a higher proportion of concentrate, dry matter intake is the main factor influencing the digestive process. Additionally, these authors suggested that the chemical characteristics of the diet may have more relevance than the retention time in terms of restricting or facilitating digestion.

Antimicrobial additives and secondary compounds in plant extracts that are added to ruminant feed interfere on digestive processes and digestibility by rumen modulations (Durmic and Blache, 2012; Nikkhah, 2014). Altered rumination can be associated with changes in the rate of degradation and fermentation induced by the presence of alkaloids, although this does not necessarily correspond to deleterious effects on microbial activity (Preeti et al., 2015; Henciya et al., 2017; Soni et al., 2018; Brito et al., 2020). The main alkaloids found in a basic chloroform extract from the pods of *P. juliflora* (mesquite) were prosoflorine, juliprosinine, juliprosine, and juliprosopine (Santos et al., 2013; Sousa et al., 2022). These alkaloids are amphiphilic compounds with calcium channel-blocking properties in cell plasmatic membranes (Choudhary et al., 2005). Piperidine alkaloids acting on the exposed cell membranes of bacteria can modify rumen fermentation, with the potential to replace monensin (MON) (Santos et al., 2013; Pereira et al., 2017; Sousa et al., 2022).

In vitro tests using rumen fluid have shown that many groups of alkaloids improved fermentation (Mickdam et al., 2016). When the extract is composed of more than one bioactive molecule, it can change the fermentation pattern to a dose-dependent threshold profile (Macheboeuf et al., 2008; Santos et al., 2013; Pereira et al., 2017). In a methane emission study, Sousa et al. (2022) tested doses of mesquite piperidine alkaloid extract (MPA) at 6.6, 17.3, and 27.8 mg/kg of diet DM in sheep and discerned methane mitigation. Santos et al. (2021) reported improved performance in lambs fed diets containing MPA (0, 2.3, 4.6, and 9.2 mg/kg diet DM) and recommended additional studies to further evaluate the interaction between MPA and different feed compositions. This study was realized to evaluate the feeding behavior, intake, digestibility, and rumen microbial synthesis in lambs fed diets containing various MPA levels (0, 2.3, 4.6, and 9.2 mg/kg diet DM) and to compare them with diets containing MON (2.1 mg/kg diet DM).

MATERIALS AND METHODS

The Ethics Committee of the State University of Southwest Bahia (UESB), Itapetinga Campus, protocol 23-2013, Brazil, approved the experimental procedures of this study, which were conducted in accordance with the Brazilian legislation for research and experimentation with animals.

LOCAL, ANIMALS, AND EXPERIMENTAL DESIGN

The experiment was conducted in the sheep sector of Southwest Bahia State University, Brazil. Five Dorper × Santa Inês non-castrated male lambs of approximately 120 days of age, with average initial body weights of 21.05 ± 0.54 kg and final average body weights of 41.03 ± 2.74 kg were kept in metabolic cages. The lambs were distributed in a 5 × 5 Latin square design during 95 days, with 14 days of adaptation and 5 days of sampling per experimental period.

ENRICHED MESQUITE PIPERIDINE ALKALOID EXTRACT (MPA)

Mature pods of *Prosopis juliflora* (SW) D.C. (mesquite) collected during the dry season from mature trees (10–15 years) in Brumado, Bahia, Brazil, were selected without changes to the pericarp, and dried for 3 days under temperature control of approximately 30 ± 0.2 ºC to avoid loss of alkaloid properties. After drying and milling, whole pod flour was used to produce a basic chloroform extract (BCE), as described by Santos et al. (2013). BCE analysis using high performance liquid chromatography mass spectrometry identified juliprosopine ($C_{40}H_{76}N_3O_2$ [M+H]+, MM = 630.54) as a minor constituent and juliprosinine ($C_{40}H_{72}N_3O_2$ [M+H]+, MM = 626.49) as a major constituent. BCE was weighed and added to the concentrate feed to obtain mesquite piperidine alkaloid extract (MPA) levels of 2.3, 4.6, and 9.2 mg/kg diet DM.

The concentration of MPA used was based on *in vitro* trials, in which 2.3 mg/kg DM for a substrate/rumen volume ratio of 0.1 g/mL is equivalent to the intermediate dose of 0.25 ppm for the same ratio, according to Pereira et al. (2017). The determination of the proportional MPA dose to the physiological volume of the rumen was based on data from lambs and the composition of the experimental diet to obtain the weight of rumen solid content, according to Weston (1984) and Purser and Moir (1966). The concentration of 2.1 mg/kg MON was used as a reference concentration, based on data reported by Richardson et al. (1976), to identify the minimum dose of MPA that resembled a MON level of 2.1 mg/kg and differed from an MPA value of 0 mg/kg. The MON dose of 2.1 mg/kg of diet DM was also used for comparison with the lowest concentration of MPA (2.3 mg/kg).

EXPERIMENTAL DIETS

The diets were balanced for estimating the requirements based on the NRC (2007) equations, considering a daily weight gain of 200 g, with 75% DM digestibility and 40% ruminal degradable protein. Experimental diets consisted of the addition of sodium monensin (MON, Rumensin, Elanco Animal Health, Indianapolis, IN, USA; 100 g/kg DM; in the proportion of 2.1 mg/kg diet DM) and MPA (0, 2.3, 4.6, and 9.2 mg/kg diet DM). The additives (MON or MPA) were mixed with the mineral mixture, which was then added to the other ingredients of the concentrate in the industrial mixer. The proportions of the diet ingredients and the chemical composition of the roughage and concentrate are shown in Table 1. Water was supplied continually in individual drinking troughs, and diets were provided daily *ad libitum* at 7:00 a.m. and 3:00 p.m. to allow orts to reach 10%. Daily voluntary intake was calculated as the difference between the total diet offered and the orts, which were weighed twice daily.

SAMPLING AND LABORATORY ANALYSES

Feeding behavior

The intake of each animal was obtained during the evaluation of ingestive behavior during each collection period. Thus, intake and feeding behavior were evaluated on the 15th day of each experimental period, in which the lambs were observed for 24 h. The times spent feeding, ruminating, and resting were recorded every 10 min by trained observers in a relay system that was strategically positioned to prevent interference with animal behavior.

Observations began at 8:00 a.m. and ended at the same time the following day. The chewing number of the rumen bolus and time spent ruminating on each bolus were counted using a digital stopwatch and measured

Ingredient		(g/kg DM)	
Tifton 85 hay		400	
Ground maize		450	
Soybean meal		130	
Mineral salt[a]		16	
Urea		4	
ME (MJ/kg DM)[b]		9.7	
Chemical composition (g/kg DM) basis)	Tifton 85 hay	Concentrate	TMR
Dry matter (g/kg NM)	915.6	879.4	894.0
Organic matter	919.7	954.9	940.8
Crude protein	68.6	163.5	125.5
Neutral detergent insoluble protein	44.0	63.5	55.7
Acid detergent insoluble protein	31.1	67.1	52.7
Ether extract	15.1	28.5	23.1
Non-fiber carbohydrates	253.0	580.0	449.2
Neutral detergent fiber[c]	810.7	201.7	445.3
Hemicellulose	103.7	264.0	199.9
Cellulose	587.3	205.0	357.9
Lignin	145.8	25.4	73.6

Table 1. Ingredients of the total mixed ration (TMR) and chemical composition of Tifton 85 hay (Bermuda grass), concentrate, and TMR.

[a]Mineral salt, composition 120 g Ca/kg, 87 g P/kg, 147 g Na/kg, 18 g S/kg, 590 mg Cu/kg, 40 mg Co/kg, 20 mg Cr/kg, 1.8 g Fe/kg, 80 mg I/kg, 1.3 g Mn/kg, 15 mg Se/kg, 3.8 g Zn/kg, 300 mg Mo/kg, 870 mg F/kg (max.), 95% solubility of phosphorus (P) in citric acid at 2% (min); [b]Metabolizable energy was calculated based on metabolizable energy (ME) tabulated values for individual feed ingredients (NRC, 2007); [c]corrected for ash and protein.

according to the methodology described by Bürger et al. (2000):

$$FT = \frac{ML}{DMI \text{ or } NDFI}$$

$$RT = \frac{RL}{DMI \text{ or } NDFI}$$

where:

FT – feeding time (min/kg);

ML – meal length (min/day);

DMI– dry matter intake (kg/day);

NDFI – neutral detergent fiber corrected for ash and protein intake (kg/day);

RT – rumination time (min/kg); and

RL – rumination length (min/day).

$$FR = \frac{DMI \text{ or } NDFI}{ML}$$

$$RR = \frac{DMI \text{ or } NDFI}{RL}$$

$$RBN = \frac{RL}{ChTRB}$$

$$DM \text{ or } NDF \text{ per bolus} = \frac{g\ DMI \text{ or } g\ NDFI}{RBN}$$

$$RChN = RBN \times ChN$$

$$RCht = \frac{ChN}{bolus} \times RChN$$

$$FCh = (FT + RT) - \frac{RCht}{DMI}$$

where:

FR – feeding rate (g DMI/h and g NDFI/h);

RR – rumination rate g DM/h and g NDF/h);

RBN – ruminated bolus number (no./day);

ChTRB – chewing time per rumen bolus (sec/bolus);

RChN – rumination chewing number (no./day);

ChN – chewing number per rumen bolus (no./bolus);

RCht – total chewing time of rumen bolus (min/day); and

FCh – feeding chewing (min/kg DM).

Intake and digestibility of nutrients

The nutrient intake for each lamb was measured from the 15th to 19th day of each experimental period by subtracting the DM of the orts from that of the roughage and concentrate supplied. To obtain the average body weight (BW), the lambs were weighed at the beginning and end of the experimental period before the morning feeding.

Feces were collected over three consecutive days using collecting bags attached to the lambs, and the daily total was measured using a 0.1 g precision digital scale. After homogenization, 10% aliquots of the daily fecal production were stored in a freezer at -20 ºC for subsequent analysis. The average value of daily excretion of fecal dry matter per animal was obtained using the dry matter values from the three collection days in each period. Aliquots were taken in proportion to the production of fecal dry matter each day to obtain a composite sample for further analysis.

The digestibility coefficients (D) of dry matter, organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and non-fibrous carbohydrates (NFC) from each diet were determined using the following calculation:

$$D = \frac{[(\text{nutrient intake} - \text{nutrient excreted in feces}) \times 100]}{\text{nutrient intake}}$$

The total digestible nutrient (TDN) content obtained using the digestibility assay was calculated according to Weiss (1999). The TDN values were converted into metabolizable energy (ME) using the equations suggested by the NRC (2001).

Samples of roughage and concentrates from each animal were collected from the 15th to 19th day of each experimental period. The contents of dry matter (method 930.15), mineral matter (MM, method 924.05), crude protein (method 984.13), and ether extract (method 920.39) (AOAC, 1990) were determined from the samples of roughage, concentrates, orts, and feces. For the analysis of NDF corrected for ash and protein, the samples were treated with thermostable alpha-amylase without sodium sulfite and corrected for residual ash according to Mertens (2002). NDF correction for nitrogen compounds and estimation of the content of insoluble nitrogen compounds in neutral (NIDN) and acid (NIDA) detergents were performed according to Licitra et al. (1996).

Lignin (method INCT–CA F–005/1) was obtained using the methodology described by Detmann et al. (2012), with the ADF residue treated with 72% sulfuric acid in roughage and concentrate samples. The non-fibrous carbohydrate (NFC) content was calculated according to Hall et al. (1999) with modifications, using NDF corrected for ash and protein (Detmann and Valadares Filho, 2010):

$$NFC = 100 - (\%CP - (\%CPU \times \%U) + \%MM + \%EE + \%NDF)$$

where:

%CP - crude protein content;

%CPU - crude protein content of urea;

%U - urea content;

%MM - mineral matter content;

%EE - ether extract content; and

%NDF - neutral detergent fiber content corrected for ash and protein.

Microbial synthesis

The 24 h total urine was collected from after the morning feeding on the 18th day until the 19th day of each experimental period. Sampling was performed by spontaneous urination of the animals using a collection container. During collection, the urine container was frequently gathered for refrigeration storage. After 24 h of collection, the urine was weighed, 100 mL of 20% sulfuric acid was added, and the sample was homogenized and gauze filtered.

Samples were prepared at an acidic pH (<3) to prevent bacterial decomposition of the urine metabolites. An aliquot of 10% of the daily volume from each animal in each period was stored at -20 ºC for subsequent analyses of creatinine, allantoin, uric acid, xanthine, and hypoxanthine. Creatinine and uric acid concentrations were determined using commercial kits (Bioclin). The concentrations of allantoin, xanthine, and hypoxanthine were analyzed by colorimetric methods according to Chen and Gomes (1992).

The excretion of total purine derivatives (PD) was obtained by summing the amounts of allantoin, uric acid, and xanthine-hypoxanthine excreted in the urine (mmol/L). The level of absorbed microbial purines (mmol/day) was estimated from the excretion of total purine derivatives (mmol/day), using the equation proposed by Chen and Gomes (1992). Microbial nitrogen synthesis (g/day) was calculated as a function of absorbed purines (AP, mmol/day), using the equation described by Chen and Gomes (1992). Microbial efficiency was obtained by dividing microbial protein synthesis (g/day) by the intake of total digestible nutrients (kg/day) (NRC, 2001; Cabral et al., 2008; Cirne et al., 2015).

STATISTICAL ANALYSIS

The feeding behavior, intake, digestibility, and microbial synthesis data were analyzed using the general procedure for linear models (PROC GLM) (version 9.4; SAS Institute Inc., Cary, NC, USA).

Orthogonal contrast was applied to compare the means observed between the diets with MON versus the control (0), MON versus MPA of 2.3 mg/kg DM, and MON versus MPA of 2.3, 4.6, and 9.2 mg/kg DM. Polynomial contrasts were performed for the linear (L) and quadratic (Q) components in the analysis of the means from dependent variables according to the MPA inclusion in the diets (0, 2.3, 4.6, and 9.2 mg/kg DM). The significance level was set at 5% probability.

The mathematical model used was:

$$Yij(k) = \mu + Per_i + An_{ij} + \tau(k) + \varepsilon ij(k)\ i,j,k = 1,\ldots,r$$

where:

Yij (k) = observation ij (k);

μ = the overall mean;

Per_i = period effect i;

An_{ij} = animal effect j;

τ (k) = treatment fixed effect k;

εij (k) = random error with an average of 0 and variance of σ^2; and

r = number of treatments, periods, and animals.

For the variables whose polynomial contrasts were significant we used the model

$$Yi = \beta 0 + \beta 1xi + \beta 2x2i + \varepsilon i$$

i = 1,..., n,

where:

Y_i = observation i of dependent variable y;

x_i = observation i of independent variable x;

β0, β1, β2 = regression parameters; and

ε_i = random error.

RESULTS

The feeding time (min/day) was not affected (P = 0.740) by the diets. The time spent ruminating (min/day) was lower (P = 0.048) and idle time was greater (P = 0.017) with the diets containing MPA than the other diets. MON tended to reduce (P < 0.10) these variables compared to those with the control diet (Table 2). The rumination time (min/kg) of DM (P = 0.016) and NDF (P = 0.009) varied quadratically with MPA levels. For these activities, minimum time was estimated at MPA 5.6 mg/kg, with lower rumination times for DM and NDF when compared to those for MON.

Feeding rate (g/h; P = 0.845) and ingestive chewing (P = 0.493) were not affected by the use of dietary additives. However, MON tended to reduce the feeding rates of DM (P = 0.090) and NDF (P = 0.086) compared to those with the various doses of MPA (Table 3). The rumination rate of DM (P = 0.016) and NDF (P = 0.023) (g/h) were influenced by MPA levels, presenting a quadratic effect with maximum points at 5.4 mg/kg MPA. When the rumination rate was compared between the additives, the amount of ruminated DM and NDF (g/h) was observed to be lower for MON, although there was no change (P = 0.595) in the number of ruminated boluses. Similar to rumination rate, the chewing number (ChN) and chewing time (sec) per bolus (ChTRB) were also changed (P < 0.001), with minimums at MPA levels of 5.4 and 4.6 mg/kg, respectively. ChN (P < 0.001) and rumination chewing (min/kg) for DM (P = 0.045) and NDF (0.020) were also lower for MPA than for MON (Table 3).

Levels of MPA and MON in the diets did not affect (P > 0.05) the intake, except for the intake of NFC, which showed a linear variation (P < 0.001) with APA levels and there was a higher intake for MPA compared to MON (P = 0.028). The digestibility of DM (P = 0.017) and OM (P = 0.017) was higher, and NDF digestibility tended to be higher (P = 0.094) in the MON diet than in the control diet (Table 4).

Microbial protein synthesis (MPS) presented quadratic variation (P =0.002), with an estimated minimum production at the MPA level of 3.3 mg/kg (Table 5). The microbial efficiency, as a consequence of MPS, followed the same quadratic variation (P = 0.002) with a minimum point at an MPA value of 3.6 mg/kg. The MON and control diets did not differ significantly (P = 0.656) in terms of microbial synthesis.

DISCUSSION

According to some studies, the reduction in particle size by rumination allows for a higher intake without compromising digestibility (Schwarm et al., 2009; Clauss et al., 2015). However, although the intake and digestibility did not differ between the diets with MPA and MON, rumination chewing was greater in sheep fed the MON diet than in those fed MPA, and the MON did not differ from that of the control. In contrast, the digestibility of DM, OM, and NDF was higher in the MON diet than in the control. Therefore, a given amount of undigested feed particles that are exposed to more chewing cycles may increase digestibility as the intake does not change (Beauchemin, 2018). The sorting mechanism in the ruminant forestomach generally ensures that large particles are regurgitated for rumination (Dittmann et al., 2015).

The relationship between intake and retention time can be modulated by gut capacity, dietary quality, and feed additive presence (Clauss et al., 2008; Boardman et al., 2020; Findeisen et al., 2021). Nevertheless, the intake corrected for body weight was not affected by the additives, and the reduction in rumination time was reflected by an increase in idle time. It is plausible to assume that this

Item	MON	MPA level (mg/kg DM)				SEM[a]	MON vs 0	MON vs 2.3 MPA	MON vs MPA	*P* Value	
	2.1	0	2.3	4.6	9.2					L	Q
Feeding											
min/day	209.7	216.0	219.5	217.5	222.4	5.16	0.721	0.560	0.463	0.740	0.951
min/kg DM[b]	222.3	214.9	208.3	193.3	206.0	11.26	0.752	0.530	0.280	0.614	0.537
min/kg NDF[c]	401.5	393.4	401.5	362.0	388.9	0.57	0.819	0.999	0.528	0.605	0.668
Ruminating											
min/day	504.8	558.0	461.1	470.8	492.7	9.14	0.064	0.109	0.178	0.048	0.006
min/kg DM	535.1	550.5	433.8	417.2	458.3	20.78	0.742	0.025	0.008	0.095	0.016
min/kg NDF	975.3	1012	837.5	785.4	860.6	20.07	0.557	0.024	0.004	0.009	0.003
Idle											
min/day	725.5	666.0	759.5	751.7	725.0	10.47	0.078	0.289	0.446	0.115	0.017

Table 2. Ingestive behavior in sheep fed diets containing monensin (MON) and various levels of mesquite piperidine alkaloid extract (MPA).

astandard error of the mean; bminute per kilogram of dry matter; cminute per kilogram of neutral detergent fiber.

Item	MON	MPA level (mg/kg DM)				SEM[a]	MON vs 0	MON vs 2.3 MPA	MON vs MPA	*P* Value	
	2.1	0	2.3	4.6	9.2					L	Q
Feeding rate (g/h)											
DM[b]	284.8	316.7	302.9	328.7	315,6	9.60	0.186	0.147	0.090	0.845	0.987
NDF[c]	153.4	163.0	154.9	176.0	166.3	4.85	0.171	0.130	0.086	0.555	0.941
FCh[d] (min/kg DM)	222.3	211.6	208.2	193.3	206.0	7.45	0.666	0.546	0.299	0.493	0.313
Rumination rate (g/h)											
DM	121.8	118.9	145.5	148.2	136.2	3.53	0.790	0.028	0.015	0.130	0.016
NDF	65.0	61.5	74.6	79.8	71.1	1.76	0.522	0.070	0.019	0.023	0.006
Rumination chew											
RBN[e] (no./day)	728.1	767.7	715.4	720.8	692.1	13.33	0.386	0.768	0.595	0.415	0.851
ChN[f] (no./bolus)	61.60	62.94	55.02	54.56	57.70	0.79	0.569	0.004	0.002	0.003	<0.001
ChTRB[g] (sec/bolus)	42.49	43.51	38.72	39.32	43.14	0.58	0.571	0.030	0.136	0.912	<0.001
RCht[h] (min/day)	714.5	773.5	680.5	688.3	715.0	10.47	0.289	0.078	0.446	0.111	0.011
RChN[i] (no./day)	44 766.2	48 151.9	39 264.7	39 240.7	39 797.6	925. 59	0.269	0.059	0.026	0.164	0.105
min/kg DM	757.4	765.4	642.0	610.5	664.3	20.07	0.900	0.059	0.018	0.167	0.045
min/kg NDF	1376.7	1405.7	1239.0	1147.5	1249.5	25.44	0.713	0.069	0.009	0.020	0.009

Table 3. Feeding, rumination rates, and chewing in sheep fed diets containing monensin (MON) and various levels of mesquite piperidine alkaloid extract (MPA).

astandard error of the mean; bdry matter; cNeutral detergent fiber; dfeed chewing; eruminated bolus number; fchewing number per rumen bolus; gchewing time per rumen bolus; htotal chewing time of rumen bolus; irumination chewing number.

Item	MON	MPA level (mg/kg DM)				SEM[a]	MON vs 0	MON vs 2.3	MON vs MPA	*P* Value	
	2.1	0	2.3	4.6	9.2					L	Q
Intake (g/kg BW)											
DM[b]	43.93	44.22	43.62	42.19	44.26	0.54	0.789	0.780	0.523	0.702	0.102
OM[c]	41.37	41.63	41.05	39.72	41.65	0.50	0.799	0.753	0.503	0.696	0.101
CP[d]	5.77	5.74	5.71	5.49	5.72	0.10	0.874	0.764	0.378	0.597	0.309
NDF[e]	18.30	18.51	18.62	17.62	18.46	0.28	0.681	0.543	0.880	0.492	0.326
NFC[f]	18.22	17.61	19.16	18.57	21.32	0.41	0.411	0.217	0.028	< 0.001	0.259
Digestibility (g/kg DM)											
DM	792.96	758.38	777.50	794.32	773.12	4.97	0.017	0.238	0.288	0.233	0.667
OM	804.97	771.41	789.52	805.35	784.91	4.79	0.017	0.227	0.260	0.212	0.703
CP	784.36	772.01	805.06	758.99	829.78	19.39	0.824	0.710	0.765	0.493	0.480
NDF	695.50	649.09	689.15	714.14	681.70	10.94	0.094	0.808	0.981	0.693	0.407
NFC	944.82	928.70	921.41	930.88	920.28	6.88	0.340	0.174	0.145	0.248	0.573
Metabolizable energy											
MJ/kg $BW^{0.75}$	1.34	1.30	1.29	1.30	1.31	0.05	0.788	0.759	0.754	0.918	0.953

Table 4. Intake of nutrients, metabolizable energy, and total digestibility in sheep fed diets containing monensin (MON) and levels of mesquite piperidine alkaloid extract (MPA).

astandard error of the mean; bdry matter; corganic matter; dcrude protein; eneutral detergent fiber; fnon-fiber carbohydrates.

Item	MON	MPA level (mg/kg DM)				SEM[a]	MON vs 0	MON vs 2.3	MON vs MPA	*P* Value	
	2.1	0	2.3	4.6	9.2					L	Q
Microbial synthesis (g/day)											
Nitrogen	13.50	12.56	10.13	10.57	17.83	0.70	0.656	0.113	0.664	0.016	0.002
CP[b]	84.38	78.48	63.29	66.07	111.46	4.36	0.656	0.113	0.664	0.016	0.002
Microbial efficiency											
g CP/kg TDN	81.51	77.88	61.55	62.79	105.64	4.37	0.786	0.137	0.668	0.047	0.002

Table 5. Synthesis and microbial efficiency in sheep fed diets containing monensin (MON) and levels of mesquite piperidine alkaloid extract (MPA).

astandard error of the mean bcrude protein; ctotal digestible nutrients.

may occur under conditions of enhanced digestion. In addition, the relationship between digestibility and retention time can be modulated by particle size reduction or chewing efficiency (Clauss et al., 2009). In ruminants, the amount of ingested feed determines the amount of feed that is actually chewed, as well as the amount of feed that is regurgitated from the forestomach. However, a low proportion of large non-ruminated particles can escape the forestomach, and this has been shown to occur after ingestion under conditions of higher forestomach fill (Kaske et al., 1992; Lauper et al., 2013; Hummel et al., 2018; Findeisen et al., 2021), although this would not be observed in the current study.

Feed intake, feeding chew-to-ingested feed ratio, and rumination chew-to-ingested feed ratio were similar between the MON and control diets, and MON showed higher OM and NDF digestibility. Santos et al. (2021) observed enhanced digestibility of OM and NFC with MPA 9.2 and an average value close to that of the MON and control diet. It is worth

noting that in the study realized by Santos et al. (2021), the main source of non-fibrous carbohydrates was maize meal, which differs from the ground whole-grain maize used in the present study. Maize meal is a by-product of flour obtained by dry maize mechanical processing and contains more fiber and crude protein than whole-grain maize (Gwirtz and Garcia-Casal, 2014).

Monensin with MPA 9.2 showed a reduced rumination rate, but it was insufficient to affect the total digestibility, which was similar to that with 2.3 and 4.6 of MPA. It is possible that the sheep adjusted the time allocated to rumination chewing or chewing frequency to achieve the targeted bolus consistency, which did not change (Findeisen et al., 2021). Different feed compositions may affect chewing time as a response to the positive correlation between fiber content or particle size and chewing time (Beauchemin, 2018).

In a study of feeding behavior in sheep, Perazzo et al. (2016) observed a positive correlation between ingestion and rumination rate. Rumination is considered a physiological action triggered at varying frequencies, depending on the quality of the diet (Perazzo et al., 2017). In the current study, the composition of the diets was the same, and voluntary intake did not vary. Therefore, differences in rumination may be related to the changes that these dietary additives cause during the digestion process in lambs (Brito et al., 2020).

The total digestibility remained unchanged even though MPA at an estimated mean dose of 5 mg/kg provided less chews per bolus than the other feeds and increased the rumination rate. Secondary compounds in plant extracts that are added to ruminant diets may modify rumen fermentation, feeding behavior, and digestive processes (Durmic and Blache, 2012). However, supplementation with 2.3 and 4.6 mg/kg of MPA showed that the decrease in the chewing intensity of rumination (increased rumination rate) was not associated with altered digestibility, although the target consistency of the bolus was more rapidly achieved with these doses (Findeisen et al., 2021). The change in the rumination pattern of sheep fed MPA may be associated with the modification of microbial growth and fermentation (Santos et al., 2013; Pereira et al., 2017; Brito et al., 2020).

A higher rumination rate could have caused increased digesta passage, despite ingestive mastication, which was not affected. Rumination quality is essential for optimizing feed use. The MPA of 9.2 may have reduced the rumination rate to a limit that did not affect microbial synthesis and digestibility because it increased the chew. Lemenager et al. (1978) in a trial with steers reported that MON decreased liquid and solid turnover rates in the rumen but did not affect cellulose disappearance.

The MON diet showed higher OM digestibility and similar microbial protein synthesis activity compared with the control diet. In contrast, MON did not differ from MPA levels for these variables, where an MPA of 9.2 mg/kg provided increased microbial synthesis and efficiency. Even at low concentrations, MON could cause changes in rumen fermentation *in vitro* and *in vivo* and reduce the rumination rate (Richardson et al., 1976; Ítavo et al., 2011; Polizel et al., 2021). Santos et al. (2021) stated that MPA modified microbial metabolism and digestion in the rumen. The dose-response effect on rumination activity and microbial synthesis allows us to infer that MPA levels may have an action of MPA levels on the rumen fermentation pattern (Pereira et al., 2017).

Altered rumination activity correlates with the rate of degradation (fermentation) induced by the presence of alkaloids, which does not necessarily correspond to deleterious

effects on the microbial population (Preeti et al., 2015). Normal physicochemical conditions in *in vitro* studies with rumen fluid have shown that many groups of alkaloids improve fermentation (Santos et al., 2013; Mickdam et al., 2016; Pereira et al., 2017).

In addition, chewing stimulates salivation, which can increase rumen turnover. Larger amounts of fluids than particles in the passage increases microbial yield from the rumen system (Clauss and Hummel, 2017), which is likely caused by an increased turnover rate and contributes to increased microbial flow to the lower gut.

For the MPA dose of 9.2 mg/kg there was an increase in microbial synthesis and efficiency which can be partially explained by the longer chewing time, similar to MON and the control diet (Clauss and Hummel, 2017). This characterizes the dose-response of digestion and microbial growth in the rumen in the presence of piperidine alkaloids.

The MPA promoted a dose-response effect on digestive processes in the rumen whereby doses of 2.3 to 4.6 mg/kg MPA resulted in lower synthesis of microbial protein, while a level of 9.2 mg/kg promoted the opposite effect.

The levels of enriched mesquite piperidine alkaloid extract (MPA) changed the rumination pattern in the lambs in association with the modification of microbial activity. The MPA between 2.3 and 4.6 mg/kg decreased the chewing intensity and microbial protein synthesis, while an MPA of 9.2 mg/kg increased these factors.

DECLARATIONS

Author contributions: RIBAS, K.P.O.: Conceptualization, Investigation, Formal analysis, and Writing - Original Draft Preparation; PEREIRA, M.L.A.: Methodology, Supervision, and Funding acquisition; SANTOS, J.R.A.: Conceptualization, Investigation, and Formal analysis; E SILVA, L.S.: Investigation, Formal analysis, and Data curation; SANTOS, O.O.: Investigation; SILVA, E.R.: Investigation; SOARES, V.P.S.: Investigation; SANTOS, E.J.: Methodology, and Investigation; SOARES, A.C.M.: Investigation; SILVA, H.G.O.: Methodology, Supervision, Formal, and Data curation. All authors read and approved the manuscript.

Funding: This study was funded by the Bahia Research Support Foundation (FAPESB) to PET0013/2013 and the Southwestern Bahia State University (UESB).

Conflict of interest: The authors declare no conflicts of interest.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Ethics approval: This study was conducted in strict compliance with the Brazilian legislation for research and experimentation with animals and was approved by the Committee of Ethics in the Use of Animals of the State University of Southwest Bahia (UESB), Itapetinga Campus, BA, Brazil (License Nº23/2013).

Consent to participate: Not applicable.

Consent for publication: Not applicable.

REFERENCES

AOAC, 1990. 'Official methods of analysis (15th edn).' Journal of AOAC International: Gaithersburg, MD.

Beauchemin, K.A., 2018. Invited review: Current perspectives on eating and rumination activity in dairy cows. Journal of Dairy Science, 101, 4762-4784.

Boardman, C.J., Wickersham, T.A., Trubenbach, L.A.and Sawyer, J.E., 2020. Effects of monensin inclusion and level of intake in limit-feeding strategies for beef cows. Translational Animal Science, 4, txaa108.

Brito, E.F., Pereira, M.L.A., Silva, H.G.O., Soares, A.C.M., Correia, G.S., Sousa, L.B., Sousa, L.B., Soares, V.P.S. and De Paula, V.F., 2020. Effects of enriched mesquite piperidine alkaloid extract in diets with reduced crude protein concentration on the rumen microbial efficiency and performance in lambs. Czech Journal of Animal Science, 65, 268-280.

Bürger, P.J., Pereira, J.C., Queiroz, A.C., Silva, J.F.C., Valadares Filho, S.C., Cecon, P.R. and Casali, A.D.P., 2000. Ingestive behavior in Holstein calves fed diets with different concentrate levels. Revista Brasileira de Zootecnia, 29, 236-242.

Cabral, L.S., Valadares Filho, S.C., Detmann, E., Zervoudakis, J.T., Souza, A.L. and Veloso, R.G., 2008. Microbial efficiency and ruminal parameters in cattle fed diets based on tropical forage. Revista Brasileira de Zootecnia**,** 37, 919-925.

Chen, X.B. and Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of technical details. Rowett Research Institute/International Feed Research Unit: Aberdeen. 21p.

Choudhary, M.I., Nawaz, S.A., Ul-Haq, Z., Azim, M.K., Ghayur, M.N., Lodhi, M.A., Jalil, S., Khalid, A., Ahmed, A., Rode, B.M., Gilani, A-Ul-H. and Ahmad, V.U., 2005. Juliflorine: a potent natural peripheral anionic-site-binding inhibitor of acetylcholinesterase with calcium-channel blocking potential, a leading candidate for Alzheimer's disease therapy. Biochemical and Biophysical Research Communications, 332, 1171-1179.

Cirne, L.G.A., Silva Sobrinho, A.G., Basso, F.C., Takahashi, R., Borghi, T.H., Viegas, C.R., Carvalho, G.G.P. and Zeola, N.M.B.L., 2015. Intake, microbial protein synthesis, and nitrogen balance in lambs fed diets containing mulberry hay. Semina: Ciencias Agrarias, 36, 4413-4422.

Clauss, M. and Hummel, J., 2017. Physiological adaptations of ruminants and their potential relevance for production systems. Revista Brasileira de Zootecnia, 46, 606-613.

Clauss, M., Kaiser, T. and Hummel, J., 2008. The morphophysiological adaptations of browsing and grazing mammals. In: I.J. Gordon and H.H.T. Prins (eds), The ecology of browsing and grazing, Heidelberg, 2008, (Springer, AG), 47-88.

Clauss, M., Nunn, C., Fritz, J. and Hummel, J., 2009. Evidence for a tradeoff between retention time and chewing efficiency in large mammalian herbivores. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology, 154, 376-382.

Clauss, M., Steuer, P., Erlinghagen-Lückerath, K., Kaandorp, J., Fritz, J., Südekum, K.H. and Hummel, J., 2015. Faecal particle size: digestive physiology meets herbivore diversity. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology, 179, 182-191.

Detmann, E., Souza, M.A., Valadares Filho, S.C., Queiroz, C., Berchielli, T.T., Saliba, E.O.S. and Azevedo, J.A.G., 2012. Métodos para análise de alimentos. Suprema: Viçosa.

Detmann, E. and Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feed and diets. Arquivos Brasileiros de Medicina Veterinária e Zootecnia, 62, 980-984.

Dittmann, M.T., Runge, U., Ortmann, S., Lang, R.A., Moser, D., Galeffi, C., Schwarm, A., Kreuzer, M. and Clauss, M., 2015. Digesta retention patterns of solutes and different-sized particles in camelids compared with ruminants and other foregut fermenters. Journal of Comparative Physiology. Biochemical, Systemic, and Environmental Physiology, 185, 559-573.

Durmic, Z. and Blache, D., 2012. Bioactive plants and plant products: Effects on animal function, health and welfare. Animal **Feed Sci**ence and Technology, 176, 150-162.

Findeisen, E., Südekum, K., Hummel, J. and Clauss, M., 2021. Increasing feed intake in domestic goats (*Capra hircus*): Measured effects on chewing intensity are probably driven by escape of few, large particles from the forestomach. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, 257, 110972.

Gwirtz, J.A. and Garcia-Casal, M.N., 2014. Processing maize flour and corn meal food products. Annals of the New York Academy of Sciences, 1312, 66-75.

Hall, M.B., Hoover, W.H., Jennings, J.P. and Webster, T.K.M., 1999. A method for partitioning neutral detergent-soluble carbohydrates. Journal of the Science of Food and Agriculture, 79, 2079-2086.

Henciya, S., Seturaman, P., James, A.R., Tsai, Y.H., Nikam, R., Wu, Y.C., Dahms, H-U. and Chang, F.R., 2017. Biopharmaceutical potentials of *Prosopis spp*. (Mimosaceae, Leguminosa). Journal of Food and Drug Analysis, 25, 187-196.

Hummel, J., Scheurich, F., Ortmann, S., Crompton, L.A., Gerken, M. and Clauss, M., 2018. Comparative selective retention of particle size classes in the gastrointestinal tract of ponies and goats. Journal of Animal Physiology and Animal Nutrition, 102, 429-439.

Ítavo, C.C.B.F., Morais, M.G., Costa, C., Ítavo, L.C.V., Franco, G.L., Da Silva, J.A. and Reis, F.A., 2011. Addition of propolis or monensin in the diet: behavior and productivity of lambs in feedlot. Animal Feed Science and Technology, 165, 161-166.

Kaske, M., Hatiboglu, S. and Von Engelhardt, W., 1992. The influence of density and size of particles on rumination and passage from the reticulo-rumen of sheep. The British Journal of Nutrition, 67, 235-244.

Lauper, M., Lechner, I., Barboza, P., Collins, W., Hummel, J., Codron, D. and Clauss, M., 2013. Rumination of different-sized particles in muskoxen (*Ovibos moschatus*) and moose (*Alces alces*) on grass and browse diets, and implications for rumination in different ruminant feeding types. Mammalian Biology, 78, 142-152.

Lemenager, R.P., Owens, F.N., Smokey, B.J., Lusby, K.S. and Totusek, R., 1978. Monensin effect on rumen turnover rate, twenty-four hour VFA pattern, nitrogen components and cellulose disappearance. Journal of Animal Science, 47, 255-261.

Licitra, G., Hernandez, T.M. and Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology, 57, 347-358.

Macheboeuf, D., Morgavi, D.P., Papon, Y., Mousset, J-L. and Arturo-Schaan, M., 2008. Dose–response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Animal Feed Science Technology, 145, 335–350.

Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. Journal of AOAC International, 85, 1217-1240.

Mickdam, E., Khiaosa-ard, R., Metzler-Zebeli, B.U., Klevenhusen, F., Chizzola, R. and Zebeli, Q., 2016. Rumen microbial abundance and fermentation profile during severe subacute ruminal acidosis and its modulation by plant derived alkaloids *in vitro*. Anaerobe, 39, 4-13.

Nikkhah, A., 2014. Review: Ruminant feed intake regulation evolution: chronophysiological rhythms perspectives. Biological Rhythm Research, 45, 563-577.

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle (7th edn), The National Academies Press: Washington DC, 405.

National Research Council (NRC), 2007. Nutrient requirements of small ruminants: Sheep, goats, cervids and New World camelids. The National Academic Press: Washington DC, 292.

Perazzo, A.F., Homem Neto, S.P., Ribeiro, O.L., Santos, E.M., Carvalho, G.G.P., Oliveira, J.S., Bezerra, H.F.C., Campos, F.S. and Freitas Júnior, J.E., 2017. Intake and ingestive behavior of lambs fed diets containing ammoniated buffel grass hay. Tropical Animal Health and Production, 49, 717-724.

Perazzo, A.F., Homem Neto, S.P., Ribeiro, O.L., Santos, E.M., Carvalho, G.G.P., Oliveira, J.S., Rodrigues, T.C.G.C. and Pinho, R.M.A., 2016. Correlations of intake, digestibility and performance with the ingestive behavior of lambs fed diets containing ammoniated buffel grass hay. Semina: Ciencias Agrarias, 37, 1551-1563.

Pereira, T.C.J., Pereira, M.L.A., Moreira, J.V., Azevêdo, J.A.G., Batista, R., De Paula, V.F., Oliveira, B.S. and Santos, E.J., 2017. Effects of alkaloid extracts of mesquite pod on the products of *in vitro* rumen fermentation. Environmental Science and Pollution Research International, 24, 4301-4311.

Polizel, D.M., Martins, A.S., Miszura, A.A., Ferraz Júnior, M.V.C., Bertoloni, A.V., Oliveira, G.B., Barroso, J.P.R., Ferreira, E.M. and Pires, A.V., 2021. Low doses of monensin for lambs fed diets containing high level of ground flint corn. Scientia Agricola, 78, e20190263.

Preeti, K., Avatar, S.R. and Mala, A., 2015. Pharmacology and therapeutic application of *Prosopis juliflora*: A review. Journal of Plant Sciences, 3, 234-240.

Purser, D.B. and Moir, R.J., 1966. Rumen volume as a factor involved in individual sheep differences. Journal of Animal Science, 25, 509-515.

Richardson, L.F., Raun, A.P., Potter, E.L., Cooley, C.O. and Rathmacher, R.P., 1976. Effect of monensin on rumen fermentation *in vitro* and *in vivo*. Journal of Animal Science, 43, 657–664.

Santos, E.T., Pereira, M.L.A., Silva, C.F.P.G., Souza-Neta, L.C., Geris, R., Martins, D., Santana, A.E.G., Barbosa, L.C.A., Silva, H.G.O., Freitas, G.C., Figueiredo, M.P., Oliveira, F.F. and Batista, R., 2013. Antibacterial activity of the alkaloid-enriched extract from *Prosopis juliflora* pods and its influence on *in vitro* ruminal digestion. International Journal of Molecular Sciences, 14, 8496-8516.

Santos, J.R.A., Pereira, M.L.A., Pereira, T.C.J., Silva, H.G.O., Santos, O.O., Carvalho, G.G.P., Almeida, J.R.F.A., Silva, R.P. and Ribas, K.P.O., 2021. Supplementation with mesquite alkaloids extract in diets for lambs fed Bermuda grass improves growth performance. Small Ruminant Research, 205, 106560.

Schwarm, A., Ortmann, S., Wolf, C., Streich, W.J. and Clauss, M., 2009. More efficient mastication allows increasing intake without compromising digestibility or necessitating a larger gut: comparative feeding trials in banteng (*Bos javanicus*) and pygmy hippopotamus (*Hexaprotodon liberiensis*). Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology, 152, 504-512.

Soni, L.K., Dobhal, S., Parasher, P. and Dobhal, M.P., 2018. Phytochemicals from *Prosopis* species. Suresh Gyan Vihar University International Journal of Environment, Science and Technology, 4, 9-32.

Sousa, L.B., Pereira, M.L.A., Silva, H.G.O., Sousa, L.B., Silva, L.S., Machado, F.S., Tomich, T.R., Oss, D.B., Ferreira, A.L., Campos, M.M., Costa, I.C. and Pereira, L.G.R., 2022. *Prosopis juliflora* piperidine alkaloid extract levels in diet for sheep change energy and nitrogen metabolism and affect enteric methane yield. Journal of the Science of Food and Agriculture, 102, 5132-5140.

Weiss, W.P., 1999. Energy prediction equations for ruminant feeds. In Cornell nutrition conference for feed manufacturers. New York State College of Agriculture & Life, Cornell University: Rochester, NY. pp. 176-185.

Weston, R.H., 1984. Rumen digesta load in relation to voluntary feed consumption and rumination in roughage-fed young sheep. Canadian Journal of Animal Science, 64, 324-325.