Enriched Mesquite Piperidine Alkaloid Extract Improves the Performance in Growing Goats

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***Abstract*—** *This study aimed to evaluate levels of enriched mesquite piperidine alkaloid extract (MPA) comparison with sodium monensin on the nutrition and growth performance of goats fed diets with high concentrate content. Thirty Anglo-Nubian crossbred goats, 120 days of age, and initial body weight 21.82 ± 0.11 kg were distributed to the following diets: 0 (no additive), with MPA 9.2, 18.4, and 27.6 mg kg^{-1} or monensin (MON) 2.7 mg kg^{-1}. The diets with MPA did not differ (P > 0.10) from the MON diet for the intake and digestibility of DM and OM. However, NDF$_{ap}$ and CP intake (g kg^{-1} BW$^{0.75}$), MON showed a higher mean compared to MPA, and their digestibility coefficients did not differ. There was a linear increase (P < 0.05) for the intake and digestibility of CP and NFC with the MPA levels. The metabolizable energy (ME) and daily weight gain (DWG) presented a quadratic effect (P < 0.05) with peaks estimated at 17.4 and 14.8. There was no difference (P > 0.10) for microbial nitrogen synthesis, and microbial efficiency decreased linearly (P < 0.05) with the MPA levels, but MPA did not differ (P > 0.05) from the MON. Nitrogen retention (NR, g day^{-1}) increased (P < 0.05) with the MPA levels due to the linear increase of N intake (NI) and digested nitrogen (DN). For the diet with 27.6 mg kg^{-1} MPA, the DWG decrease occurred due to the lower digestible energy intake and microbial protein synthesis efficiency.*

***Keywords*— Growth Promoter, Performance, Phytogenic Additive, Prosopis Juliflora, Rumen Fermentation.**

I. INTRODUCTION

The efficiency of energy and protein utilization in ruminants can be improved by manipulating the rumen microbial population or by the digestion process [1-2]. Ionophore antibiotics are classic supplements in diets showing proven effectiveness in increasing feeding efficiency and reducing the incidence of disorders digestive in diets with high concentrate content [3-4]. For goats maintained in confinement, dose of monensin at 20 mg kg^{-1} in the feed, recommended by manufacturers, is indicated only for the prevention and control of coccidiosis and the dose of 11 mg kg^{-1} of feed for three weeks or 8 mg kg^{-1} of body weight for five days showed no signs of hepatotoxicity [5].

However, many countries have banned the use of antibiotics as growth promoters as a food safety precaution against the occurrence of antibiotic residues in the carcasses [6-7]. In this sense, the use of new technologies is being investigated based on the premise of public health and sustainably increased productivity.

Vegetable secondary compounds extracts, when included in the diet, can affect a wide range of rumen microorganisms [8, 4, 9, 10]. *In vitro* studies carried out with extracts from *Prosopis juliflora* (Sw.) DC (mesquite) pods or leaves have demonstrated the bactericidal potential and antimethanogenic [11-12]. Also, bioactive actions as fungicide, nematicide, and cytotoxic [13-24]. These properties were attributed to the presence of piperidine alkaloids with the greatest pharmacological importance, such as juliprosopine and juliprosine [12].

Mesquite piperidine alkaloids (MPA) are amphoteric molecules of character basic, acting as blockers of Ca^{2+} channels (proteins binding calcium, CaBPs) or as inhibitors of acetylcholinesterase (AChE) in eels and tick [13, 25]. The first action can inhibit sensitive strains to antibiotics similar to ionophores [26]. The binding of piperidine alkaloids to these membrane CaBPs could affect the transport and storage of Ca^{2+} in the bacterial cell, reducing its survival [27]. The second action is a consequence of pH-dependent interactions that prosopine, juliprosopine, and juliprosinine had at the active site of the AChE, mainly relating to hydrogen bonds and cation-pi interactions [13].

The MPA showed positive effects on *in vitro* rumen fermentation products and improved the performance without affecting health in sheep fed diets with levels ranging from 2.3 to 31.5 mg kg^{-1} DM [11-12, 28-29]. Because of the scarcity of information with goats, the present study aimed to evaluate the effect of MPA levels (0; 9.2; 18.4; 27.6 mg kg^{-1}) compared to sodium monensin in diets with high concentrate content for growing goats on nutrient intake, digestibility, growth performance, rumen microbial synthesis, and nitrogen balance.

II. MATERIALS AND METHODS

2.1 Ethical Considerations, management, treatments and diets

All the animal care and handling procedures were approved by the Ethics Committee on Animal Use of the State University of Southwest Bahia (UESB), with protocol number 23/2013.

This experiment was conducted in the goat's farming sector of UESB, Itapetinga Campus, State of Bahia, Brazil. Thirty Anglo-Nubian crossbred goats, 120 days of age, and initial body weight 21.82 ± 0.11 kg were housed in individual (1.5 × 1.0 m) stalls equipped with feeders and drinkers and provided free access to water, feed *ad libitum*.

Animals were distributed in a completely randomized experimental design, with five diets: Diet 1: sodium monensin (MON) 2.7 mg kg^{-1} DM; Diet 2: No additives; Diet 3: enriched mesquite piperidine alkaloid extract (MPA) 9.2 mg kg^{-1} DM; Diet 4: MPA 18.4 mg kg^{-1} DM and Diet 5: MPA 27.6 mg kg^{-1} DM and six animals/treatment. The acclimation period lasted 14 days for adaptation to roughage: concentrate ratio of 20:80 and more 14 days for adaptation to experimental diets. The experimental period was 89 days divided into three sub-periods for sample collection over 5 days. The total dose of monensin was approximately 278 mg kg^{-1} of feed DM per 103 days.

TABLE 1
INGREDIENTS OF THE DIETS AND CHEMICAL COMPOSITION OF TIFTON 85 HAY (BERMUDA GRASS) AND CONCENTRATE

Item	(g kg^{-1} DM)	
Tifton 85 hay	205.3	
Milled corn	202.8	
Soybean meal	571.8	
Mineral salt[1]	20.6	
TDN[2]	755.0	
ME (MJ kg^{-1} DM)[3]	11.8	
Chemical composition (g kg^{-1} DM)	Tifton 85 hay	Concentrate
Dry matter (g kg^{-1} NM)	830.5	879.1
Organic matter	944.4	951.1
Crude protein	97.9	189.3
Neutral detergent fiber corrected for ash and protein	761.3	208.9
Acid detergent insoluble protein	498.5	154.7
Ether extract	31.9	34.1
Non-fiber carbohydrates	36.5	496.9
Hemicellulose	346.2	177.7
Cellulose	356.6	66.1
Lignin	168.6	11.7

[1] 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.).

[2] Total digestible nutrients and [3]Metabolizable energy were calculated on tabulated values for individual feed ingredients according NRC (2007).

The diets were formulated following the recommendations of the National Research Council [30] to meet the nutritional requirements of growing goats with an estimated weight gain of 180 g day^{-1}. The diets were supplied twice a day, at 0600 h and 1600 h, with a ratio of 20% Tifton 85 hay and 80% concentrate (total mixed ration, TMR). Tifton-85 hay was used as roughage and chopped to 5.0 mm. Samples of the ingredients and formulated diets were collected and examined for analysis of their chemical compositions (Table 1). The concentrate portion of the diet was a combination of milled corn, soybean meal, and mineral mixture.

2.2 Obtaining enriched mesquite piperidine alkaloid extract

Mature pods of *Prosopis juliflora* (SW) D.C. were obtained from located in the Brumado municipality, Bahia State, being manually harvested from June to July 2017. The pods were sun-dried for three days consecutive and then processed in a Wiley knife mill (A. H. Thomas, Philadelphia, PA, USA) with a 1-mm sieve. 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 45°C for obtaining the crude ethanol extract (CEE). The CEE was partitioned using acid-base solutions and organic solvents according to the methodology of [31].

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 sulfate (Na_2SO_4), homogenized, and allowed to stand for 2 hours.

Next, the BAS-III containing the piperidine alkaloids was transferred to a round bottom flask after filtration, and chloroform was evaporated on a rotary evaporator at 57°C to produce the solid basic chloroform extract (BCE) of piperidine alkaloids from mesquite [32]. The BCE analysis by HPLC-MS identified juliprosopine ($C_{40}H_{76}N_3O_2$ $[M+H]^+$, MM = 630.54) as major constituent and juliprosinine ($C_{40}H_{72}N_3O_2$ $[M+H]^+$, MM = 626.49) as minor constituent. The BCE was weighed and added to the concentrate feed to obtain the 9.2, 18.4, and 27.6 mg kg^{-1} diet DM. The levels of enriched mesquite piperidine alkaloid extract (MPA) used were based on *in vivo* experiments with lambs where concentrations in the diets ranged from 2.3 to 31.5 mg kg^{-1} DM.

2.3 Sample processing and laboratory analyses

Chemical analyses of the ingredients, experimental diets, residual feed, and feces were carried out according to the analytical procedures of the Association of Analytical Communities [33] following the grinding of the samples in Wiley knife mills (A.H. Thomas, Philadelphia, PA) with a 1-mm sieve. The dry matter (DM, method 967.03), crude protein (CP, method 981.10), ether extract (EE, method 920.29), ash (method 942.05), and total nitrogen contents were determined.

The levels of neutral detergent fiber (NDF), samples were treated with thermostable alpha-amylase, without the use of sodium sulfite and corrected for residual ash according to the methodology proposed by [34]. For the determination of acid detergent lignin (ADL), the ADF residue was treated with 72% sulfuric acid based on the methodology described by [35] in which ADF residue was obtained by sequential analysis. The hemicellulose content was determined as the difference between NDF and ADF, and the cellulose content was determined as the difference between ADF and ADL. The levels of nonfibrous carbohydrates were calculated according to the methodology proposed by [36].

The total digestible nutrients (TDN), digestible (DE), and metabolizable energy (ME) contents to formulate the diet was estimated according to equations described by [37]. The TDN obtained in the digestibility assay was calculated according to [38]. The TDN values were converted into DE and ME, using the equations suggested by [37].

2.4 Evaluation of intake, digestibility, and live weight gain

The daily individual intakes (DMI) were measured over 89 days of supply of the experimental diets, by subtracting the amount of feed offered and refused. The amount of feed was adjusted daily, with the acceptable refusal amount corresponding to 10% of the total amount supplied to ensure *ad libitum* intake. The animals were weighed at the start, every 29 days, and at the end of the experiment. At the beginning of the experimental period, the animals were subjected to a 16-h solid fast and weighed to determine initial body weight (IBW). Total weight gain (TWG) was estimated as the difference between final body weight (FBW) and initial body weight (IBW): TWG = (FBW - IBW). Average daily gain (ADG) was calculated by dividing TWG by the total number of days in the experiment: ADG = TWG/Days in feedlot. Finally, feed conversion ratio was calculated as the ratio between dry matter intake (kg day^{-1}) and TWG (kg day^{-1}).

Apparent total digestibility of nutrients was estimated by total collection of feces during 3 days for each range of 25 days, in growing goats with a fecal bag attached to them. The feces were sampled after homogenization. A composite sample of feces, based on dry weight, from collection days for each animal and sub-period was prepared for chemical analysis. During the total tract digestibility trial representative samples of supplied hay and concentrate, and residual feed was collected within 3 days and composed for each animal and sub-period for chemical analysis. The nutrient digestibility coefficients (DC) were calculated as proposed by [39], as the ratio between the ingested amount of a nutrient and its excretion in the feces: DC (%) = ((Nutrient intake, in g – nutrient excreted in the feces, in g) / Nutrient intake, in g) × 100.

2.5 Microbial protein synthesis and N balance

On the 25th day of each experimental sub-period, urine was collected as a urine spot sample by spontaneous urination from each animal, approximately 4 h after the morning meal [40]. These samples were intended for the quantification of the urinary concentrations of urea, creatinine, and uric acid, using commercial kits of Bioclin® (Delft, the Netherlands). Xanthine-hypoxanthine and allantoin were determined according to procedures described by [41]. The urine volume of each animal was estimated with daily creatinine excretion (DCE) divided by creatinine concentration determined in urine spot samples. DCE in the different experimental diets was obtained in another assay with five animals of the same genetic group, in metabolic cages, distributed in a 5 × 5 Latin square, and feeding the same experimental diets. The values used were 16.03, 14.05, 17.59, 18.4, 21.26, and 14.87 mg kg^{-1} BW, respectively for MON, 0, 9.2, 18.4, 27.6 mg kg^{-1} MPA diets.

The amount of absorbed microbial purines (mmol day^{-1}) and the intestinal flow of microbial nitrogen (g MN day^{-1}) were estimated from the excretion of total purines (mmol day^{-1}), using equations proposed by [42], for growing goats. The microbial efficiency was obtained by dividing the microbial protein synthesis (g day^{-1}) by the intake of TDN (kg day^{-1}) [43-44, 37].

2.6 Statistical analysis

Data analysis was performed using the GLM procedure of SAS statistical software version 9.1 (SAS Institute, Inc. Cary, NC). The average data from the three sampling sub-periods were used for apparent digestibility, microbial protein synthesis, and nitrogen balance, and the normality of variance was verified. The intake data for each animal were obtained by dividing the total ingested nutrients amount by the days of the experiment duration (89 days).

The contrast was applied to compare the means observed between the diets with MON *versus* MPA concentrations. The polynomial contrasts were performed for the linear (L) and quadratic (Q) components. For the dependent variables whose polynomial contrasts were significant, regression analysis of linear (L) or quadratic (Q) effects was performed according to

the MPA concentrations in diets (0; 9.2, 18.4, and 27.6 mg kg^{-1} DM). The critical level of significance adopted was $P < 0.05$ and for tendency was $0.05 < P < 0.10$.

III. RESULTS

3.1 Evaluation of intake, digestibility, and live weight gain

The intakes of dry matter (DM), organic matter (OM), and neutral detergent fiber corrected for ash and protein (NDF_{ap}) were not affected ($P > 0.05$) by levels of enriched mesquite piperidine alkaloid extract (MPA) in diets. There was a tendency ($P = 0.051$) to reduce the NDF_{ap} (g kg^{-1} BW) in diets with MPA compared to the diet with monensin (MON) (Table 2).

The levels of MPA increased 0.086 g kg^{-1} $BW^{0.75}$ the crude protein (CP, $P = 0.0004$) intake for each unit of MPA inclusion, which showed lower ($P = 0.004$) mean (10.64 g kg^{-1} BW from 9.2 to 27.6 mg kg^{-1} MPA) than MON diet (11.57 g kg^{-1} BW). The intake of non-fiber carbohydrates (NFC, $P = 0.035$) increased 0.083 g per kg $BW^{0.75}$ for each unit of MPA inclusion in the diets, and the mean (9.2 to 27.6 mg kg^{-1}) MPA was similar ($P > 0.10$) to the MON diet.

There was a quadratic response for the intakes (g day^{-1}) of ether extract (EE, $P = 0.023$), total digestible nutrients (TDN, $P = 0.008$), digestible (DE, $P = 0.008$) and metabolizable energy (ME, $P = 0.007$), with a maximum at 10.5, 15.3, 17.3, and 17.4 mg kg^{-1} MPA, respectively. These variables did not differ from MON (Table 2).

There was no difference ($P > 0.10$) among diets with MPA when compared to MON for the digestibility of DM, OM, CP, and NDF_{ap}. For EE, MPA diets provided the highest mean compared to MON diet ($P = 0.001$) and quadratic effect ($P = 0.034$) with the MPA levels.

The CP and NFC digestibilities increased with the MPA levels showing an increment of 0.22 g 100 g^{-1} ($P = 0.003$) and 0.14 g 100 g^{-1} ($P = 0.040$) for each unit of MPA inclusion in the diet, respectively (Table 3). The average CP digestibility did not differ ($P > 0.10$) between diets with MPA and MON and the NFC digestibility tended to be higher for diets with MPA ($P = 0.093$).

Diets supplemented with MPA changed the daily weight gain (DWG) in growing goats ($P = 0.044$) with the maximum point at 14.8 mg kg^{-1} MPA, showing a 0.2 g reduction for each unit of MPA inclusion after the peak. The average DWG of growth goats fed diets supplemented with MPA did not differ from the diet with MON, and both additives promoted a 28% increase in DWG compared to diet without additives (Table 3). The feeding efficiency (FEF) tended ($P = 0.067$) to be maximum with 15.6 mg kg^{-1} MPA in the diet.

3.2 Microbial protein synthesis and N balance

There were no differences ($P > 0.10$) among diets containing MPA compared to the diet with MON for the synthesis of microbial nitrogen (Table 4). But, the efficiency of microbial protein synthesis (MEF) showed a decreasing linear effect ($P = 0.043$), in which the mean for diets with MPA was equal to 87.35 g kg^{-1} of TDN. However, there was no difference ($P > 0.10$) between MPA and MON.

There was a linear increase of the nitrogen intake (NI, $P = 0.022$) and of the amount of digested N (DN, $P = 0.017$) with the MPA levels and both variables did not differ from the MON diet. The excretion of N in the feces (FN) varied quadratically ($P = 0.025$) with a minimum point at 13.3 mg kg^{-1} MPA. The urinary N (UN) was no changed ($P > 0.10$) according to the MPA levels and it was greater ($P = 0.038$) for MPA diets than the diet with MON (Table 4). The plasma urea N (PUN) concentration peaked ($P = 0.019$) at 16.9 mg kg^{-1} MPA. There was an increase ($P = 0.006$) in the amount of retained N (RN) with MPA levels and the diets with MPA were lower ($P = 0.040$) than the MON diet.

The proportion between the amounts of RN and N intake (g RN 100 g^{-1} NI) tended ($P = 0.057$) to be lower for MPA diets in comparison with MON diet. The RN to digested N ratio (g RN 100 g^{-1} DN) was lower ($P = 0.020$) for MPA diets compared to MON diet (Table 4). However, there was an improvement ($P = 0.043$) in the conversion of RN in DWG (g 100 g^{-1}) with the MPA in comparison with MON diet.

TABLE 2

INTAKE AND DIGESTIBILITY OF NUTRIENTS IN GROWING GOATS FED DIETS WITH CONCENTRATIONS OF ENRICHED MESQUITE PIPERIDINE ALKALOID EXTRACT (MPA) OR MONENSIN (MON)

Item	Experimental diet (mg kg^{-1} DM)					*SEM*	*P*-Value		
	MON	MPA							
	2.7	0	9.2	18.4	27.6		MON *vs* MPA	L	Q
DM (kg day^{-1})	0.91	0.77	0.86	0.90	0.86	0.02	0.497	0.087	0.053
OM	0.88	0.74	0.77	0.84	0.75	0.03	0.252	0.772	0.402
CP	0.15	0.11	0.12	0.15	0.14	0.004	0.260	0.002[1]	0.095
EE	0.03	0.03	0.03	0.03	0.02	0.001	0.977	0.185	0.023[2]
NDFap	0.28	0.23	0.22	0.26	0.22	0.006	0.011	0.847	0.222
NFC	0.38	0.31	0.37	0.38	0.39	0.009	0.977	0.005[3]	0.201
TDN	0.64	0.56	0.65	0.66	0.63	0.01	0.909	0.037	0.008[4]
DE (MJ day^{-1})	11.88	10.13	12.05	12.18	11.67	0,06	0,909	0,037	0,008[5]
ME	10.29	8.79	10.54	10.63	10.17	0.05	0.801	0.033	0.007[6]
DM (g kg^{-1} BW)	30.22	27.76	29.36	30.32	29.26	0.69	0.719	0.231	0.161
NDF_{ap}	9.31	8.23	7.41	8.98	7.80	0.23	0.051	0.857	0.635
CP (g kg^{-1} $BW^{0.75}$)	11.57	8.87	9.55	11.53	10.84	0.28	0.004	0.0004[7]	0.151
EE	2.05	2.25	2.43	2.28	1.88	0.80	0.448	0.116	0.081
NFC	29.35	26.08	29.84	29.38	30.54	0.71	0.743	0.035[8]	0.324
TDN	49.69	45.43	51.31	51.98	50.11	0.10	0.547	0.069	0.018[9]

$^{1}Y = 0.11 + 0.0013\ X$; $^{2}Y = 0.027 + 0.00063\ X - 0.00003\ X^2$; $^{3}Y = 0.32 + 0.0028\ X$; $^{4}Y = 0.56 + 0.0122\ X - 0.0004\ X^2$; $^{5}Y = 10.19 + 0.2497\ X - 0.0072\ X^{26}Y = 8.85 + 0.2261\ X - 0.0065\ X^2$; $^{7}Y = 9.01 + 0.0858\ X$; $^{8}Y = 29.09 + 0.0834\ X$; $^{9}Y = 45.56 + 0.7917\ X - 0.0229\ X^2$

NDFap, Neutral detergent fiber correct for ash and protein; NFC, Non-fiber carbohydrates; TDN, Total digestible nutrients; DE, digestible energy; ME, metabolizable energy; SEM, standard error of the mean; L, linear; Q, quadratic.

TABLE 3

DIGESTIBILITY OF NUTRIENTS AND PERFORMANCE OF GROWING GOATS FED DIETS WITH CONCENTRATIONS OF ENRICHED MESQUITE PIPERIDINE ALKALOID EXTRACT (MPA) OR MONENSIN (MON).

Item	Experimental diet (mg kg^{-1} DM)								
	MON	MPA				*SEM*	*P*-Value		
	2.7	0	9.2	18.4	27.6		MON *vs* MPA	L	Q
Apparent digestibility (g 100 g^{-1} DM)									
DM	85.11	83.13	82.84	80.65	85.59	0.85	0.275	0.414	0.136
OM	85.15	81.02	88.02	83.19	83.57	1.14	0.815	0.807	0.204
CP	82.77	77.75	79.60	79.49	84.08	0.80	0.341	0.003[1]	0.429
EE	65.45	68.54	72.24	74.75	71.75	0.82	0.001	0.067	0.034[2]
NDFap	66.65	64.34	68.12	69.14	69.06	1.20	0.567	0.196	0.406
NFC	83.85	83.62	85.05	88.37	86.81	0.66	0.093	0.040[3]	0.285
TDN	69.74	72.93	76.79	74.78	72.45	9.16	0.065	0.691	0.158
Growth performance (kg)									
IBW	22.56	21.98	21.92	21.78	21.92	-	-	-	-
FBW	37.74	33.07	37.36	37.26	36.03	0.93	0.742	0.354	0.196
TWG	15.18	11.09	15.44	15.47	14.11	0.71	0.926	0.190	0.070[4]
DWG	0.171	0.125	0.173	0.174	0.159	0.01	0.944	0.140	0.044[5]
FEF (g 100 g^{-1})	21.44	19.36	24.16	23.88	21.72	0.84	0.429	0.412	0.067[6]

[1]Y= 77.52 + 0.2199 X; [2]Y= 68.41 + 0.6397 X – 0.0186 X^2; [3]Y= 84.02 + 0.140 X; [4]Y = 11.24 + 0.5643 X – 0.0169 X^2; [5]Y= 0.127 + 0.0059 X – 0.0002 X^2; [6]Y = 19.52 + 0.6413 X – 0.0206 X^2

IBW, Initial body weight; FBW, Final body weight; TWG, Total weight gain; DWG, Daily weight gain; FEF, feeding efficiency: daily weight gain ÷ digestible dry matter intake; SEM: standard error of the mean; L, linear, Q, quadratic.

TABLE 4

NITROGEN BALANCE AND MICROBIAL SYNTHESIS IN GROWING GOATS FED DIETS WITH CONCENTRATIONS OF ENRICHED MESQUITE PIPERIDINE ALKALOID EXTRACT (MPA) OR MONENSIN (MON).

Item	Experimental diet (mg kg^{-1} DM)								
	MON	MPA				*SEM*	*P*-value		
	2.7	0	9.2	18.4	27.6		MON *vs* MPA	L	Q
Microbial nitrogen (g day^{-1})	8.01	9.35	8.92	10.47	7.79	0.35	0.296	0.333	0.123
MEF (g CP kg^{-1} TDN)	80.85	111.2	84.75	98.87	78.42	3.83	0.702	0.043[1]	0.534
Nitrogen intake (NI, g day^{-1})	24.37	17.35	19.72	23.75	22.11	0.86	0.240	0.022[2]	0.245
Nitrogen excretion (g day^{-1})									
Feces	4.30	4.10	5.16	5.70	3.79	0.29	0.454	0.883	0.025[3]
Urine	1.22	3.23	2.00	2.85	2.16	0.29	0.038	0.751	0.865
Plasma urea nitrogen (mg dl^{-1})	14.79	9.43	13.12	14.18	11.97	0.65	0.416	0.150	0.019[4]
Digested nitrogen (DN)									
g day^{-1}	20.07	13.25	14.56	18.05	18.32	0.83	0.138	0.017[5]	0.752
g 100 g^{-1} NI	81.44	75.03	63.36	74.88	82.46	1.85	0.069	0.004[6]	0.725
Retained nitrogen (RN)									
g day^{-1}	18.85	10.02	12.56	15.20	16.17	0.87	0.040	0.006[7]	0.619
g 100 g^{-1} NI	75.56	62.50	72.65	63.67	72.96	1.78	0.057	0.136	0.898
g 100 g^{-1} DN	93.86	86.10	84.49	87.02	88.35	1.20	0.020	0.427	0.564
RNC (g 100 g^{-1})	11.83	9.21	7.21	8.83	10.13	0.57	0.043	0.168	0.131

[1]Y= 105.94 – 0.9154 X; [2]Y= 17.99 + 0.199 X; [3]Y= 4.06 + 0.236 X – 0.0089 X^2; [4]Y= 14.02 + 0.199 X; [5]Y= 13.24 + 0.2033 X; [6]Y= 68.86 + 0.368 X; [7]Y= 10.32 + 0.229 X

MEF, Microbial efficiency; CP, microbial crude protein; TDN, total digestible nutrients intake; RNC, retained nitrogen conversion: retained nitrogen ÷ daily weight gain; SEM, standard error of the mean; L, linear; Q, quadratic

IV. DISCUSSION

The supplementation of the diets with MON or MPA did not affect the DM intake. However, both additives increased the intakes of CP, NFC, TDN, DE, ME and the supplementation with MON increased the NDF_{ap} intake, indicating selective behavior. The MPA diet increased CP, EE, and NFC digestibilities resulting in greater TDN intake than the diet without additives.

The maintenance of the digestibility of DM and OM in diets with MON or MPA levels may have been the factor that contributed to the similarities in DM and OM intakes of diets with additives. On the other hand, when considering the diet without additives, the similar response for DM intake did not correspond to the composition of the ingested diets with MON or MPA due to feed selection. The use of a high concentrate proportion may cause digestive disorders, resulting in decreased intake, a fact that did not occur since the DM intake was reached due to the adaptation of the animals to the roughage and concentrate ratio (20: 80).

The polynomial contrast showed a significant quadratic component of the effects of MPA levels on the EE digestibility, consistent with the effect on its intake. Likewise, the linear increase in the digestibility of CP and NFC with the use of MPA levels reflects the increased intake of these nutritional components. The intake composition expresses as TDN and protein ratio (g kg^{-1} of diet DM) was: MON = 4.2; without additive = 5.1; MPA 9.2 mg kg^{-1} = 5.5; 18.4 mg kg^{-1} = 4.5, and 27. 6 mg kg^{-1} = 4.4. The higher intake of CP and NFC indicates that there was a change in the roughage and concentrate ratio of the diet consumed, which justifies the greater intake and digestibility of these components. The variation in the composition of the consumed diet is probably due to the ability of goats to select during prehension [45]. [28] observed that MPA at 9.2 mg kg^{-1} increased the utilization of NFC in lambs fed diets with MPA levels (2.3; 4.6 and 9.2 mg kg^{-1} of diet DM) composed of 60 % concentrate and 40 % roughage.

The fact that MON and MPA did not affect the NDFap digestibility, associated with the stable DM and OM intake and digestibility indicates that the amounts used of the additives maintained the conditions for fiber degradation in the rumen. This is an important aspect of evaluating an additive, especially using low-quality roughage. Thus, it indicates responses quite similar to monensin, which are maintenance of rumen pH more favorable to cellulolytic bacteria and amylolytic bacteria that provide degradation products used by gram-negative cellulolytic bacteria [2, 46-47].

In ruminants fed high concentrate diet, the fiber digestibility has often been increased by the inclusion of additives, and this increase may be the result of changes in the microbiome activity and/or the longer retention of fiber in the rumen, which favors the extension of microbial digestion [2]. Conversion of rumen digested energy to microbial protein is less efficient with reduced rumen turnover, but the increased extent of rumen digestion may partially compensate to maintain microbial protein output from the rumen [48].

The estimated CP intake for growing goats with average body weight (BW) of 25 kg is approximately 0.115 kg day^{-1}, with an estimated daily gain of 180 g [30]. In diets with MON and MPA at 18.4 mg kg^{-1}, there was an average daily CP intake of 0.150 kg, and in growing goats showed similar daily weight gain (DWG). For the diet containing MPA at 9.2 mg day^{-1}, the daily intake of CP was 0.120 kg and the goats also reached similar DWG. This response showed that the digestion and/or utilization of diet protein depend of the MPA dose and the minimum concentration effective of MPA for maximum DWG was 9.2 mg kg^{-1}.

In contrast, the diet without additives was no efficient, causing a CP intake of 0.110 kg day^{-1} with DWG of 0.125 kg, although the ME intake was closer to the recommended by [30]. The ME requirement recommended is 7.82 MJ day^{-1} by the [30] for crossbred kids goats with 25 kg BW and an estimated daily gain of 180 g.

Commonwealth Scientific and Industrial Research Organisation (CSIRO) defined the potential for food intake as the amount of food eaten when offered *ad libitum*, and the animal can select a diet with a minimum DM digestibility of 80 % or with a minimum concentration of 11 MJ kg^{-1} of DM [49]. In this study, it was observed the average value of DM digestibility above 80%, and the ME content, which averaged 11.25 MJ kg^{-1} for diets with MPA. Consistently, there was a linear increase of the CP and NFC intakes with MPA levels indicating diet selection, probably, as a consequence of a change in the rumen digestion [50].

The feeding efficiency (FEF) tended to achieve the peak at approximately 15 mg kg^{-1} MPA and the higher daily weight gain (DWG) at this point confirms the potential use of MPA as an additive for growing goats fed the diet with a high concentrate proportion.

In this study, the response of DWG was due to the use of additives, mainly as a reflection of the improvement in the intake of digestible and metabolizable energy from diets. It was demonstrated that the addition of MPA was effective to increase DWG in goats consuming 20% Tifton 85 hay. There was a plateau for DWG between 9.2 and 18.4 mg kg^{-1} MPA levels, demonstrating that this range of MPA inclusion in diets promotes the best response in growing goats.

The 27.6 mg kg^{-1} MPA did not promote an increase in DWG, probably in response to factors affecting the digestive processes that changed the energy and crude protein intakes. [13] reported that juliprosinine and juliprosopine interact with catalytic site residues of enzymes in pH 8.0, however, the rumen and gut have a lower pH than this resulting in less stable interactions. The lower pH could favor the action of piperidine alkaloids of molecular mass above 600 Da to act as an ionophore in the membrane of gram-positive bacteria and not of gram-negative bacteria which present the outermost membrane barring the access of ionophores to the plasmatic membrane [11, 51].

The MPA ranging from 9.2 to 18.4 mg kg^{-1} tended to increase the feeding efficiency (FEF). [28] reported that MPA (2.3 to 9.2 mg kg^{-1}) improved the feed conversion compared to MON in lambs fed diets composed of 40% Tifton 85 hay and 60% concentrate. Hence, the authors suggested more studies with higher concentrations would be promising.

In high concentrate diets, MON generally maintains or increases the weight gain and improves feed conversion [52]. In this study, even though there was a tendency to increase the FEF in growth goats fed diets with MPA levels, it was found that the increased DWG with the same DM intake as a consequence of the improvement in the energy and protein utilization of the diets.

For MON and MPA at 27.6 mg kg^{-1}, there was a higher amount of dietary retained nitrogen (RNC) for every 100 g of weight gain. Possibly, the largest fraction of the amount of retained N was used in other metabolic routes that use amino acids than the protein synthesis to be deposited in the gain of BW due to the ME intake does not to follow the increase of CP intake. Among the major factors affecting the post-rumen N efficiency is the amount of energy available [53]. Increasing the supply of post-rumen energy substrates while maintaining protein supply improves the efficiency of utilization of amino acid for gain; and thus reduces the output of N in urine [54].

There was a decrease in the concentration of plasma urea nitrogen (PUN) and the feces N excretion reduced in the highest level of MPA, without observing a change in the excretion of N in the urine. Thus, the increase in the amount of digested N and retained N showed that probably there was a reduction in the degradation of dietary protein in the rumen, and greater use of dietary amino acids in the intestine with 27.6 mg kg^{-1} MPA [55]. [56] reported that monensin decreased bacterial N and more dietary protein reached the abomasum of steers adapted to monensin. Other reports support a higher ruminal escape effect of dietary protein by monensin [57; 2].

The MPA at 27.6 mg kg^{-1} showed an effect of less protein degradation in the rumen, due to the lower PUN value, and the diets with MPA at 9.2 and 18.4 mg kg^{-1} were more efficient in the synthesis of microbial protein, consistent with that observed by [28] using the maximum level of 9.2 mg kg^{-1} MPA for sheep. The increase in microbial efficiency allows an increase in the availability of microbial protein to be absorbed in the intestine, supplying, thus, the requirements of growing animals [58]. The higher microbial efficiency of diets with MPA at 9.2 and 18.4 mg kg^{-1} compared to 27.6 mg kg^{-1} may have contributed to the higher DWG in growing goats fed with the first two diets.

The MON diet provided a rise of 33% in retained nitrogen (RN) when compared with the 9.2 mg kg^{-1} MPA diet, however, both showed similar DWG. [28] observed that the N intake was not changed in lambs, as it was consistent with similar CP levels in the diets. At that study, for the diet with MPA in the highest dose of 9.2 mg kg^{-1}, the effect of greater feces N excretion caused a reduction in the proportion of retained N relative to ingested N. This finding was supported in the present study, which was noted a tendency of decrease in the RN proportion (g 100 g^{-1} NI) in MPA diets compared to MON diet.

The RN proportional to the ingested N can predict the efficiency of the use of N in the animal organism [59]. It is more strongly associated with the supply of N than with the energy content of the diet, and it is amplified by the improvement in the conditions of protein status in the animal organism [60]. There was a linear increase in the N intake with the MPA levels, consequently, the DN and RN proportional to the ingested N also increased. In addition, these variables did not differ from MON diet. The DN proportional to the N intake was similar between the MON diet and 27.6 mg kg^{-1} MPA diet, indicating that both take action equally in the protein digestion.

The protein status refers to the qualitative and quantitative availability of nitrogen compounds for all physiological functions in tissue metabolism, including functions associated with energy metabolism [60]. However, the RN proportional to the

digested N and the RN conversion (RNC) was lower with MPA at 9.2 mg kg^{-1} than with MON in the diet, possibly, due to the dose-response effect to the MPA. However, both additives were comparables to nitrogen excretion (feces and urine) and DWG. The 18.4 mg kg^{-1} MPA increased the N intake and in comparison with MON, it showed higher urinary N excretion, a tendency to reduce RN proportional to the ingested N, and similarity in DWG. In contrast, the 27.6 mg kg^{-1} MPA increased the N intake, digested nitrogen, retained nitrogen and it reduced the microbial efficiency and also DWG.

Probably, in the rumen environment, the additives performed as modulators of the fermentation with metabolic routes that produce less methane and deamination of amino acids resulting in lower energy loss or spilling energy [1]. The effectiveness of an additive is measured using productive and metabolic parameters. The monesin reduces protein degradation with the decrease of rumen N-NH_3 concentrations and increases the intestinal digestion of dietary protein [2, 61]. As long as it does not compromise the efficiency of microbial synthesis, it can be beneficial, since the excess of rumen N-NH_3 has a high energy cost in the transformation of urea in the liver [62], reducing the diet energy yield [63]. Also, the reduction in rumen concentration of N-NH_3 can occur due to the availability of energy in the rumen, which allows greater use of ammonium for microbial growth, with a consequent reduction in urine urea loss [59].

The excretion rates of nitrogen compounds in the urine and feces of ruminants are associated with the amount of DN [64]. [54] related that the variation in dietary N intake will particularly affect the excretion of urinary N, which is much more vulnerable to losses than is fecal N. The quantity of N excreted in urine varies widely. Urinary N excretion, in particular, that of urea N, is decreased upon reduction of dietary N intake or an increase in the supply of energy to the rumen microorganisms and to the host animal itself.

The 27.6 mg kg^{-1} MPA showed a reduction in the concentration of PUN possibly due to the action to limit available ammonia for bacterial growth as a consequence of increased escape of dietary protein from the rumen. Hence, the greater N intake has resulted in a value like MON for retained nitrogen conversion per 100 g weight gain (RNC). Possibly, the growth goats fed the diet with MPA at 27.6 mg kg^{-1} showed lower DWG response than MON and 9.2 to 18.7 mg kg^{-1} MPA as a result of a decrease in DE and ME intake. On the other hand, the lower dose of MPA showed better efficiency of energy and protein utilization due to greater microbial efficiency and daily weight gain with lower CP intake. Consistently, [29] proposed a CP reduction, from 16% to 13%, in the diet for lambs fed diet constituted of 33.3% Buffelgrass hay and 66.7% concentrate with 31.5 mg kg^{-1} MPA due to the rise of microbial efficiency in the rumen.

V. CONCLUSIONS

The doses of MPA between 9.2 to 18.4 kg^{-1} of feed dry matter for growing goats improve metabolizable energy intake and daily weight gain with the lower conversion of retained nitrogen than MON. To achieve a better description of the action mechanism of main alkaloids obtained from the alkaloid-rich fraction (juliprosopine and juliprosinine), additional studies on enzyme activity in the rumen and small intestine and microbial population are required.

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