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Intake, Digestibility, and Rumen Metabolism of Feedlot Lambs Supplemented either Monensin or Increasing Doses of Copaiba (*Copaifera* spp.) Essential oil

Research Article

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ABSTRACT

This trial aimed to evaluate increasing levels of copaiba oil on dry matter intake and digestibility, rumen fermentation, microbial protein synthesis, and thermal regulation in lambs. Ten lambs (32.4±2.86 kg body weight and 6.1±0.4 months of age) were assigned to two concurrent 5 × 5 latin square design trials, in which the following treatments were randomly distributed to lambs: control (CON), a basal diet with no feed additives; basal diet plus monensin (MON, 25 mg/kg dry matter (DM)); and the basal diet pluscopaiba oil (CO), added at 0.5, 1.0, and 1.5 g/kg DM. The supplementation of copaiba oil (CO) did not influence the intake and digestibility of dry matter and nutrients. A quadratic effect was observed for the concentrations of propionate for lambs supplemented with CO. Lambs fed 1.0 g/kg DM of CO had a higher concentration of purine derivatives and microbial nitrogen and protein compared to lambs fed the ionophore. Copaiba oil (CO) positively influenced rumen fermentation and microbial protein synthesis, without altering dry matter consumption and digestibility. From these results, we suggest the use of 1.0 g/kg DM in growing lambs' diets.

KEY WORDS antibiotic-free, cleaner production, essential oil, feedlot lambs.

INTRODUCTION

An ionophore is a feed additive used in ruminants' diets to improve feed efficiency and animal performance. Ionophores improve fermentation characteristics in the rumen, which leads to improved production efficiency (Russell and Strobel, 1988; Yang and Russell, 1993). The search for natural alternatives to this antibiotic to modulate ruminal fermentation is extremely important within the context of clean animal production and free antibiotics aiming at security in the production of animal protein.

Essential oils are characterized by reducing the rates of amino acid deamination ammonia production, and the number of ammonia-hyperproductive bacteria, together with an increase in the amount of N passing from the rumen into the intestine (Calsamiglia *et al.* 2007). Essential oils consist of substances, whose components include terpenic hydrocarbons, simple alcohols, aldehydes, ketones, phenols, esters, and fixed organic acids, in different concentrations, in which a pharmacologically active compound is in the majority (Simões and Spitzer, 2000). Most of the antimicrobial activity of functional oils appears

to be associated with phenolic compounds. The antimicrobial effect is mainly related to changes in the permeability and integrity of the bacterial cell membrane (Lambert *et al*. 2001).

Copaiba oil (CO) is a natural product extracted by tapping the trunk of copaiba trees (*Copaifera* spp.) which are largely distributed in northern South America, especially in the Brazilian Amazonas rain forest (Veiga Junior and Pinto, 2002). Copaiba oil has been studied as an alternative because it is a natural product and a potential rumen manipulator. It is known that the main groups of active substances in existing plants are: alkaloids, glucosides, phenolic compounds, saponins, mucilages, flavonoids, tannins, essential oils, etc. (Martins *et al*. 2000).

Copaiba oil exhibit's potent antimicrobial activity against several species of gram-positive bacteria, causing cell wall disruption and release of cytoplasmic components (Santos *et al*. 2008). Moura *et al*. (2018) supplemented feedlot lambs with increased levels of CO in the diet and observed better weight gains for inclusion between 0.5 to 1.0 g/kg DM, without affecting dry matter and nutrients intake.

Based on the evidence presented above, we hypothesized that CO levels in the diet improve the digestion and ruminal metabolism of lambs, being able to replace sodium monensin as a modular rumen fermentation with performance equal to or similar to ionophore. This trial aimed to evaluate the benefits obtained from a range of CO inclusion on the intake and digestibility of dry matter and nutrients, rumen fermentation, microbial protein synthesis, and thermal regulation in lambs.

MATERIALS AND METHODS

Animal care

The experimental protocol was reviewed and approved by the Animal Care and Use Committee of the Federal University of Grande Dourados, Dourados, Brazil. This study was conducted at the Animal Science sector of the Faculty of Agrarian Science, University Federal of Grande Dourados.

Animals, treatments, and management

Ten castrated and cannulated lambs (32.4±2.86 kg body weight and 6.1±0.4 months of age) were installed in metabolic cages for a total collection of feces and urine. They were assigned to two contemporary 5 × 5 latin square, design trials, consisting of 19-d periods, with the last 5 d for data record and sampling, were assigned to a randomized design experiment. The five treatments were: 1) control (CON), a basal diet with no feed additives; 2) MON, sodium monensin (Rumensin®, Elanco Eli Lilly and Company, Sao Paulo, Brazil) to the control diet at 25 mg/kg DM; and (3, 4, and 5) CO, the addition of copaiba oil added to the control diet at 0.5, 1.0, and 1.5 g/kg DM, diluted into isopropyl alcohol (7 mL). Diets were formulated according to (NRC, 2007) the needs of growing crossbred Suffok-Santa Inês lambs. The animals were fed a total mixed ration (Table 1). Monensin and the diluted CO were mixed into 100 g of concentrate and supplied before feeding the main diet to ensure the total intake of additives. Copaiba oil is extracted from the trunk of the copaiba tree and is a transparent, yellow to light-brown liquid (Table 2). Copaiba oil was extracted by cold pressing. The chemical composition of copaiba oil (Table 2) was performed using mass spectrometry.

Intake and digestibility

Samples of all diet ingredients and orts from each lamb were collected during the last five days of each period and combined into one composite sample of orts for each animal and one composite sample of hay. Samples were analyzed to determine the dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, lignin, and ash according to AOAC (2000). Total feces were collected on days 15, 16, and 17 of each experimental period from each lamb, and then feces were homogenized and aliquots of 10% (wet basis) were frozen at −20 ˚C until analyses. The total apparent digestibility was calculated through the total collection of feces by metabolic cages.

Ruminal fermentation and microbial protein synthesis

Rumen digesta samples (from five different sites) were collected on day 19 of each experimental period before offering concentrates (time 0), and then at intervals of 2, 4, 6, and 8 h. Rumen digesta samples were composited and strained through four layers of cheesecloth. The pH of the rumen liquor was measured, and the samples were mixed with methanoic acid before centrifuging at 7000 × g for 15 min at 4 ˚C. The supernatant of each sample was then frozen for posterior short-chain fatty acid (SCFA) analysis. Rumen juice aliquots (2 mL) were mixed with sulfuric acid (1 mL; 0.5 N) and stored at −20 ˚C for subsequent analysis of ammonia nitrogen by the colorimetric phenol-hypochlorite method (Broderick and Kang, 1980). Rumen SCFA concentrations were measured using a gas chromatograph (model GC-2104, Shimadzu, Tokyo, Japan) according to the method described by Erwin *et al*. (1961) and adapted by Getachew *et al*. (2002).

The counting of rumen protozoa and the differential counts of ciliated protozoa per milliliter of rumen liquid were performed using the technique described by Dehority (1977), using the Sedgwick-Rafter chamber and an eyepiece with a 0.4323 mm^2 reticulum of area.

Table 1 Percentage and chemical composition of the concentrate and experimental diet (% DM)

Ingredients	Concentrate	Diet
Soybean meal	22.00	10.34
Wheatmeal	21.00	9.87
Cornmeal	53.00	24.91
Tifton 185 hay	-	53.00
Mineral mix[1]	2.00	0.94
Salt	2.00	0.94
Chemical (% DM)		
Dry matter	87.09	88.14
Crude protein	18.16	11.66
Neutral detergent fiber	18.96	49.44
Acid detergente fiber	7.70	23.54
Lignin	3.16	6.42
Fat	2.31	1.47
Ash	7.62	7.06
Net energy (Mcal/kg)	1.82	1.40

[1] Each kg (DM basis) of mineral mix contained: Calcium (min): 111.00 g/kg; Cobalt: 50.00 mg/kg; Sulfur: 11.99 g/kg; Iron: 4.42 mg/kg; Phosphorus (min): 72.00 g/kg; Iodine: 75.00 mg/kg; Magnesium: 9.00 g/kg; Manganese: 1550.00 mg/kg; Selenium: 13.50 mg/kg; Sodium: 174.00 g/kg; Zinc: 7200.00 mg/kg and Fluorine (max): 720.00 mg/kg.

Table 2 Chemical characterization (sesquiterpenes, diterpenes and fatty acids) of copaiba oil

Sesquiterpenes	%
β-cariophylene	9.78
β-bisabolene	8.15
α-humulene	8.08
β-selinene	7.76
α-bisabolol	7.14
β-element	6.19
γ-cadinene	5.98
α-cadinol	5.67
Diterpenes	**%**
Hardwickicacid	5.78
Colavenol	3.03
Copaifericacid	2.99
Copaiferolicacid	2.65
Calavenicacid	2.34
Patagonianacid	2.22
Copalicacid	2.03
Fattyacids	**%**
14:0	1.67
16:0	3.67
18:0	2.98

The counts made in 100 independent fields were performed in duplicate and the average obtained was considered.

Microbial protein synthesis

Total urine was collected on days 17 and 18 of each experimental period. Was collect for metabolic cage by total urine collection. A 10-mL aliquot of the urine was diluted in 40 mL 0.036 N sulfuric acid, and after this process, the pH was adjusted, if necessary, to below 3, by adding droplets of concentrated sulfuric acid. This was done to prevent bacterial destruction of the purine derivatives and precipitation of the uric acid.

Samples were stored at –18 ˚C for later analyses of the purine derivatives allantoin, uric acid, xanthine, and hypoxanthine. The determination of allantoin was described by Young and Conway (1942), cited by Chen and Gomes (1992), and is based on the alkaline hydrolysis at 100 ˚C of the allantoin to allantoic acid, which, later, is converted into urea and glyoxylic acid in acidic solution. The glyoxylic acid then reacts with phenylhydrazine hydrochloride to produce phenylhydrazone from the acid. The product forms an unstable chromogen, with potassium ferricyanide, which can be dosed, colorimetrically, at 522 nm. The method described by Fujihara *et al*. (1987), for the determination of uric acid, is based on the treatment of the urine sample with

uricase, resulting in allantoin, which does not absorb ultraviolet (UV) at 293 nm. Then, the reduction in the absorbance reading at 293 nm was correlated with the concentration of uric acid. To determine xanthine and hypoxanthine, these were converted to uric acid by xanthine oxidase and measured as uric acid at 293 nm, as described by Chen and Gomes (1992). Absorbed microbial purines (X, mmol/d) were calculated from the excretion of purine derivatives (Y, mmol/d), by the following equation:

$$Y= 0.84X + (0.150\ BW0.75\ e–0.25X)$$

Where:
0.84: recovery of absorbed purines as urinary purine derivatives.
0.150 BW0.75 e -0.25X: endogenous contribution to the excretion of purines (Verbic *et al*. 1990).

The intestinal flow of nitrogen compounds (Y, g N/d) was calculated as a function of the absorbed microbial purines (X, mmol/d), using the following equation:

$$Y= (70X) / (0.83\times0.116\times1000)$$

Where:
70: N content in the purines (mg N mmol).
0.83: digestibility of the microbial purines.
0.116: purine N: total bacterial N ratio.

Microbial production was expressed as g microbial N (grams of microbial N) and g microbial P (grams of microbial protein).

Infrared thermal images and heat stress

Infrared thermal images were performed on days 15, 16, and 17 of each experimental period before (time 0) and 2, 4, 6, and 8 hours after the morning feeding using a thermal camera (Testo 880, Brandt Instruments, Prairieville, LA, USA). The anatomical regions assessed by the thermal camera were left and right flanks, rump, and head (Martello *et al*. 2009); (Figure 1).

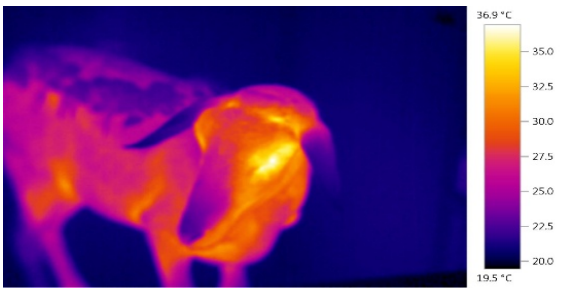

Figure 1 Thermal image of growing lambs

The emissivity value used was 0.98 and images were recorded approximately 1.5 m from the animals (Gomes *et al*. 2016). Total sensible heat loss (Q) was calculated as a function of heat loss by radiation (Qr) and by convection (Qc), as suggested by Yahav *et al*. (2004) and Van Brecht *et al*. (2005), respectively, by using the following equations:

(1) $Q= Qr + Qc$
(2) $Qr= \varepsilon\sigma A\ (Ts4-Tair4)$
(3) $Qc= hA\ (Ts-Tair)$
(4) $h= 0.336 \times 4.184 \times (1.46+\sqrt{}vair-100)$

Where:
Q: total sensible heat (W).
e: lamb emissivity (0.98).
σ: Stefan-Boltzman constant (5.67m-2×10-8, W m-2 K-4).
A: lamb surface area (m2).
h: heat transfer coefficient is given by Eq. 4 (15 W m-2).
Vair: air velocity.
Qr: heat loss by radiation (W).
Qc: heat loss by convection (W).
Ts: heifer's surface temperature (˚C).
Tair: air temperature (˚C).

The area (A, m2) in Eq. 2 and Eq. 3 was estimated as the average area of a spherical form exposed to convective and radiant heat transfer.

Statistical analyses

Data for nutrient intake and digestibility and microbial protein synthesis were submitted to analyses of variance using the MIXED procedure of SAS 9.1. (SAS, 2003) according to the model:

$$Y_{ijlm}= \mu + T_i + P_j + S_l + A_m + e_{ijlm}$$

Where:
Y: dependent variable.
μ: overall mean.
T: fixed effect of treatment.
P: fixed effect of the period.
S: random effect of a square.
A: random effect of animal.
e: residual error.

Data e ruminal fermentation and thermoregulation were analyzed as repeated measures. The differences among treatments were tested for linear and quadratic contrasts and the significance level was set at 0.05. The means were adjusted by the LSMEANS procedure and analyzed by the

Dunnett test between the copaiba levels and monensin of PROC MIXED.

RESULTS AND DISCUSSION

Supplementation with copaiba oil (CO) did not influence (P≥0.245) the intake and digestibility of dry matter and nutrients. In addition, no differences were observed between animals supplemented with monensin and CO (Table 3). Lambs supplemented with monensin had a lower pH and higher rumen N-NH_3 concentration than those supplemented with CO (Table 4).

A quadratic effect (P=0.006) was observed for the concentrations of propionate ($Y=21.20+6.07X–4.59X^2$; $r^2=0.67$) for lambs supplemented with CO. The optimal level of CO inclusion was 0.67 g/kg DM. Additionally, animals fed monensin had higher concentrations of propionate and total SCFA compared to those that received CO, except for those supplemented with 1.0 g CO/kg DM.

A quadratic effect (P≤0.035) was observed for viability ($Y=63.241+37.96X^2–23.87X$; $r^2=0.40$) and count of *Diplodiniinaesp* ($Y=208.69+33.58X–21.72X^2$; $r^2=0.20$), with an optimal inclusion point of 0.79 and 0.77 g/kg DM of CO, respectively. In addition, a decreasing linear effect (P=0.018) was observed for large protozoa ($Y=27.24–10.89X$; $r^2=0.25$). Finally, lambs supplemented with 1.5 g CO/kg showed a large reduction of viability and large protozoa compared with monensin.

A quadratic effect (P=0.006) was observed for microbial nitrogen ($Y=8.372+3.907X–1.356X^2$; $r^2=0.26$) and protein ($Y=52.327+24.421X–8.478X^2$; $r^2=0.27$) for animals fed CO, where the optimal level of CO inclusion was 1.44 g/kg DM (Table 5).

Finally, animals fed 1.0 g CO/kg DM showed higher concentration of purine derivatives, microbial nitrogen and protein compared to lambs fed the ionophore. Increased levels of CO and monensin supplementation did not influence (P≥0.789) the emission and losses of heat by growing lambs (Table 6). It is important to note that plant extracts and essential oils are not standard substances, thus presenting a great variation in the active substances and their concentrations. Therefore, it is expected that the results are contradictory and inconsistent. The roughage:concentrate ratio contributed to avoiding differences in dry matter intake and digestibility. Ionophores and functional oils have a greater impact on intake and digestibility when the proportion of starch in the diet is high, which did not occur in this study. Also, in lambs receiving non-antibiotic rumen fermentation modulators, the effects vary with additive source and type of diet, which in turn influence effects and, or, adaptation of the rumen microbial population (Geraci *et al.* 2012). In ruminants, a small additives may stimulate intake, whereas a higher level may adversely affect intake in ruminants (Patra, 2011).

Ribeiro *et al.* (2019) did not observe differences in dry matter intake (DMI) and total digestibility in lambs supplemented with 25 mg of monensin/kg DM or doses of 1.25, 2.50, or 3.75 g of thyme/kg DM. Chaves *et al.* (2008) supplementing growing lamb with carvacrol and cinnamaldehyde reported similar effects. However, Abeer *et al.* (2019) observed increased digestibility of dry matter and nutrients by feeding ewes with an essential oil blend.

The effects of the MON on microbial fermentation in the present experiment, where it was regarded as a positive control, was similar to that found in other trials (Russell and Strobel, 1988; Yang and Russell, 1993).

Table 3 Intake and digestibility of dry matter and nutrients of lambs fed diets supplemented with monensin or copaiba oil

Item	MON	Copaibaoil (g/kgDM)[1]				SEM	P-value	
		0	0.5	1	1.5		L	Q
Intake (kg/d)								
Drymatter	0.878	0.865	0.991	0.854	0.895	0.038	0.889	0.565
Organicmatter	0.796	0.781	0.897	0.771	0.810	0.001	0.889	0.565
Crudeprotein	0.174	0.172	0.196	0.169	0.178	0.124	0.891	0.607
Neutral detergente fiber	0.511	0.502	0.576	0.495	0.521	0.114	0.902	0.558
Digestibility (g/kg)								
Drymatter	647.56	652.56	655.56	652.23	657.56	2.554	0.548	0.245
Organicmatter	674.54	679.75	682.88	679.41	684.96	2.444	0.552	0.255
Crudeprotein	803.03	809.23	812.95	808.82	815.43	1.894	0.587	0.288
Neutral detergente fiber	550.43	554.68	557.23	554.40	558.93	2.578	0.568	0.284

[1] MON (25 mg/kg DM of sodium monensin), 0, 0.5, 1.0 and 1.5 g/kg DM of copaiba oil in diet.
SEM: standard error of the means.
L: linear effect and Q: quadratic effect.

Table 4 Rumen fermentation of lambs supplemented with monensin or copaiba oil in diets

Item	MON	Copaibaoil (g/kgDM)[1]				SEM	P-value	
		0	0.5	1	1.5		L	Q
pH	6.51[a]	6.49[a]	6.74[b]	6.79[b]	6.89[b]	0.002	0.123	0.221
N-NH_3 (mg/dL)	24.37[a]	25.48[a]	21.58[b]	20.56[b]	20.44[b]	1.547	0.547	0.332
				mmol/L				
Acetate	58.96	58.19	55.09	59.30	53.52	1.243	0.526	0.698
Propionate	24.60[a]	21.52[b]	22.14[b]	23.63[a]	19.65[b]	0.514	0.548	0.006
Butyrate	9.61	10.83	10.90	10.09	9.42	0.345	0.292	0.724
Total	89.19[a]	90.55[a]	88.13[a]	93.04[b]	82.60[a]	1.893	0.383	0.409
Acetate:propionate	2.95	2.80	2.68	2.69	2.86	0.052	0.818	0.416
				Protozoa				
Viability (%)	75.60[a]	64.11[b]	71.20[a]	82.40[a]	64.80[b]	2.943	0.627	0.035
Small (%)	26.00	18.06	15.60	18.00	21.80	2.445	0.574	0.558
Medium (%)	52.60	54.27	63.60	64.40	67.80	3.013	0.201	0.676
Large (%)	21.80[a]	27.40[a]	20.80[a]	17.60[a]	10.40[b]	2.195	0.018	0.956
				Count/mL				
Isotrichidae spp.	48	63	57	49	54	3.568	0.385	0.552
Entodinium spp.	276	271	265	273	278	2.075	0.294	0.263
Diplodiniinae spp.	214[a]	209[a]	219[a]	221[b]	210[a]	3.776	0.833	0.050

[1] MON (25 mg/kg DM of sodium monensin), 0, 0.5, 1.0 and 1.5 g/kg DM of copaiba oil in diet.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
L: linear effect and Q: quadratic effect.

Table 5 Excretion of purine derivatives and microbial protein synthesis in lambs fed diets supplemented with monensin or copaiba oil

Item	MON	Copaibaoil (g/kgDM)[1]				SEM	P-value	
		0	0.5	1	1.5		L	Q
				mmol/d				
Allantoin	17.98[a]	28.64[a]	24.86[a]	43.44[b]	31.86[a]	0.01	0.529	0.189
Uricacid	3.20[a]	2.98[a]	4.34[a]	5.43[b]	4.98[a]	0.04	0.517	0.226
Xanthine	0.39[a]	0.50[a]	0.46[a]	0.61[a]	0.75[b]	0.04	0.563	0.720
Total purines	21.58[a]	32.13[a]	29.66[a]	49.49[b]	37.60[a]	0.01	0.466	0.670
Purine absorbed	8.09[a]	12.44[a]	10.96[a]	17.80[b]	14.45[a]	2.95	0.460	0.809
				g/d				
Microbial nitrogen	5.88[a]	9.04[a]	7.96[a]	12.94[b]	10.50[a]	0.01	0.460	0.006
Microbial protein	36.78[a]	56.53[a]	49.80[a]	80.88[b]	65.67[a]	0.01	0.460	0.006

[1] MON (25 mg/kg DM of sodium monensin), 0, 0.5, 1.0 and 1.5 g/kg DM of copaiba oil in diet.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
L: linear effect and Q: quadratic effect.

Table 6 Heat emission and heat losses by infrared thermography of lambs supplemented with monensin or copaiba oil in diets

Item	MON	Copaibaoil (g/kgDM)[1]				SEM	P-value	
		0	0.5	1	1.5		0	0.5
				Surface temperature (˚C)				
Left flank	34.55	34.59	35.02	35.04	34.55	0.17	0.776	0.196
Right flank	32.65	32.44	32.50	32.39	32.65	0.23	0.265	0.287
Rump	34.31	34.29	34.32	34.27	34.31	0.17	0.457	0.827
Head	37.13	36.87	36.99	36.99	37.13	0.17	0.187	0.867
				Heatloss (w/m^2)				
Radiation	0.071	0.069	0.072	0.073	0.074	0.01	0.587	0.887
Convection	62.17	62.48	62.56	61.99	61.56	1.13	0.237	0.823
Total	62.24	62.55	62.63	62.06	61.63	0.03	0.654	0.321

[1] MON (25 mg/kg DM of sodium monensin), 0, 0.5, 1.0 and 1.5 g/kg DM of copaiba oil in diet.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.
L: linear effect and Q: quadratic effect.

Lambs supplemented with CO had a pH compared to those receiving control and monensin supplemented diets. Data reporting variation in ruminal pH after supplementation with phytorumenl fermentation modulators are highly variable (Morsy *et al*. 2012; Vakili *et al*. 2013). One possible explanation may be related to a higher frequency of feeding due to reduced acceptability without reducing DMI as seen in this trial.

Copaiba oil considerably reduced rumen deamination by about 18% compared to monensin, and 20% about the control diet (Table 4). Copaiba has strong antimicrobial activity against gram-positive and gram-negative bacteria, thereby increasing the accumulation of amino acids, and a reduction in branched-chain fatty acids, ammonia N, and peptidolysis (Castillejos *et al*. 2008). The quality and proportion of hay used in the trial affected the highest concentration of N-NH_3 observed in the control and monensin diets. Another important point to note is that CO may have been efficient than MON in reducing rumen deamination and thereby reducing the concentration of ammonia.

The inclusion of 1 g/kg DM of CO gave a higher concentration of propionate and a quadratic effect between CO levels. Monensin use in ruminant diets has the capacity of increasing the molar proportion of propionate (Ribeiro *et al*. 2019). Before a level of supplementary copaiba oil was identified that showed better concentrations of rumen fermentation, the addition of monensin was widespread. However, the use of CO in high doses resulted in a lower molar proportion of propionate, which could be explained by the toxic effect of CO on protozoa (Table 4). Lambs supplemented with 1.5 g/kg DM showed a strong reduction of propionate and total SCFA (about 20%) compared to those fed 1 g CO/kg DM.

Lambs fed diets with 1 g CO/kg DM had the same concentration of total SCFA as those receiving MON, suggesting both with potential as modulators of rumen fermentation. However, the action of plant-based modulators of rumen fermentation is extremely inconsistent: several studies show positive results (Newbold *et al*. 2004; Castillejos *et al*. 2005; Nazzaro *et al*. 2013; Abeer *et al*. 2019; Ribeiro *et al*. 2019), but several are negative (Evans and Martin, 2000; McEwan *et al*. 2002; Calsamiglia *et al*. 2006; Castillejos *et al*. 2006).

The protozoan counts observed for lambs fed CO are in accordance with the SCFA rumen profile presented previously, especially for the viability (%) and Diplodiniinaesp count. However, large protozoa counts were reduced as the level of CO increased. Lambs without rumen protozoa often show an increase in bacterial numbers, a decrease in ammonia and volatile fatty acid (VFA) production, and a decrease in organic matter digestibility, depending on the quality of the diet consumed (Franzoli and Dehority, 2010).

Our results for rumen fermentation and protozoa numbers reflect the significant improvement in microbial protein production shown by lambs supplemented with 1g CO/kg DM. These lambs had N and microbial protein production levels 2.19 times higher than those that received MON in the diet. However, the dose of 1.5 g CO/kg DM proved to be toxic to the rumen microbial population.

The supplementation of 1 g CO/kg of DM increased suggests that diets lower in starch with more fiber may result may be led to an increase in potentially fermentable organic matter (Table 5), without alteration of dry matter intake (Table 3). Energy availability is the limiting factor for microbial growth, and the manipulation of the diet, through the change in the proportions of roughage and concentrate, increases the amount of fermented organic matter and, consequently, protein synthesis. The availability of energy for microbial growth depends on the composition of the diet and the extent of rumen fermentation.

Animals fed copaiba oil (CO) did not show changes in their heat emissions. This result may be related to the profile of the basal diet (roughage:concentrate ratio). Generally, diets with a low starch content do not have the capacity to alter the rumen calorific increase, so it was not possible to obtain relevant results about the thermoregulation in this trial (Araki *et al*. 2018). As the nutritional density of the diets is the same, the addition of CO or MON was not able to alter the heat production of the animals, since they were under the same installation, management, and feeding conditions.

CONCLUSION

Copaiba oil (CO) appears to have the potential to be used as a modulator of rumen fermentation. However, more studies should be directed to changing diets with a high content of starch and lipids. Copaiba oil (CO) positively influenced rumen fermentation and microbial protein synthesis, without altering dry matter consumption and digestibility. We suggest the addition of 1.0 g CO/kg DM in the diet of growing lambs.

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