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Effect of copaiba oil on intake, apparent nutrient digestibility, ruminal fermentation, nitrogen balance, and growth performance of lambs in confined conditions

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ABSTRACT - Two experiments were carried out aiming to evaluate the effect of copaiba oil on growth performance, apparent digestibility of nutrients, and rumen fermentation in lambs on feedlot. The treatments were: no additive inclusion (negative control); 8 mg/kg of sodium monensin (MS; positive control); and 250, 500, and 750 mg of copaiba oil/kg dry matter added to a diet with 70% concentrate and 30% forage. In experiment I, five lambs (Dorper × Santa Inês) cannulated in the rumen, 8 ± 1.0 months old and 51.4 ± 0.7 kg of initial body weight (BW) (mean ± SD), were assigned in a 5 × 5 Latin square design to assess ruminal parameters, apparent digestibility of nutrients, and nitrogen balance. In experiment II, 40 lambs (Dorper × Santa Inês), 8 ± 1.0 months old and 37.7 ± 1.6 kg of initial BW, were registered. The experimental design was in completely randomized blocks (five treatments and eight blocks). The treatments were: no additive inclusion (negative control); 8 mg/kg of sodium monensin (MS; positive control); and 250, 500, and 750 mg of copaiba oil/kg dry matter added to a diet with 70% concentrate and 30% forage. The experimental trial lasted 84 days, divided into three sub-periods of 28 days. Additives did not affect growth performance. Total dry matter intake, individual nutrient intakes, and apparent digestibility were not influenced by feed additives. The additives also did not affect the short-chain fatty acids molar concentration, ruminal pH, and ruminal ammonia nitrogen. Feed additives, in the dosages of 250, 500, and 750 mg of copaiba oil/kg of DM used in this experiment, are not effective to modify growth performance, apparent digestibility, and ruminal fermentation in lambs.

Keywords: additives, digestibility, metabolism, performance, sheep

1. Introduction

Sheep farming in tropical regions is a promising activity to attend the growing human demand for food, especially meat (Balaro et al., 2014). The major constrains in the current sheep farming system are the limited production of meat from young animals and the lack of constant supply of meat throughout the year caused, in most cases, by the low performance of animals in this region,

since diets of sheep in tropical regions are mainly composed of low energy-dense feeds (Kennedy and Charmley, 2012). The use of feed additives is one of the alternatives used to increase feed efficiency in animal production (Ferraz Junior and Carvalho, 2022), among which ionophores have been reported as effective feed additives for modulation of ruminal fermentation and increase of growth performance (Polizel et al., 2021; Oliveira et al., 2022). Studies in the literature show that monensin improved feed efficiency, decreased methane production, and reduced the risk of digestive and metabolic diseases such as ruminal acidosis (Duffield et al., 2012; Polizel et al., 2021; Oliveira et al., 2022).

Faced with the issues of economic, environmental, and social aspects, there is a growing scientific interest in alternatives that provide similar effects to ionophores. In this sense, researchers have explored strategies for manipulating rumen fermentation using biomolecules from plant sources with antimicrobial properties, showing great potential for use in animal nutrition (Calsamiglia et al., 2007).

Copaiba oil-resin is one of the main natural products in the Amazon region and can be extracted sustainably by drilling the stems of the trees of the Copaifera genus (Medeiros and Vieira, 2008). The well-known properties reported in literature for copaiba oil-resin are: antimicrobial (Mendonça and Onofre, 2009) and anti-inflammatory (Veiga Junior et al., 2007) activities, antitumor (Ohsaki et al., 1994), fungitoxic (Deus et al., 2011), and activity against parasites, as an example of *Leishmania amazonensis* (Santos et al., 2008). However, little is known about the effect of copaiba oil-resin on sheep growth performance. Thus, the hypothesis of this study was that adding copaiba oil as feed additive in the diet of feedlot lambs would enhance beneficial processes in rumen fermentation, increasing apparent digestibility of nutrients and growth performance.

This study aimed to evaluate the effect of copaiba oil-resin on rumen fermentation, apparent digestibility of nutrients, and growth performance of lambs in feedlot.

2. Material and methods

This study was carried in Piracicaba, São Paulo, Brazil (22°42'24" S and 47°37' 53" W). Research on animals was conducted according to the institutional committee on animal use (no. #901.02.90420).

2.1. Experiment I: Intake, apparent nutrient digestibility, ruminal fermentation, nitrogen balance, and ruminal pH

2.1.1. Animal, experimental design, and diet

Five castrated lambs (Dorper × Santa Inês) cannulated in the rumen with initial BW of 51.4 ± 0.7 kg (mean ± SD) and 8 ± 1.0 months old were used. All lambs were housed individually in metabolism crates (1.30 × 0.55 m), provided with feeders, water troughs, and a system for collecting feces and urine. The metabolism crates were kept indoors, protected from rain and direct sunlight.

The lambs were distributed in a 5 × 5 Latin square design, with five treatments and five experimental periods. The experiment lasted 105 days, divided into five periods of 21 days, of which 15 days were used for lamb adaptation to the experimental diets, five days for measuring the dry matter intake (DMI) and collecting feces and urine, and one day for collecting ruminal fluid.

The experimental treatments were defined as: CON - negative control (no additive added); MON - positive control (addition of 8 mg/kg DM of sodium monensin - Rumensin 100, Elanco Animal Health, São Paulo, SP, Brazil); CO250 - addition of 250 mg/kg DM of copaiba oil; CO500 - addition of 500 mg/kg DM of copaiba oil; and CO750 - addition of 750 mg/kg DM of copaiba oil. The copaiba oil-resin used in this experiment was purchased from a trade located in the municipality of Parintins, AM, Brazil.

The experimental diets were formulated to be isoenergetic and isonitrogenous (Table 1), using the Small Ruminant Nutrition System (Cannas et al., 2004). The ingredients and chemical composition of the experimental diets are shown in Table 1.

Table 1 - Proportion of ingredients and chemical composition of experimental diets (% DM)

Item	Diet[1]				
	CON	MON	CO250	CO500	CO750
Ingredient (%)					
Coast cross hay (*Cynodon* sp)	30	30	30	30	30
Ground corn	56	56	56	56	56
Soybean meal	10	10	10	10	10
Urea	0.5	0.5	0.5	0.5	0.5
Limestone	1.0	1.0	1.0	1.0	1.0
Ammonium chloride	0.5	0.5	0.5	0.5	0.5
Mineral	2.0	2.0	2.0	2.0	2.0
Monensin (mg/kg of DM)[2]	-	8	-	-	-
Copaiba oil (mg/kg of DM)	-	-	250	500	750
Chemical composition (%)[3]					
Dry matter	94.14 ± 0.2	90 ± 0.0	94.16 ± 0.0	94.14 ± 0.1	94.11 ± 0.3
Crude protein	17.7 ± 0.2	17.5 ± 0.2	17.7 ± 0.2	17.7 ± 0.0	17.5 ± 0.1
Neutral detergent fiber	28.9 ± 0.1	29.1 ± 0.0	29.1 ± 0.2	29.1 ± 0.0	29.1 ± 0.2
Acid detergent fiber	9.0 ± 0.2	10.0 ± 0.0	10 ± 0.1	10 ± 0.0	10 ± 0.1
Non-fibrous carbohydrates	47.9 ± 0.0	47.6 ± 0.1	47.9 ± 0.0	47.4 ± 0.3	47.9 ± 0.2
Ether extract	2.9 ± 0.3	2.9 ± 0.1	2.8 ± 0.1	2.9 ± 0.2	2.96 ± 0.2
Ash	6.8 ± 0.3	7.0 ± 0.2	6.9 ± 0.1	7.1 ± 0.0	7.0 ± 0.3

[1] CON: basal diet, no additive added (negative control); MON: addition of 8 mg/kg DM of sodium monensin (positive control); CO250: addition of 250 mg/kg DM of copaiba oil; CO500: addition of 500 mg/kg DM of copaiba oil; CO750: addition of 750 mg/kg DM of copaiba oil.
[2] Rumensin 100 (sodium monensin, Elanco Animal Health, São Paulo, São Paulo, Brazil).
[3] Considering three samples per treatment (n = 3).

2.1.2. Characterization of the compounds present in the essential oil

The volatile compounds were identified by gas chromatography/mass spectrometry (HS-GC/MS) on a GCMS-QP2010 Plus (Shimadzu Corp., Tokyo, Japan). An Rtx-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm; 5% methyl silicone as stationary phase; Bellefonte, PA, USA) was used. The carrier gas used was helium at a flow rate of 1.0 mL/min. The mass spectrum was acquired via ionization at 70 V in the range of 40 to 500 m/z. Samples of 1 µL were injected in split mode. The temperature of the injector and detector was 220 to 230 °C. The temperature ramp started at 50 °C and was maintained for 1.5 min, followed by 200 °C at 4 °C/min, and 240 °C at 10 °C/min, maintained for 7 min. For the identification of volatile compounds, the data (retention time, and area on TIC) were processed using GCMS Solution software (version 4.20; Shimadzu, Tokyo, Japan). Identification was done by similarity with library data (Wikey 8.lib, and FFNSC1.3.lib), and by calculation of the linear retention index (LRI) by running the C7-C30 alkane series (Supelco, Bellefonte, PA, USA).

2.1.3. Feed management and sampling

Samples of the experimental diets (n = 5) were collected at each feed. Coast cross hay (*Cynodon* sp) and corn were ground using a grinder (Nogueira DPM - 4, Itapira, São Paulo, Brazil) equipped with a 10-mm pore size sieve.

For the CON diet, hay was mixed with ground corn, soybean meal, ammonium chloride, limestone, and mineral premix using a horizontal mixer with a 500-kg capacity (Lucato, Limeira, São Paulo, Brazil). For the MON diet, all the ingredients of the CON diet plus sodium monensin (Elanco Animal Health, São Paulo, São Paulo, Brazil) were mixed in the same way as the CON. The sodium monensin was premixed using in a portion of ground corn, and then this premix was added to the other ingredients in the mixer.

For diets CO250, CO500, and CO750, a premix (containing only concentrate) was prepared using a horizontal mixer with a capacity of 500 kg (Lucato, Limeira, São Paulo, Brazil), and CO was weighed

daily on an analytical balance with a precision of 0.0001 g (Sartorius BA11OS, Gottingen, Germany) and mixed into the premix. Then, the hay was weighed separately on an electronic scale with an accuracy of 1 g (Marte, LC 100, São Paulo, Brazil), added and homogenized manually into the premix plus CO, and offered daily in the form of total mixed ration (TMR).

Daily, the diets were offered *ad libitum* at 07:00 h as TMR, and the orts were weighed. Daily feed was assigned according to previous DMI, and adjustments were made if necessary, so that the orts would not exceed 10% of the daily intake.

2.1.4. Orts and fecal collection

From day 16 to 20 of the experimental period, at 07:00 h, the orts were weighed, sampled (10%), and stored at −18 °C for later analysis and determination of DM and chemical composition. On the same days and times, the total fecal production of the lambs was quantified. The collection of feces was performed using canvas collection bags. The bags were attached to the lambs by harnesses. The feces were weighed on an electronic scale (Marte LC 100, São Paulo, São Paulo, Brazil), and a sample that was representative of 10% of the daily fecal production was collected and stored at −18 °C for later analysis.

2.1.5. Nitrogen balance

On days 16 through 20 of the experimental period, at 07:00 h, the total urine production was collected by using plastic recipients containing HCl (6N), to prevent ammonia volatilization, maintaining pH below 3.0. Urine pH was measured twice a day with pH indicator strips (MQuant Merck, Darmstadt, Germany). The total urine production was quantified every day, and a sample was collected and stored at −18 °C. The urine collected was used to calculate the nitrogen retention.

2.1.6. Ruminal fluid collection

Ruminal fluid samples were collected on day 21 of the experimental period. Samples were carried out at 0, 3, 6, 9, 12, 18, and 24 h after feeding. At each interval, approximately 200 mL representative samples of ruminal fluid were collected from each lamb via a rumen cannula, rapidly filtered on a 150-micron nylon cloth, and used for measurement of ruminal pH with a digital pH meter (Digimed DM20, São Paulo, São Paulo, Brazil). The solid fraction of the ruminal content that remained in the nylon cloth after filtration was returned to the rumen. Aliquots of ruminal fluid were taken, stored in microtubes (Eppendorf, São Paulo, São Paulo, Brazil), and frozen at −20 °C for future determination of the short-chain fatty acids (SCFA; acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate) and N-NH_3.

2.2. Experiment II: Performance of female lambs fed diets containing copaiba oil

2.2.1. Animal, experimental design, and diet

Forty crossbred (Dorper × Santa Inês) female lambs, 37 ± 6.0 kg of BW and 8 ± 1.0 months old (mean ± SD), were blocked according to BW and assigned to one of five treatments. The experimental design was completely randomized blocks (five treatments and eight blocks according to initial body weight). The experimental trial lasted 84 days. The animals were kept in a tie-stall system, in covered stalls (one animal/pen) with a slatted floor, provided with feeders, drinkers and salt shakers for mineral supplementation. The experimental treatments (diets) were the same as those presented in Experiment I.

In a daily basis, the diet was weighed on a 1-g precision electronic scale (Marte®, LC 100, São Paulo, Brazil). The female lambs received their respective experimental diets; however, to ensure *ad libitum*

intake, orts were maintained at approximately 10% of the amount offered, based on the intake of the previous day. Once a week, the orts from each experimental unit was weighed to calculate the DMI, sampled (10%) and composed by experimental unit and by treatment. At each feed beating, a sample was collected and stored at −18 °C for further analysis of the chemical composition.

The female lambs were kept in s 16-h fasting from solids to assess BW on days 0, 28, 56, and 84 of the experimental period to calculate feed efficiency using DMI and ADG data (FE = ADG/DMI).

2.3. Laboratory analyzes and calculations of experiment I and II

After thawing, feed, orts, and feces samples were dried in a forced-air oven at 55 °C for 72 h. All samples were ground with a Wiley-type mill (Marconi, Piracicaba, São Paulo, Brazil) to pass a 1-mm screen. Dry matter was determined by oven-drying at 105 °C for 24 h (AOAC, 1990; #930.15). Mineral matter (MM) was determined by incinerating the samples in a muffle furnace at 550 °C for 4 h (AOAC, 1990; #942.05) and organic matter (OM) was obtained by subtracting the MM from 100. The total nitrogen concentration was measured using a Leco TruMac N (Leco Corporation, St. Joseph, Michigan, USA; AOAC, 1997; #990.03). Crude protein (CP) was calculated by multiplying the total N content by 6.25. Neutral detergent fiber (NDF) was performed by the sequential method, using α-amylase thermostable and sodium sulfite according to Van Soest et al. (1991) and acid detergent fiber (ADF) according to Goering and Van Soest (1970), using an Ankom A2000 apparatus (Ankom Tech. Corp., Fairport, New York, USA). The ether extract (EE) content was determined using an Ankom XT15 extractor (Ankom Tech Corp., Macedon, New York, USA) according to AOAC (1990; #920.39). Non-fibrous carbohydrates (NFC) were estimated according to the equation: NFC (%) = 100% − (% NDF + % CP + % EE + % MM). The total digestible nutrients (TDN) were calculated according to Weiss et al. (1992) using the equation: TDN (%) = % CP digestible + (% EE digestible × 2.25) + % NDF digestible + % NFC digestible. Apparent nutrient digestibility was calculated by the difference between each nutrient consumed (DM, OM, CP, EE, NDF, NFC, starch) and its fecal excretion.

For determination of SCFA, 1.6 mL of ruminal fluid was added with 0.4 mL metaphosphoric acid:formic acid (3:1; 250 mL/L metaphosphoric acid: 980 −1000 mL/L formic acid) and 0.2 mL of 100 mM 2-ethyl butyric acid (internal standard) were centrifuged at 15,000 *g* (Sorvall Superspeed RC2-B, Newton, Connecticut, USA) for 15 min at 4 °C. After centrifugation, 1.2 mL of supernatant was transferred to a chromatographic vial. The analysis of the SCFA and NH_3 concentration was performed according to procedures described by Ferreira et al. (2016) and Broderick and Kang (1980), respectively. For statistical analysis, the data were transformed to the molar ratio (mM/100 mM), i.e., the ratio between the amount of a particular SCFA and the observed total.

2.4. Statistical analysis

All data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, 1999).

2.4.1. Experiment I

Five lambs were distributed in a 5 × 5 Latin square design, with five treatments and five experimental periods. Nutrient intake and apparent digestibility data were analyzed according to the statistical model:

$$Y = \mu + A_i + T_j + P_k + E_{ijk}, \quad (1)$$

in which μ = overall mean, A_i = animal effect (i = 1 to 5), T_j = treatment effect (j = 1 to 5), P_k = period effect (k = 5), and E_{ijk} = residual error.

Ruminal fermentation data were analyzed as repeated measures, according to the following statistical model:

$$Y = \mu + A_i + T_j + P_k + H_l + (TH)_{jl} + E_{ijkl}, \quad (2)$$

in which μ = general average, A_i = animal effect (i = 1 to 5), T_j = treatment effect (j = 1 to 5), P_k = period effect (k = 5), H_i = time (h) after feeding effect, $(TH)_{jl}$ = effect of interaction between treatment and time after feeding, and E_{ijkl} = residual error. The covariance structure was first-order autoregressive, which provided the best fit for the analyses, according to lowest corrected Akaike Information Criteria. The effect of copaiba oil concentration in the diet was evaluated using linear and quadratic orthogonal polynomial contrasts. Period and interaction effects between treatments and periods were determined by the F test of analysis of variance.

2.4.2. Experiment II

The animals were considered the experimental unit. The trial was carried out using a completely randomized block with five treatments and eight blocks. Animal performance data were analyzed according to the following statistical model:

$$Y = \mu + B_i + T_j + P_k + (TP)_{jk} + E_{ijk}, \quad (3)$$

in which μ = overall mean, B_i = block effect (i = 1 to 8), T_j = treatment effect (j = 1 to 5), P_k = period effect (k = 4), $(TP)_{jk}$ = interaction between treatment and experimental period, and E_{ijk} = error residual. Effects were declared significant when $P<0.05$.

3. Results

The results of the analysis showed that Caryophyllene (41.1%) is the major secondary compound, followed by α-cis-Bergamotene (11.61%) and α-Copaene (10.35%) (Table 2).

There was no difference ($P>0.05$) in BW, DMI, ADG, and FE among treatments (Table 3). Dry matter and nutrient intake were not influenced by the inclusion of additives (monensin or CO) in the experimental diets when compared with the CON diet. Regarding the apparent digestibility of nutrients, there was also no difference in the apparent digestibility of DM and OM of the experimental diets (P = 0.50 and P = 0.48, respectively) (Table 4).

Table 2 - Composition of copaiba oil used in the trial

Compound[1]	%
Caryophyllene	41.15
α-cis-Bergamotene	11.61
α-Copaene	10.35
α-Humulene	7.34
Germacrene	6.89
β-Bisabolene	4.13
trans-Ocimene	3.56
β-Elemene	3.07
Δ-Cadinene	1.85
α-Amorphene	1.77
β-Selinene	1.55
α-Cubebene	1.39
α-Selinene	1.23
trans-β-Caryophyllene	0.72
Alloaromadendrene	0.63
α-Muurolene	0.6
β-Germacrene	0.58
Cyperene	0.55
B-Farnesene	0.47

[1] Relative amounts of identified compounds based on the area of each peak in the chromatogram.

Table 3 - Performance of lambs fed diets containing different levels of copaiba oil in experiment II

Item	Diet[1]					SEM	P-value
	CON	MON	CO250	CO500	CO750		
Initial BW (kg)	38.29	38.20	37.20	37.70	37.20	1.67	0.98
Initial $BW^{0.75}$ (kg)	15.35	15.34	15.06	15.21	15.05	0.50	0.99
Final BW (kg)	49.38	49.55	49.70	49.50	49.55	2.39	1.00
Final $BW^{0.75}$ (kg)	18.60	18.60	18.70	18.60	18.70	0.67	1.00
ADG (g/day)	132	135	149	140	150	0.10	0.84
DMI (g/day)	1.010	1.075	1.134	1.070	1.21	0.09	0.61
DMI (kg/LW)	2.29	2.44	2.59	2.42	2.78	0.13	0.13
DMI $BW^{0.75}$ (g/kg)	58.86	62.62	66.64	62.42	71.36	3.83	0.21
FE (ADG/DMI)	0.131	0.126	0.131	0.131	0.12	0.01	0.76

BW - body weight; $BW^{0.75}$ - metabolic BW; ADG - average daily gain; DMI - dry matter intake; FE - feed efficiency; SEM - standard error of the mean.
[1] CON: basal diet, no additive added (negative control); MON: addition of 8 mg/kg DM of sodium monensin (positive control); CO250: addition of 250 mg/kg DM of copaiba oil; CO500: addition of 500 mg/kg DM of copaiba oil; CO750: addition of 750 mg/kg DM of copaiba oil.

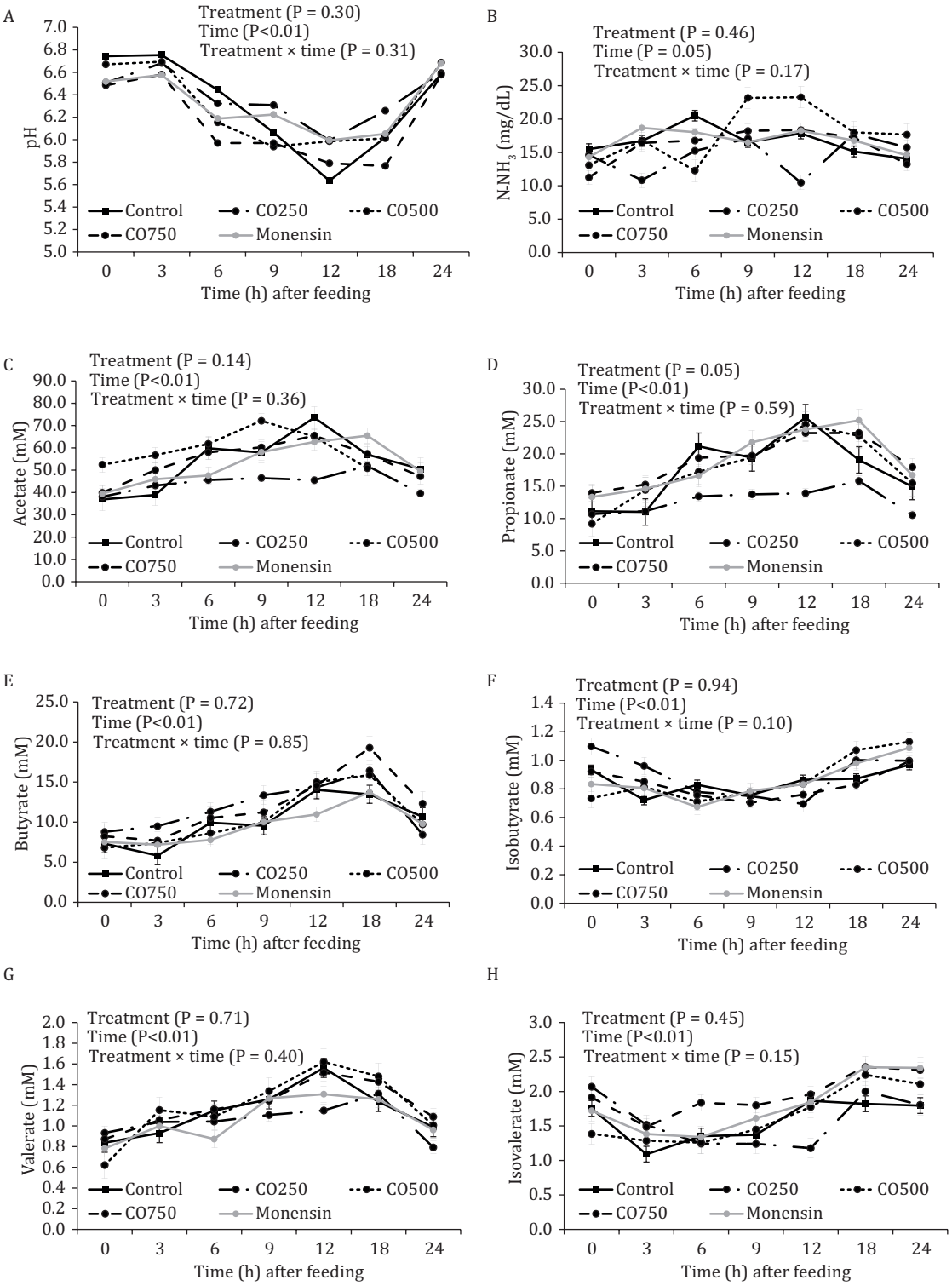
Table 4 - Effects of copaiba oil on intake and apparent nutrient digestibility of lambs in experiment I

Item	Diet[1]					SEM	P-value		
	CON	MON	CO250	CO500	CO750		Treatment	Animal	Period
Intake (kg/day)									
DM	1.29	1.25	1.07	1.32	1.21	0.07	0.17	0.002	0.240
OM	1.20	1.17	1.00	1.22	1.12	0.06	0.16	0.002	0.234
CP	0.26	0.25	0.22	0.26	0.24	0.01	0.15	0.002	0.258
NFC	0.60	0.57	0.50	0.59	0.54	0.02	0.10	0.002	0.307
NDF	0.27	0.30	0.25	0.30	0.29	0.02	0.48	0.003	0.132
ADF	0.09	0.10	0.08	0.11	0.10	0.00	0.16	0.001	0.076
EE	0.09	0.02	0.02	0.03	0.03	0.02	0.47	0.540	0.448
Digestibility coefficient (%)									
DM	58.61	61.64	60.64	53.59	56.66	3.42	0.50	0.793	0.747
OM	55.63	58.85	57.99	50.09	53.49	3.67	0.48	0.795	0.773
CP	62.50	65.44	64.63	57.57	58.46	3.02	0.29	0.938	0.675
NDF	60.47	72.14	68.53	66.76	66.89	4.29	0.46	0.429	0.159
ADF	63.78	68.56	66.19	63.08	64.57	3.52	0.81	0.422	0.305
NFC	53.61	53.90	54.52	42.58	44.91	4.14	0.17	0.335	0.829
EE	79.29	78.85	77.38	75.29	75.29	3.32	0.85	0.703	0.455

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDF - neutral detergent fiber; ADF - acid detergent fiber; NFC - non-fibrous carbohydrate; SEM - standard error of the mean.
[1] CON: basal diet, no additive added (negative control); MON: addition of 8 mg/kg DM of sodium monensin (positive control); CO250: addition of 250 mg/kg DM of copaiba oil; CO500: addition of 500 mg/kg DM of copaiba oil; CO750: addition of 750 mg/kg DM of copaiba oil.

The CO used also did not affect (P = 0.30) the ruminal pH in the different treatments, nor was there any interaction between treatment and time of collection. However, there was a difference (P<0.01) in the ruminal pH of the animals in relation to the time of collection. It was possible to observe that the highest ruminal pH values showed greater stability until the third hour of harvest, followed by a decrease of one of these values until the 12th hour, followed by an increase in the pH of the rumen fluid from this hour until the 24th hour (Figure 1A).

No effect of treatment, time, and interaction between treatment and time was observed on the concentration of $N\text{-}NH_3$ in the rumen fluid (Figure 1B). Likewise, N balance was not affected by treatments (Table 5). The molar concentration of acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate was also not influenced by the treatments (P>0.05). Differently than pH, the molar concentration of acetate, propionate, butyrate, and valerate increased over time, reaching a maximum concentration 18 h after intake of the diet (Figures 1C-1H).



Control: basal diet, no additive added: (negative control); Monensin: addition of 8 mg/kg DM of sodium monensin (positive control); CO250: addition of 250 mg/kg DM of copaiba oil; CO500: addition of 500 mg/kg DM of copaiba oil; CO750: addition of 750 mg/kg DM of copaiba oil.

Figure 1 - Effect of experimental diets on rumen pH, N-NH_3 concentration, and molar concentration of short-chain fatty acids in lambs in experiment I.

Table 5 - Effects of copaiba oil on nitrogen balance of lambs in experiment I

Item	Diet[1]					SEM	P-value
	CON	MON	CO250	CO500	CO750		
Nitrogen (g/day)							
Intake	42.13	41.22	35.15	42.88	39.52	2.26	0.18
Fecal	15.82	14.17	12.35	18.87	14.89	1.80	0.25
Urinary	14.95	15.77	15.25	16.38	15.8	1.05	0.83
Absorbed	26.43	26.72	22.79	24.41	23.43	1.37	0.22
Nitrogen retention							
g/day	11.47	10.94	7.54	8.03	8.54	1.28	0.17
Intake (%)	71.67	81.34	57.90	53.41	52.93	12.85	0.46
Absorbed (%)	42.57	40.70	31.30	32.62	31.04	4.08	0.18

SEM - standard error of the mean.
[1] CON: basal diet, no additive added (negative control); MON: addition of 8 mg/kg DM of sodium monensin (positive control); CO250: addition of 250 mg/kg DM of copaiba oil; CO500: addition of 500 mg/kg DM of copaiba oil; CO750: addition of 750 mg/kg DM of copaiba oil.

4. Discussion

The main objective of this study was to evaluate whether copaiba oil-resin could be used to modulate rumen fermentation to increase energy efficiency and growth performance in lambs on feedlot. However, the copaiba oil used was not able to affect the growth performance variables, apparent nutrient digestibility, and SCFA production in the lamb rumen. Based on these results, the hypothesis initially proposed was rejected, even though it is described in the literature that copaiba oil has antimicrobial effects (Pacheco et al., 2006). Another important result to be highlighted was the similarity of the results between the diets without additives and with sodium monensin, which represented the positive control in this experiment. The effects of monensin on performance, apparent digestibility, and SCFA are already widely studied. The main effect of monensin reported in the literature for ruminants is the increase in the energy efficiency of the animal (Duffield et al., 2012; Polizel et al., 2021). In most cases, monensin reduces DMI without decreasing body weight gain (Duffield et al., 2012). Another result widely reported in the literature is the increase in the molar concentration of propionate (Polizel et al., 2021; Duffield et al., 2012), which makes the animal more energy-efficient.

Despite reports that copaiba oil has greater microbial activity against Gram-positive bacteria than against Gram-negative bacteria (Pacheco et al., 2006), similar to ionophores (Russell and Houlihan, 2003), no effect was observed in ruminal fermentation variables. We speculated that copaiba oil used in this trial for 15 days of adaptation was not enough for the effects in the rumen. The time required for essential oils to exert their effects on rumen fermentation can vary, but adaptation periods may be crucial for achieving consistent results. Soltan et al. (2018) demonstrated that a progressive adaptation period allowed for the stabilization of ruminal fermentation parameters and improved nutrient digestibility. Similarly, Joch et al. (2019) showed that essential oil blends require a period of adaptation to optimize their effects on rumen fermentation. These findings suggest that gradual adaptation to essential oils is necessary to fully perceive their benefits.

The use of essential oils has been proposed as an alternative to ionophores; however, the results have been quite inconsistent. Several studies have shown specific results in growth performance, apparent nutrient digestibility, and rumen fermentation, but it is not uncommon to find studies without the effects of essential oils (Ribeiro et al., 2020). Lima et al. (2018) and Oliveira et al. (2020) also did not find effects of the inclusion of copaiba oil on DMI and apparent nutrient digestibility diets for cattle under grazing conditions and lambs in feedlot, respectively. Much of this divergence may be due to the lack of standardization of essential oil and dosage used. For example, Moura et al. (2017) did not observe effect in body weight and DMI in lambs receiving mixed diets (i.e., 53% forage and 47% concentrate) containing different levels of copaiba oil; however, they found higher ADG and FE for animals fed diets at 0.5 g/kg DM. Lima et al. (2018) related a quadratic effect of copaiba oil (0, 0.5, 1.0, and 1.5 g/kg DM) on the DMI of pasture-fed cattle.

The sheep presents a high capacity for diet selection; however, it is important to mention that the doses of copaiba oil used were not able to negatively influence the DMI of the lambs, not causing any episode of repulsion of the lambs to the experimental diets. Since voluntary intake of DM is one of the main components of the production process, it is considered the main determinant of the intake of digestible nutrients and the efficiency with which such nutrients are used in the animal's metabolic processes (Valadares Filho and Marcondes, 2009).

The observation of the pH data clearly shows that there was a peak intake of the diet after its supply (hour 0), causing the pH to drop in the following hours until 12 hours after the feeding of the diet. This pH is caused by the higher production of SCFA produced in the rumen, which can also be confirmed by the higher molar concentration of SCFA 12 to 18 h after feeding. These data analyzed together show the fermentation profile of the diet used, which was rapidly fermented, but without causing episodes of subclinical acidosis.

5. Conclusions

Copaiba oil at doses of 250, 500, and 750 mg of copaiba oil/kg of DM does not change the performance of lambs and does not affect dry matter intake, apparent nutrient digestibility, and short-chain fatty acids in lambs compared to diets without additive and with sodium monensin. Importantly, in this study, monensin also shows no effect compared to the diet without additive.

Data availability

The data generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author upon reasonable request.

Author contributions

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Conflict of interest

The authors declare no conflict of interest.

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