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Effects of enriched mesquite piperidine alkaloid extract in diets with reduced crude protein concentration on the rumen microbial efficiency and performance in lambs

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Abstract: The objective of this study was to evaluate the effects of enriched mesquite piperidine alkaloid (MPA) extract at 31 mg/kg of dry matter (DM) in diets with 16% or 13% of crude protein (CP) compared to a diet with 16% CP without additive (control) and diets with monensin (MON) at 31 mg/kg DM and 16% or 13% CP. The intake, ingestive behaviour, apparent digestibility, body weight gain, microbial protein synthesis and nitrogen balance were evaluated. A total of 30 uncastrated crossbred Santa Inês × Bergamasca lambs were used and allocated in a completely randomized design. No effects of additives on dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), and non-fibre carbohydrate (NFC) intake were observed in diets with 16% and 13% CP compared to the control diet. The total digestible nutrient intake (g/kg BW) for the diet with MPA 13% CP was lower than for the control diet, while the metabolisable energy intake (MJ/kg BW) did not differ between diets. The DM feeding rate (g/min) was reduced for the diet with MON 13% CP compared to the control diet. There was no difference between MON or MPA with CP 16% or 13% and the control diet in the digestibility of DM, OM, NFC, and NDF. MON in the diet with 13% CP reduced the BW gain, which differed from the control diet, while MPA 13% CP did not differ from the control diet. The microbial protein synthesis efficiency was higher for diets with MPA compared to MON 13% CP and the control diet. Dietary nitrogen retention (g/kg metabolic weight) was lower in diets with 13% CP that differed from the diets with 16% CP. The nitrogen retained as a percentage of ingested and digested nitrogen was unchanged with the use of MPA or MON 13% CP due to lower urinary nitrogen excretion. The MPA 13% CP diet does not affect the performance of lambs by increasing the microbial synthesis efficiency in the rumen.

Keywords: body weight gain; ionophore; phytogenic additives; *Prosopis juliflora*; sheep

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Supplementation with antimicrobial additives as ruminal fermentation modulators and animal performance enhancers is a common feeding management practice in feedlots. One of the most commonly used additives for ruminants is the ionophore monensin, having the main objective to control ruminal acidosis and improve the efficiency of dietary energy utilization by reducing the ratio of acetate to propionate, in addition to improving dietary protein utilization (Appuhamy et al. 2013).

However, due to the emergence of antibiotic resistant bacteria which are used to treat human and animal infections, the European Commission banned the inclusion of growth promoting antibiotics in animal feed in accordance with Regulation (EC) No. 1831/2003 and by the precautionary principle. Since then, researchers have sought to develop natural additives which are secondary metabolic plant products, being extracted without changes in their chemical structure and which have biological activity in the rumen.

Complex plant extracts containing several secondary metabolites can have an advantage over conventional antibiotics which are based on a single active principle. Thus, the presence of various compounds with complementary functions can have a beneficial synergistic effect which prevents the development of microbial resistance.

An enriched mesquite piperidine alkaloid (MPA) extract obtained from the species *Prosopis juliflora* (Sw) D.C. with the main constituents juliprosopine (juliflorine), prosoflorine and juliprosine can be a viable alternative to replace monensin (MON) in ruminant diets. It has an inhibitory effect on gram-positive bacteria, in addition to acting as a modifier of rumen fermentation and in mitigating *in vitro* methane production (Santos et al. 2013; Pereira et al. 2017). MPA doses ranging from 2.3 mg/kg to 27.6 mg/kg dry matter (DM) of the diet with 60% concentrate showed improved energy and dietary protein utilization in lambs. This was demonstrated by a higher efficiency of rumen microbial synthesis, increased body weight (BW) gain and effectiveness in mitigating enteric methane production (Santos 2017; Sousa 2018).

MPA can also possibly contribute to a lower dietary protein supply without affecting productive performance (Santos 2017). Therefore, the objective of this study was to evaluate the effects of MPA or MON (31.5 mg/kg DM) in diets with 16% and 13% of crude protein on the intake, ingestive behaviour, apparent digestibility, body weight gain, microbial protein synthesis and nitrogen balance in lambs.

MATERIAL AND METHODS

Experiment location

The experiment was conducted in the sheep sector of the State University of Southwest Bahia, Brazil. All experimental procedures were performed according to the Animal Use Ethics Committee, protocol No. 23-2013 of the State University of Southwest Bahia.

Obtaining enriched mesquite piperidine alkaloid extract

Mature pods of *Prosopis juliflora* (SW) D.C. were obtained from Brumado/BA, being manually harvested from June to July 2016. The pods were sun dried for three days, and then processed in a mill (Wiley mill, A. H. Thomas, Philadelphia, PA, USA) using a 1-mm mesh screen. The whole pod meal was macerated with 99.5% ethanol over 72 h in a sealed container. The macerate was then percolated and the extracted solution was concentrated in a vacuum evaporator (rotary Fisatom Evaporator – model 802; São Paulo, Brazil) at –600 mmHg and a controlled temperature of 40 °C for obtaining the crude ethanol extract (CEE). The CEE was partitioned using acid-base solutions and organic solvents according to the methodology of Ott-Longoni et al. (1980).

Part of the CEE (100 g) was subsequently solubilized in 1.6 M acetic acid aqueous solution (AcOH, 200 ml) and the resulting solution was filtered to obtain acidic aqueous solution I (AAS-I). The AAS-I was extracted with chloroform ($CHCI_3$) in two successive 150 ml washes, thereby obtaining acidic aqueous solution II (AAS-II). The AAS-II was alkalized with sodium hydroxide (NaOH) until pH 9.0, and called basic aqueous solution I (BAS-I). The BAS-I was triple-washed with 100 ml of $CHCI_3$, obtaining basic aqueous solution II (BAS-II). The BAS-II was subjected to double washing with sodium chloride solution (NaCl), resulting in basic aqueous solution III (BAS-III) which was subsequently dehydrated with 5 g of sodium sulphate (Na_2SO_4), homogenized and allowed to stand for 2 hours.

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Next, the BAS-III containing the piperidine alkaloids was transferred to a round bottom flask after filtration, and the chloroform was evaporated on a rotary evaporator at 57 °C to produce the solid basic chloroform extract (BCE) of piperidine alkaloids from mesquite (Pereira and Batista 2014). The nuclear magnetic resonance spectrum of the BCE showed signals of prosoflorine, juliprosopine and juliflorine from the mesquite pod meal. The BCE was weighed and added to the concentrate feed to obtain the dose of 31.5 mg/kg diet DM.

Design and experimental description

A total of 30 uncastrated crossbred Santa Inês × Bergamasca male lambs at 150 days of age and with initial body weight of 23 ± 4 kg were used. The animals were initially identified by earrings, vaccinated against clostridiosis, wormed and adapted under experimental conditions for a period of 14 days. They were housed in individual stalls equipped with troughs for feed and water. A completely randomized design with five diets and six replications was implemented. The experimental period lasted 89 days subdivided into three equidistant periods of sample collection lasting 3 days each.

The diets consisted of 33.3% Buffel grass hay and 66.7% concentrate formulated with ground maize, soybean meal, urea, ammonium sulphate and mineral supplement for sheep, and 31.5 mg/kg DM sodium monensin (MON) or an enriched mesquite piperidine alkaloid (MPA) extract. Two crude protein levels (16% and 13% CP) were evaluated with MPA or MON compared to the control diet (16% CP without additives). The proportion of ingredients and chemical composition of the experimental diets, roughage and concentrate are presented in Table 1.

The diets were provided daily at 7:00 and 16:00 h *ad libitum* to allow 5% to 10% of leftovers adjusted daily. Voluntary DM intake was measured for 89 days and it was obtained by the amount of hay and concentrate supplied as total mixed ration (TMR) minus the amount of leftovers.

Experimental sample collections, laboratory analyses and calculations

Ingestive behaviour was evaluated on the first day of each collection period in which animals were observed for 24 h. The times spent for feeding, rumination and idleness were recorded by trained observers visually observing the animals every 10 min in a relay system and strategically positioned so as not to interfere with animal behaviour.

Observations started at 8:00 h and ended at the same time the following day. The number of rumination chews and the time spent on ruminating

Table 1. Dietary ingredients and chemical composition of Buffel grass hay and concentrates (g/kg DM)

Ingredient	Diets	
	16% CP	13% CP
Buffel grass hay	333	333
Ground corn	498	579
Soybean meal	144	66.3
Urea + Ammonium sulphate (9 : 1)	5	5
Mineral salt[a]	20	20
Monensin or MPA (mg/kg DM)	31.5	31.5

Item	Buffel hay	Concentrates	
		16% CP	13% CP
DM (g/kg NM)	799	893	894
OM	934	943	955
CP	48	196	167
EE	28	35	41
NFC	156	629	660
NDF	721	93	110
Mineral matter	66	50	45
Cellulose	369	172	171
Hemicellulose	303	89	105
Lignin	79	29	29
NDIP	24	68	45
ADIP	19	41	27
DE (MJ/kg DM)	11.8	12.3	12.4
ME (MJ/kg DM)	4.0	10.5	10.6

ADIP = acid detergent insoluble protein; CP = crude protein; DE = digestible energy; DM = dry matter; EE = ether extract; ME = metabolizable energy; MPA = mesquite piperidine alkaloid; NDIP = neutral detergent insoluble protein; NDF = neutral detergent fibre corrected for ash and protein; NFC = non-fibre carbohydrates corrected for ash and protein; NM = natural matter; OM = organic matter
[a]120g Ca; 87g P; 147 g Na; 18 g S; 590 mg Cu; 40 mg Co; 20 mg Cr; 1 800 mg Fe; 80 mg I; 1 300 mg Mn; 15 mg Se; 3 800 mg Zn; 300 mg Mo; 870 mg F (max.); P solubility in Citric acid 2% (min.) – 95%

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each bolus were counted using a digital stopwatch during this period. Observations of three ruminal boluses at three different periods of the day (morning, afternoon, and evening) were made in order to obtain the chewing and time averages. The time and number of chews for each ruminal bolus per animal were subsequently quantified. It is worth mentioning that observation of the environment was maintained by artificial lighting during the night.

Individual intake of dry matter (DM) and neutral detergent fibre corrected for ash and protein (NDF) by animals during ingestive behaviour on the first day of each collection period was considered for calculating the following variables based on the equations described by Burger et al. (2000):

$$FT = ML/DMI \text{ or } NDFI \quad (1)$$

$$RT = RL/DMI \text{ or } NDFI \quad (2)$$

where:

FT – feeding time (min/g);
ML – meal length (min/day);
DMI (g/day) – dry matter intake;
NDFI (g/day) – neutral detergent fibre corrected for ash and protein intake;
RT – rumination time (min/g);
RL – rumination length (min/day).

$$FR = DMI \text{ or } NDFI/ML \quad (3)$$

$$RR = DMI \text{ or } NDFI/RL \quad (4)$$

$$RBN = RL/ChTRB \quad (5)$$

$$DM \text{ or } NDF \text{ per bolus} = g \text{ DMI or } g \text{ NDFI}/RBN \quad (6)$$

$$RCht = ChN/bolus \times RChN \quad (7)$$

where:

FR (g DMI/min and g NDFI/min) – feeding rate;
RR (g DM/min and g NDF/min) – rumination rate;
RBN (no./day) – ruminal bolus number;
ChTRB (s/bolus) – chewing time per ruminal bolus;
RCht (min/day) – total chewing time of bolus;
ChN (no./bolus) – chewing number per ruminal bolus;
RChN (no./day) – rumination chewing number.

The digestibility coefficients (DC) of DM, CP, EE, NDF and NFC were calculated as proposed by Berchielli et al. (2011):

$$DC = \frac{(\text{nutrient ingested} - \text{nutrient excreted}) \times 100}{(\text{nutrient ingested})} \quad (8)$$

Faeces collections were performed using collection bags attached to the animals' bodies three days prior to adaptation to the bag. The faeces were collected at the end of each day after morning feeding, weighed and then pre-dried in a 65 °C forced-ventilation oven for 72 hours. After milling, composite samples were obtained using equal weight of faeces from the three days of collection per animal and stored for further chemical analysis.

Dry matter (DM) (method INCT-CA G-003/1), organic matter (OM), mineral matter (MM) (method INCT-CA M-001/1), crude protein (CP) (method INCT-CA N-001/1) and ether extract (EE) (method INCT-CA G-004/1) concentrations in roughage, concentrate, leftovers and faeces samples were analysed according to Detmann et al. (2012). The samples were treated with thermostable alpha amylase, without sodium sulphate, and corrected for residual ash (Mertens 2002) for the analyses of neutral (NDF) and acid (ADF) detergent fibre. The neutral (NDIN) and acid (ADIN) detergent insoluble nitrogen compounds were carried out according to Licitra et al. (1996), and the NDF correction for nitrogen compounds was also performed.

Lignin (method INCT-CA F-005/1) in roughage and concentrate samples was obtained from the ADF fraction treated with 72% sulphuric acid based on the methodology described by Detmann et al. (2012) in which ADF residue was obtained by sequential analysis.

The non-fibre carbohydrate (NFC) content was calculated according to Hall et al. (1999) with modifications, using neutral detergent fibre corrected for ash and protein (NDF) (Detmann and Valadares Filho 2010):

$$NFC = 100 - [(\%CP - \%CPU + \%U) + \%MM + \%EE + \%NDF] \quad (9)$$

where:

%CP – crude protein content;
%CPU – crude protein content of urea;
%U – urea content;
%MM – mineral matter content;
%EE – ether extract content;
%NDF – neutral detergent fibre content corrected for ash and protein.

Total digestible nutrients (TDN) and metabolizable energy (ME) were determined by digestibility assay performed in three periods of total faeces col-

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lection during three days each. The total digestible nutrients (TDN) were calculated according to Weiss (1999), using NDF and NFC corrected for ash and protein by the following equation:

$$TDN = DCP + DNDF + DNFC + 2.25\ DEE \quad (10)$$

where:
DCP – digestible crude protein;
DNDF – digestible neutral detergent fibre;
DCNF – digestible non-fibre carbohydrates;
DEE – digestible ether extract.

The TDN values were converted into digestible energy and thus the metabolisable energy was estimated using the equations suggested by the NRC (2001). However, they were multiplied by 4.184 to convert to megajoules.

$$DE\ \left(\frac{Mcal}{kg}\right) = 0.044\ 09 \times TDN \quad (11)$$

$$ME\ \left(\frac{Mcal}{kg}\right) = 1.01 \times DE\ Mcal/kg - 0.45 \quad (12)$$

where:
DE – digestible energy;
ME – metabolisable energy;
TDN – total digestible nutrients.

For performance evaluation, the animals were weighed at the beginning and at the end of the experiment, and solid fasting for 16 h was performed to evaluate body weight gain and daily nutrient intake (g/kg BW). The feeding efficiency was obtained from body weight gain divided by dry matter intake.

A spot urine sample was collected 4 h after the morning feeding by spontaneous urination on the third day in each one of the three collection periods. To do so, a 10 ml aliquot of urine was diluted with 40 ml of 0.036 N sulphuric acid. Then the pH was measured and adjusted to below 3.0 with small drops of sulphuric acid when necessary in order to prevent ammonia volatilization and bacterial decomposition of purine derivatives.

Urine samples were analysed for creatinine, urea, allantoin, xanthine-hypoxanthine and uric acid concentrations. Analyses of uric acid (K139-1), urea (K047-1) and creatinine (K016-1) were performed by Bioclin® commercial kits. Allantoin and xanthine-hypoxanthine were determined according to the methodologies presented in Chen and Gomes (1992).

Urinary volume was estimated using the mean daily creatinine excretion of two experimental lambs fed each experimental diet in each collection period. Daily creatinine excretion (mg/kg BW) was divided by the spot urine creatinine concentration (mg/l) of each lamb to calculate the daily volume of urine produced per animal.

Total purine derivative (TPD) excretion was obtained by summing the amounts of allantoin, uric acid and xanthine-hypoxanthine excreted in the urine. The amount of absorbed microbial purines (mmol/day) was estimated from TPD (mmol/day) using the equation proposed for sheep by Chen and Gomes (1992).

The intestinal flow of microbial nitrogen (g MN/day) was estimated from the amount of absorbed purines (mmol/day) according to the equation of Chen et al. (1992).

The microbial CP synthesis was obtained by multiplying the MN by 6.25, whereas the microbial efficiency was determined by the formula of NRC (2001):

$$MEF\ \left(\frac{g}{kg}\right) = MCP/TDNI \quad (13)$$

where:
MEF – microbial efficiency;
MCP – microbial CP synthesis;
TDNI – total digestible nutrient intake.

The nitrogen compound balance was obtained by the difference between the total nitrogen ingested and the total nitrogen excreted in faeces and urine. Total nitrogen in faeces and urine was determined according to the methodology described by Detmann et al. (2012).

Statistical analysis

Data analysis was performed using the MIXED procedure of the SAS statistical computer program (SAS Institute Inc., Cary, NC). The average data from the three periods were used for ingestive behaviour, apparent digestibility, microbial protein synthesis and nitrogen balance. The ratio between the total ingested nutrients by the animal and the days of the experiment duration was used

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to calculate the intake, without considering the period. Normality of variance was verified and then the comparison of the means observed between diets with and without MON or MPA and different CP concentrations was performed by Tukey's test. The variables of feeding time in DM min/g and NDF min/g were non-parametric and thus the data were inversely transformed. Initial body weight was the covariate to analyse the weight gain parameters.

RESULTS

The addition of MON or MPA to diets with 13% and 16% crude protein (CP) did not affect ($P > 0.05$) dry matter intake (DMI), organic matter intake (OMI), neutral detergent fibre intake (NDFI) or non-fibre carbohydrate intake (NFCI). The crude protein intake (CPI, g/kg BW) was lower ($P < 0.000\ 1$) in diets with 13% CP with MON and MPA. The lower CPI for 13% CP diets with additives compared to diets containing 16% CP is consistent with the CP content in the diet composition (Table 1). There was a 23% and 21% reduction in CPI for MON (13% CP) and MPA (13% CP), respectively.

The effect of MON and MPA on the ether extract intake (EEI, g/kg BW) was observed which showed a lower value in diets with 16% CP compared to the control diet. However, MON and MPA in diets with 13% CP did not affect the EEI, presenting the same mean from the control diet (Table 2). The EEI increased in diets with 13% of

Table 2. Nutrient intake by lambs fed diets containing 13% and 16% crude protein with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	*P*-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
g/day							
DMI	1 107.7	1 104.0	1 094.3	937.5	1 019.6	26.686	0.056
OMI	1 045.6	1 026.6	1 036.9	889.3	965.5	25.985	0.100
CPI	169.3^{a}	164.2^{a}	165.1^{a}	121.3^{bc}	136.2^{b}	6.438	< 0.000 1
EEI	30.9^{a}	23.2^{b}	25.7^{b}	26.8^{b}	29.3^{ab}	0.735	0.002
NDFI	310.2	306.7	306.0	283.3	292.3	7.134	0.704
NFCI	543.4	530.6	539.3	483.2	519.4	12.805	0.409
TDNI	749.6	734.8	737.8	654.4	667.2	19.300	0.217
ME (MJ/day)	10.67^{ab}	10.63^{ab}	10.71^{ab}	11.09^{a}	10.29^{bc}	0.109	0.049
g/kg BW							
DMI	30.6	30.3	28.7	28.0	28.1	0.562	0.178
OMI	28.9	27.9	27.2	26.6	26.6	0.526	0.284
CPI	4.67^{ab}	4.47^{ab}	4.33^{b}	3.64^{c}	3.76^{c}	0.167	< 0.000 1
EEI	0.85^{a}	0.63^{c}	0.67^{c}	0.80^{ab}	0.81^{a}	0.205	< 0.000 1
NDFI	8.6	8.3	8.0	8.4	8.1	0.153	0.627
NFCI	15.0	14.4	14.1	14.5	14.3	0.293	0.789
TDNI	23.2^{a}	23.0^{ab}	22.3^{ab}	20.8^{ab}	20.3^{bc}	0.359	0.023
ME (MJ/kg BW)	0.34	0.35	0.33	0.37	0.33	0.008	0.703

CP = crude protein; CPI = crude protein intake; DMI =dry matter intake; EEI = ether extract intake; ME = metabolisable energy; MON = monensin; MPA = mesquite piperidine alkaloids; NDFI = ash and protein corrected neutral detergent fibre intake; NFCI = non-fibre carbohydrate intake; OMI = organic matter intake; SEM = mean standard error; TDNI = total digestible nutrient intake; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

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ether extract (EE) due to the higher EE content in those diets which were prepared with a higher proportion of ground maize grain, since the DMI was not changed (Table 1).

The diets affected ($P = 0.023$) the total digestible nutrient intake (TDNI, g/kg BW) and the MPA 13% CP diet had the lowest value with respect to the control; however, the metabolisable energy intake (MJ/kg BW) was not changed by the diets (Table 2).

There was no difference ($P > 0.05$) in DMI and NDFI observed for ingestive behaviour (Table 3). The results obtained for feeding time (min/day) and number of meal periods (frequency of trough visits) did not show any difference ($P > 0.05$) between diets. There was a difference ($P = 0.015$) in DM feeding time (min/g), and the diet with MON 13% CP showed a greater value compared to the control diet (Table 3).

The rumination length (min/day) and rumination time of DM and NDF (min/g) did not present any differences ($P > 0.05$) with the use of additives in diets with different CP concentrations. Moreover, the idle time did not differ ($P > 0.05$) between the diets with MON and MPA when compared with the control diet. The lambs in the present study remained idle for an average of 56% of the observation period. There were no differences ($P > 0.05$) in the variables of total chewing time of bolus (RCht), chewing number per bolus (ChN) and chewing time per ruminal bolus (ChTRB) (Table 3).

Table 3. Ingestive behaviour parameters in lambs fed diets containing 13% and 16% crude protein with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	*P*-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
Intake							
DM (g/day)	1 042.65	1 043.74	1 077.80	888.05	965.68	27.126	0.170
NDF (g/day)	294.46	287.42	303.66	270.52	276.80	7.247	0.640
Feeding							
min/day	180.94	191.11	191.83	213.89	216.00	6.693	0.440
min/g DM	0.18^{b}	0.18^{b}	0.18^{b}	0.24^{a}	0.22^{ab}	0.008	0.015
F (no. of trough visits)	17.16	19.11	19.11	21.39	21.61	0.750	0.310
Rumination							
min/day	401.44	452.22	448.00	425.00	423.33	9.918	0.390
min/g DM	0.39	0.44	0.40	0.49	0.44	0.017	0.420
min/g NDF	1.39	1.61	1.42	1.62	1.53	0.058	0.660
Idleness							
min/day	866.94	796.67	827.17	801.11	800.67	11.918	0.300
Rumination chewing							
RCht (min/day)	391.95	445.63	448.0	425.0	443.0	9.918	0.310
ChN (no./bolus)	67.67	61.56	64.38	54.78	64.59	1.705	0.160
ChTRB (s/bolus)	44.23	45.15	46.28	40.20	45.31	1.207	0.720
Full chewing							
min/day	582.39	643.33	612.84	638.89	639.33	11.565	0.420
min/g DM	0.57	0.63	0.58	0.73	0.67	0.021	0.110
min/g NDF	2.01	2.28	2.06	2.41	2.31	0.069	0.320

ChN = chewing number per bolus; ChTRB = chewing time per ruminal bolus; CP = crude protein; DM = dry matter; F = frequency; MON = monensin; MPA = mesquite piperidine alkaloids; NDF = neutral detergent fibre; RCht = total chewing time of bolus; SEM = mean standard error; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

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The DM feeding rate (FR) (g/min) differed (P = 0.012) and there was a lower DM FR for MON 13% CP with respect to the control diet. The rumination rate (RR) of DM (g/min) and NDF (g/min), ruminal bolus number (RBN) (no./day) and the DM and NDF amount per bolus (g/bolus) did not differ between diets (Table 4).

The digestibility coefficients did not show any difference (P > 0.05) between diets in dry matter (DMD), organic matter (OMD), neutral detergent fibre (FDND) corrected for ash and protein, non-fibre carbohydrate (NFCD) or in total digestible nutrient content (TDN) (Table 5). There was lower CPD in diets with lower CP concentration. However, the MON 16% CP diet showed higher CPD compared to the control diet. The MPA in the 13% and 16% CP diet did not differ compared to the control for CPD. On the other hand,

Table 4. Feeding and rumination rate in lambs fed diets containing 13% and 16% crude protein with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	P-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
Feeding rate							
DM (g/min)	5.77^{a}	5.65^{ab}	5.62^{ab}	4.19^{b}	4.71^{ab}	0.189	0.012
NDF (g/min)	1.62	1.54	1.59	1.27	1.35	0.046	0.051
Rumination rate							
DM (g/min)	2.62	2.33	2.65	2.15	2.31	0.096	0.420
NDF (g/min)	0.74	0.64	0.74	0.66	0.66	0.026	0.610
RBN (no./day)	557.15	616.59	549.02	657.06	598.65	25.668	0.690
g DM/bolus	1.95	1.76	2.03	1.47	1.76	0.099	0.470
g NDF/bolus	0.55	0.48	0.57	0.45	0.50	0.027	0.640

CP = crude protein; DM = dry matter; MON = monensin; MPA = mesquite piperidine alkaloids; NDF = neutral detergent fibre; RBN = ruminal bolus number; SEM = mean standard error; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

Table 5. Digestibility of nutritional components (g/kg DM) in lambs fed diets containing 13% and 16% crude protein with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	P-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
DMD	67.39	68.62	67.86	65.75	64.55	0.536	0.096
OMD	68.00	69.13	68.42	66.40	65.10	0.525	0.090
CPD	65.84^{bc}	69.69^{ab}	71.19^{a}	59.15^{d}	62.42^{cd}	0.986	< 0.000 1
NDFD	49.09	52.00	48.55	47.44	47.55	0.704	0.239
NFCD	82.53	82.37	82.58	82.92	79.43	0.001	0.229
EED	63.59^{b}	56.30^{b}	57.41^{b}	67.22^{a}	66.78^{a}	1.428	0.022
TDN	66.99	66.73	67.21	68.98	65.27	0.265	0.078

CP = crude protein; CPD = crude protein digestibility; DMD = dry matter digestibility; EED = ether extract digestibility; MON = monensin; MPA = mesquite piperidine alkaloids; NDFD = neutral detergent fibre corrected for ash and protein digestibility; NFCD = non-fibre carbohydrate digestibility; OMD = organic matter digestibility; SEM = mean standard error; TDN = total digestible nutrients; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

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there was an increase (P = 0.022) of EE digestibility in diets with 13% CP, probably as a consequence of the higher EE concentration in these diets being formulated with a higher proportion of maize in relation to soybean meal (Tables 1 and 5).

The initial body weight was similar between diets and it was a significant covariate on final body weight (FBW, P < 0.000 1), average body weight (ABW, P < 0.003), total and average daily weight gain (TWG and ADG, P = 0.030). An effect of additives and CP content (P = 0.049) was observed.

The weight gain did not differ in the diet with MPA 13% CP from the control diet. On the other hand, the diet with MON 13% CP diet showed lower body weight gain compared to the control diet. However, no differences were observed in feeding efficiency (FE) (Table 6).

Differences (P = 0.013; P = 0.000 1) in synthesis and microbial efficiency were observed between diets. The diet with 16% CP and MPA showed higher microbial nitrogen synthesis differing from the diet with 13% CP and MON. The diet containing 16% CP with MPA provided higher microbial protein synthesis efficiency than the control, MON 16% and 13% CP diets. The 13% CP with MPA diet presented greater microbial efficiency than the control and MON 13% CP diets (Table 7).

The nitrogen intake (NI, g/day) was affected (P < 0.000 1) by the diets, which was reduced in the diets with 13% CP. There was a 28% and 21% reduction in NI for MON and MPA, respectively (Table 8). The faecal nitrogen (FN, g/day) differed (P = 0.025) with respect to the diets, with the MON 13% CP diet being 27% lower than the control.

Table 6. Performance of lambs fed diets containing 13% and 16% crude protein with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	P-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
IBW (kg)	22.0	22.9	24.1	22.4	24.6	–	–
FBW (kg)	41.8^{a}	41.0^{a}	40.3^{a}	37.5^{b}	39.6^{ab}	1.055	0.049
ABW (kg)	31.9	31.4	34.8	29.9	30.8	0.881	0.396
TWG (kg)	18.9^{a}	18.0^{ab}	17.4^{ab}	14.6^{b}	16.7^{ab}	0.551	0.049
ADG (g)	212.4^{a}	203.3^{ab}	196.0^{ab}	163.7^{b}	187.5^{ab}	6.191	0.049
FE (%)	19.5	18.7	18.1	18.0	17.7	0.350	0.516

ABW = average body weight; ADG = average daily gain; CP = crude protein; FBW = final body weight; FE = feeding efficiency; IBW = initial body weight; MON = monensin; MPA = mesquite piperidine alkaloids; SEM = mean standard error; TWG = total weight gain; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

Table 7. Microbial nitrogen and crude protein synthesis, microbial efficiency in lambs fed diets containing 13% and 16% CP with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	P-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
MN	16.50^{ab}	22.22^{a}	17.87^{ab}	14.41^{b}	17.53^{ab}	0.767	0.013
MCP	103.18^{ab}	138.90^{a}	111.69^{ab}	90.09^{b}	109.58^{ab}	4.797	0.013
MEF (g/kg TDN)	135.37^{c}	195.63^{a}	149.62^{bc}	132.32^{c}	176.09^{ab}	6.058	0.000

CP = crude protein; MCP = microbial crude protein; MEF = microbial efficiency; MN = microbial nitrogen; MON = monensin; MPA = mesquite piperidine alkaloids; SEM = mean standard error; TDN = total digestible nutrients ; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

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Table 8. Nitrogen balance in lambs fed diets containing 13% and 16% CP with mesquite piperidine alkaloids or monensin

Item	Diets					SEM	*P*-value
	16% CP			13% CP			
	WA	MPA	MON	MON	MPA		
NI (g/day)	26.05^{a}	25.93^{a}	26.37^{a}	18.73^{b}	20.64^{b}	0.791	< 0.000 1
FN (g/day)	9.13^{a}	8.48^{ab}	8.11^{ab}	6.66^{b}	7.74^{ab}	0.258	0.025
DN (g/day)	16.91^{a}	17.45^{a}	18.25^{a}	12.07^{b}	12.90^{b}	0.601	< 0.000 1
UTN (g/day)	3.19	3.01	2.48	1.56	1.81	0.255	0.169
UUN (g/day)	1.77	1.65	1.66	1.04	1.07	0.126	0.182
RN (g/day)	13.82^{ab}	14.30^{ab}	15.75^{a}	10.45^{b}	11.04^{b}	0.543	0.002
RNMW (g/day)	1.05^{ab}	1.07^{ab}	1.16^{a}	0.83^{c}	0.83^{c}	0.038	0.004
RN%NI	53.11	54.20	59.52	54.70	52.37	0.198	0.374
RN%DN	82.58	80.76	86.66	85.75	85.54	0.803	0.835
DN%NI	64.58^{ab}	67.13^{ab}	69.14^{a}	64.11^{ab}	62.00^{b}	0.661	0.005

CP = crude protein; DN = digested nitrogen; FN = faecal nitrogen; MON = monensin; MPA = mesquite piperidine alkaloids; NI = nitrogen ingested; RN = retained nitrogen; RNMW = retained nitrogen per metabolic weight; UTN = urinary total nitrogen; UUN = urinary urea nitrogen; SEM = mean standard error; WA = without additive

Means followed by the same letter on the same line do not present significant differences at the 5% significance level by Tukey's test

The digested nitrogen (DN, g/day) was similar between the 16% CP diet with additives and the control diet. However, a reduction of 29% and 24% in DN was observed in 13% CP diet with MON and MPA, respectively, in relation to the control diet. Similarly, a decrease for the MON and MPA diets occurred as a result of the CP reduction. The total and urea nitrogen excreted in the urine (UTN and UUN) did not differ significantly between diets. However, the diets with 13% CP, the UTN and UUN were numerically lower than the diets with 16% CP. The retained nitrogen (RN, g/day) changed (P = 0.002) between diets. In comparing the diets with 13% CP (MON and MPA) and the MON 16% CP, there was a reduction of 31% and 26% in dietary RN, respectively. No difference (P > 0.05) was observed between diets in the retained nitrogen and ingested ratio (RN%NI), retained nitrogen and digested (RN%DN). The 13% and 16% CP diets with additives were similar to the control diet for DN%NI. However, there was a difference between the diets containing MPA 13% CP and MON 16% CP. The MPA 13% CP reduced the DN%NI by 10%.

DISCUSSION

The hypothesis that the addition of MPA to a diet with 16% to 13% reduction in CP does not affect productive performance by improving dietary energy and protein utilization was not rejected. The results indicated that it is possible to reduce the CP content of the MPA diet by not reducing fibre digestibility and by increasing the rumen microbial protein synthesis efficiency. The improvement in dietary energy and protein utilization by MPA, even in a diet with lower CP concentration, provided a body weight gain which was equivalent to diets with 16% CP. Therefore, it is possible to use MPA as an alternative to MON as a nutritional additive.

Neither of the additives affected the NFC (g/kg BW), NDF (g/kg BW) or ME (g/kg BW) intakes, observing a similar effect to the control diet, and even associated to a lower protein concentration. The lower CP intake observed in lambs fed diets with 13% of CP supplemented with MPA or MON is justified by the CP levels which were lower than in the other diets. Even though there were no differences in DMI and NDFI, MON with 13% CP diet increased the feeding time of DM (min/g) in comparison with the control diet. MON could have possibly affected the palatability and/or activity of cellulolytic and ammonia-hyperproducing gram-positive bacteria (HAP rumen bacteria). The reduced fibre digestion contributes to rumen filling; therefore ruminant animals can increase their frequency of trough visits to avoid a reduction

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in daily dietary intake. Poor palatability contributes to reduce the chew mass, consequently increasing the trough frequency (Salinas-Chavira et al. 2010).

The numerical increase in trough frequency may have influenced the increased feeding time in min/g DM, thus avoiding the decrease of DM and NDF intake.

Miranda et al. (1999) described rumination as a physiological resource to reduce the fibre particle size for optimal use of food, being triggered and paced by the diet supply time. The time spent on rumination is influenced by the nature of the diet and is certainly proportional to the cell wall content of roughage. Thus, the result obtained in the present study can be explained by the similarity of the diet characteristics, since NDF content, quality and fibre particle size did not vary among the experimental diets.

The fibre sources are influential in chewing activities, so the results obtained with no significant difference in rumination and chewing time are explained by the same fibre source from roughage which presented an equal particle size. According to Mertens (1997), increasing the amount of fibre in diets stimulates chewing activity, and particle size is the most important factor to stimulate this activity. According to Alves et al. (2010), bolus number/day is influenced by rumination time and time spent to cud each bolus. This is consistent with the results found herein, since the rumination time and the time spent to cud each bolus were not different, and thus there was no difference in the number of boluses/day.

The reduction of CP content in diets caused an apparent CP digestibility reduction, possibly due to nitrogen interference from rumen microbial synthesis, squamation and gastrointestinal tract secretions. The same TDN content observed among diets reflected the unchanging apparent digestibility coefficient of most nutrients.

The use of MPA or MON in the diet with 16% and 13% of CP provided similarities in total digestible nutrient intake (TDNI), being that MPA 13% of CP was the only diet with lower TDNI per kg BW compared with the control diet. However, it did not affect MEI per kg BW, indicating that the reduction of CP from 16% to 13% with MPA contributed to the improved dietary energy efficiency (Sousa 2018). This can be confirmed by the ADG and TWG of lambs fed this diet, which did not differ from the control diet with 16% CP. In contrast, the diet with MON and 13% CP provided lower ADG and TWG when compared to the control diet, although there was no change in MEI compared with 16% CP diet.

These results are consistent with Javed et al. (2010), in which a higher ADG (152 g) was observed in Thalli lambs fed a diet with 14% CP and low energy when compared to lambs fed a diet with low CP content (13%) and high energy, thus observing 125 g ADG in this condition. The efficacy of the MPA on lamb performance is proven in the present study, since no difference in DMI (g/kg BW) or MEI (g/kg BW) was observed between diets with and without this additive. The body weight gain observed in the 13% CP with MPA diet did not significantly differ from the control diet. However, a lower body weight gain was observed in lambs fed MON 13% CP in the diet.

Diets with low protein which do not provide enough N-ammonia and branched short-chain fatty acids to meet the requirements of cellulolytic bacteria limit microbial growth and negatively affect fibre digestibility and DMI, and consequently animal performance (Russell and Martin 1984). There was probably rumen microbiome modification as there was no reduction in DMI and body weight gain did not differ when comparing MPA 13% CP with the control diet.

The effect of dietary additives and protein levels on the rumen microbial protein synthesis efficiency was observed. MPA can probably alter the syntrophic relationships of rumen microbes in a different way than MON, as rumen gram-positive bacteria are inhibited by both antimicrobial agents (Pereira et al. 2017). This statement can be confirmed by the higher efficiency of microbial synthesis obtained in MPA diets when compared with MON for both protein levels. Monensin acts on rumen protein metabolism by decreasing the HAP rumen bacteria growth and thereby reducing the degradation of rumen dietary protein (Santos et al. 2013; Pereira et al. 2017).

The largest amount of undegraded protein fraction which reaches the duodenum to be digested can increase the amino acid supply to the body metabolism. An increase of digestible non-degraded rumen protein and an increase in the intestinal flow of digestible microbial protein can additionally result in excess amino acids in the body, contributing to increased catabolism and elimination of total N in the urine (TUN) (Russell and Martin 1984).

No differences in TUN and urinary urea nitrogen (UUN) were observed with the use of MPA or MON, but TUN decreased numerically when

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associated with the lower dietary protein concentration which did not differ from the control diet. The results for the faecal nitrogen excretion (FN) reflected the effects of MON and MPA and the protein content of the diets, being that MON 13% CP was lower in comparison with the control diet.

It can be observed that the lower amount of digested nitrogen (DN) for diets with MON and MPA with 13% CP also caused lower retained nitrogen (RN, mg/kg $BW^{0.75}$) compared to diets with 16% CP, but within the same proportion of the nitrogen intake (NI). This suggests that the amount of digested protein decreased only due to the effect of dietary protein concentration and not to the additives. In contrast, MPA increased the microbial protein flow in the gut. However, digestible dietary protein amino acids were used more efficiently for tissue deposition in diets with additives, despite the reduction in the amount of digested protein, as the MON and MPA with 13% CP diets presented a proportion of dietary N retention in the gain equal to the control diet with 16% CP. The proportion of N retention in the weight gain was higher than in the control diet for both additives with 16% CP. This can be demonstrated by the percentages of N retained in the body weight gain which were 6.6, 7.0, 7.8, 6.4, and 6.2% for the control (16% CP), MPA (16% CP), MON (16% CP), MON (13% CP) and MPA (13% CP) diets, respectively.

CONCLUSION

The mesquite piperidine alkaloid extract as an alternative additive to monensin enables reducing the crude protein supply from 16% to 13% in diets for lambs, does not affect the ingestive behaviour or body weight gain, and also increases microbial efficiency in the rumen.

Conflict of interest

The authors declare no conflict of interest.

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