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Digestibility of nutrients, ruminal parameters and nitrogen balance in lambs fed with diets containing high concentrate and concentrations of essential oil of (*Cymbopogon flexuosus*) lemongrass

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ABSTRACT. The objective of this experiment was to evaluate the effects of inclusion of essential oil of Lemongrass (*Cymbopogon flexuosus*) in rumen fermentation parameters, nutrients digestibility and nitrogen balance in lambs feeding. The treatments were: negative control or CN (no additives); positive control or MON (addition of 25 mg of monensin kg^{-1} of dry matter); and 1.25, 2.5 and 3.75 mL essential oil kg^{-1} of diet as feed. Thirty crossbred Dorper x Santa Ines lamb cannulated in the rumen with 59.66 ± 10.03 kg of body weight (BW) were allocated into randomized complete blocks design with five replicates per treatment. Based on the total data of collections, the treatment 1.25 mL of essential oil of lemon grass was able to positively manipulate ruminal fermentation in which it was observed higher concentration of total short-chain fatty acids (SCFA), higher concentrations of acetate and propionate -together with lower acetate/propionate ratio, but with higher methane concentration. The treatments did not influence dry matter intake, but the its digestibility. Organic matter and ether extract were higher for the three doses of essential oil of lemon grass compared to the CN and MON treatments, respectively. The lemon grass essential oil did not affect the nitrogen balance in sheep. Thus, it is concluded that the best dosage was 1.25 mL in terms of total SCFA, propionate and decreased acetate/propionate ratio, however, these same treatments can be evaluated in sheep performance and thus ensure the best dose of this essential oil so that it can have a better feed efficiency in sheep.

Keywords: nutrients digestibility; additives; rumen; fermentation; sheep; feed intake.

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Introduction[1]

For centuries, compounds extracted from vegetables have been used by the most diverse human civilizations, for medicinal, industrial and even in the food industry. Many plant extracts have proven antimicrobial activity (Calsamiglia et al., 2007) and, simultaneously, most of them have GRAS status ('generally recognized as safe') for human consumption by FDA (U.S Food and Drug Administration), which gives them a natural and safe alternative to antibiotics. The antimicrobial activity of this essential oil is cited in the literature in which several authors suggest that citral is the main responsible for such activity (referencia excluída como solicitado).

Verified the capacity of this essential oil to reduce the production of CH_4 in an *in vitro* gas production system on the highest dosage used, causing only reduction in the concentration of C_2 and C_3, proving that in high doses, this essential oil can inhibit both C_2 and C_3 producers. For the lowest dose used, this same author observed an increase in the concentrations of C_2 and C_3, consequently, in the production of CH_4.

Lemon Grass (*Cymbopogum flexuosus*) is a plant with another same genus called cidreira grass, but rarer in Brazil. It is internationally named East Indian Lemon grass. Most often, both plants (lemon grass or cidreira grass) are given the same denomination (May et al., 2008). The mixture of geranial and neral makes its essential oil rich in citral, with concentration close to 60% of the total oil.

[1] Abbreviations: CTL, negative control; MON, positive control; BW, body weight; SCFA, Short chain fatty acids; CH4, methane gas; C2, Acetate; C3, Propionate; DM, dry matter; DATT, apparent digestibility of the nutrients in the total digestive tract; HCL, hydrochloric acid; OM, organic matter; NDF, neutral detergent fiber; NH3-N, ammoniacal nitrogen.

Moderate antifungal and antibacterial effects have already been found in lemon grass oil, being attributed to citral (Oussalah et al., 2006; Kumar et al., 2009). Essential oil of lemon grass from southeast Asia has antifungal, antibacterial, diuretic, anticarcinogenic, gastric protection mechanisms and parasite protection properties.

The objective of this experiment was to evaluate the effect of the addition of essential oil of lemon grass (*Cymbopogon flexuosus*) on nutrient intake and digestibility in the total digestive tract, nitrogen balance and ruminal fermentation in sheep.

Materials and methods

Animals, experimental design, diets and feed management

This study was conducted in the research facilities with sheep from the Sheep and Goat Intensive Production System (SIPOC) of the Department of Animal Science, 'Luiz de Queiroz' College of Agriculture, University of São Paulo, located in Piracicaba, state of São Paulo, Brazil. All animal procedures followed the guidelines recommended by the Animal Care and Use Committee at the same University (Comitê de Ética no Uso de Animais – CEUA – Number 3045/2013).

Thirty crossbred (½Dorper x ½ Santa Inês) castrated male (initial body weight 53.08 ± 11.40 kg and approximately 210 d of age) were used. All lambs were vaccinated against clostridia (Sintoxan T, Merial Animal Health, Campinas, SP, Brazil) before weaning and were dewormed with 1.0% moxidectin (Cydectin®, Fort Dodge Animal Health, Campinas, São Paulo, Brazil) at a dosage of 1 mL 50^{-1} kg BW and 5 g de Cloridrato de Levamisol (Ripercol®, Fort Dodge Animal Health, Campinas, São Paulo, Brazil). The animals were housed in individual pens during the first 15 days for adaptation to diets. Between 15 and 21 days they were placed in metabolic cages (1.30 x 0.55 m) provided by feed bunks, waterers and a system to collect feces and urine.

Lambs were blocked by initial body weight and randomly assigned within 10 blocks. Diets were composed by 10% of coast cross hay and 90% of concentrate. The experimental diets were: CN – negative control diet (no inclusion of fed additives); MON – positive control diet adding of 25 mg of monensin (Rumensin® 100, Elanco Brazil, São Paulo, SP, Brazil) kg^{-1} of DM; and inclusion of 1.25, 2.50 and 3.75 mL of essential oil of *Cymbopogon flexuosus* kg^{-1} as feed.

Corn and coast cross hay used in the diets were ground using a mill (Nogueira DPM - 4, Itapira, SP, Brazil) with 10 mm sieves. Later, corn and coast cross hay were mixed with soybean meal, limestone, ammonium chloride and mineral mix using a horizontal mixer with capacity of 500 kg (Lucato, Limeira, SP, Brazil) (Table 1). On MON experimental diet the sodium monensin was added at the rate of 25 mg kg^{-1} of DM. Essential oil were mixed with diets every time that feed was offered to the animals. The experimental diets were weight daily in an electronic scale with 5.0 g accuracy and offered, ad libitum, 07:00 and 19:00 h. The refused feed was collected every day from each animal to calculate the dry matter intake.

The animals were weighted on day 0 and day 21, without fasting, to monitor the body weight.

Table 1. Composition of basal diet with differing additives and essential oil doses (mL kg^{-1}) as feed.

Item	Diet [1]				
	CN	MON	1.25	2.50	3.75
Ingredients, % of dry mater					
Coast cross hay	10.0	10.0	10.0	10.0	10.0
Soybean meal	16.0	16.0	16.0	16.0	16.0
Ground corn	70.0	70.0	70.0	70.0	70.0
Ammonium chloride	0.35	0.35	0.35	0.35	0.35
Limestone	1.65	1.65	1.65	1.65	1.65
Mineral[2]	2.0	2.0	2.0	2.0	2.0
Essential oil (mL 100 kg^{-1} MS)	0	0	125.0	250.0	375.0
Monensin (ppm)	0	25.0	0	0	0
Chemical composition					
DM (% of MO)	87.5	87.5	87.5	87.5	87.5
Organic matter	95.0	95.0	95.0	95.0	95.0
Crude protein	15.7	15.7	15.7	15.7	15.7
Ether extract	2.83	2.83	2.83	2.83	2.83
NDF[3]	14.5	14.5	14.5	14.5	14.5

[1] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2] Composition: Ca 13.4%. P 7.5%. Mg 1%. S 7%. Cl 21.8%. Na 14.5%. Mn 1100 mg kg^{-1}. Fe 500 mg kg^{-1}. Zn 4600 mg kg^{-1}. Cu 300 mg kg^{-1}. Co 40 mg kg^{-1}. I 55 mg kg^{-1}. Se 30 mg kg^{-1}. [3] NDF = Fiber insoluble in neutral detergent.

Data recording and sample collection: sample ruminal fluid, nutrient digestibility and nitrogen balance

On days 0 (before start offering experimental diets), 7, 14 and 21 ruminal fluid was collected at zero (before feeding), 3, 6 and 9 hours after feed being offered. Ruminal contents were collected via rumen cannula. Ruminal fluid pH was measured immediately by using a pH meter (DM20, Digimed, São Paulo, SP, Brazil). Two 25 mL aliquots of ruminal fluid were stored at -20 °C in plastic containers at each time of collection for future determinations of short-chain fatty acids (SCFA) and ammonia-N.

The animals were placed in metabolic cages between days 15 and 21 for total collection of feces, urine and diets offered in the previous day. Feces were collected using collection bags to avoid urine contamination. The total fecal production of the animals was quantified (Marte, AC 10K, São Paulo, SP, Brazil) at 7:00 a.m., and a sample that was representative (10%) of the daily production was collected and stored at -18°C. The feces collected were used to determine the nutrient digestibility in the total digestive tract.

The apparent digestibility of the nutrients in the total digestive tract (DATT) and nitrogen balance were calculated according to the following formulas: DATT % = ((nutrient intake – nutrient excreted in feces)/nutrient intake) x 100. Nitrogen retained was calculated as follow: $N_{retained}$ (g/d) = N_{intake} - N_{feces} - N_{urine}.

The urine was collected with plastic recipients containing HCl (6*N*), to prevent ammonia volatilization, maintaining pH below 3.0. Urine pH was measured twice a day. 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.

Laboratory analysis

The composition of the essential oil of lemon grass (*Cymbopogon citratus*) was determined in the Laboratory of Chemistry and food analysis of the School of Agriculture Luis de Queiroz of University of São Paulo in a chromatograph equipped with RTX 5MS columns with 30 m of length and 0.25 mm thick with and initial temperature of 40°C for 8 minutes (ramp 1) increasing by 3°C per minute up to 180°C (ramp 2) and 20°C up to 230°C (ramp 3) for a total period of 77.17 minutes (Table 2).

Table 2. Composition of essential oil of lemon grass (*Cymbopogon flexuosus*).

Compound	Lemon grass % relative
α-Pineno	0.21
2,2- Dimetil	1.61
6 Metil	2.44
Micreno	1.70
α-Limoneno	0.37
Propil Cetona	0.87
α-Naginateno	0.14
Citronelal	0.60
β Linalol	0.77
Furan Oxide	0.34
Neral	37.07
Isogeranial	0.57
Neril acetate	0.82
2,6 Octadieno dimetil	0.72
Cariofileno Oxide	1.13
2,6 Octadienal dimetil	49.95
Cariofileno	1.50

Samples of the offered feed, orts and feces were thawed, dried in a forced-air oven at 55°C for 72h and ground with a Wiley mill (Marconi, Piracicaba, SP, Brazil) to pass a 1-mm screen. DM content was determined by drying the samples at 105°C for 24h, and ash content was obtained by incinerating the samples in an oven at 550°C for 4h (Association of Official Analytical Chemists, 1990). The OM was calculated by the difference between the DM and ash. Total N of offered feed, orts and feces was determined using the Leco FP528 instrument (Leco Corporation, St. Joseph, MI, USA) according to Association of Official Analytical Chemists (1997). The ether extract was determined using the Leco TFE 2000 (Leco Corporation, St. Joseph, MI, USA). NDF was determined according to Van Soest et al. (1991) using α-amylase and sodium sulfite in a 2000 Ankom system (Ankom Tech. Corp., Fairport, NY, USA). Total N of urine was determined by Microkjeldahl method (Association of Official Analytical Chemists, 1997).

Short chain fatty acid profile was determined using Agilent 7890A gas chromatograph equipped with flame ionization detector (7683B) and a fused-silica capillary column (J&W 19091 F-112, Agilent Technologies, Santa Clara, CA, USA), 25 m in length and 320 µm internal diameter. Sample extraction was carried out by withdrawing 1.6 mL of ruminal fluid sample added with 0.4 mL of 3:1 solution of 25% metaphosphoric acid with 98-100% formic acid and 0.2 mL of internal standard, centrifuged in a Sorvall Superspeed RC2-B, Newton, CT, USA device, for 15 minutes at 40°C. After centrifugation, 1.2 mL of the supernatant from each sample was transferred to chromatographic vials. From the obtained extract 1 µL was injected into the gas chromatograph. The injection was performed automatically, and the entrainment gas was H_2, maintained at a flow of 31.35 mL $min.^{-1}$. The temperature of the injector and detector was 260 °C. The total time of the chromatographic run was 16.5 minutes per sample divided into three heating ramps, as follows: 80°C (1 min.), 120°C (20°C $min.^{-1}$) and 205°C (100°C $min.^{-1}$).

The NH_3-N concentration was determined with a colorimetric method that was described by Chaney and Marbach (1962), adapted for a microplate reader (EON, BioTek Instruments, Winooske, VT, USA) with a 550 nm absorbance filter.

The methane production was calculated using the equation proposed by Abdl-Rahman (2010) based in stoichiometry of Wolin (1960), as follows:

Fermentative $CO_2 = A/2 + P/4 + 1.5*B$

Fermentative $CH_4 = (A + 2*B) - CO_2$

A = mole of acetate.

P = mole of propionate.

B = mole of butyrate.

Statistical analysis

The animal was the experimental unit used to perform all statistical analysis. Statistical analyses were performed using the MIXED procedure of the SAS statistical software program (Statistical Analysis System, 2002). All data were submitted to the Shapiro-Wilk test to verify the regularity of the residuals, the removal of 'outliers', and homogeneity of variances using the Levene test. The data that did not respect those premises was subjected to logarithmic, inverse or square root transformation.

The data for nutrient intake, nutrient digestibility and nitrogen balance were analyzed using the model: $Y_{ij} = \mu + D_i + b_j + e_{ij}$, where μ = overall mean; D_i = fixed effect of diet; b_j = random effect of block; and e_{ij} = random error. The means were obtained by the LSMEANS command. For the data analyzed as repeated for days only, measures over time as VFA profile, ruminal pH, and ammonia. The statistical model used was: $Y_{ijk} = \mu + D_i + b_j + e_{ij} + T_k + (DT)_{ik} + (bT)_{jk} + e_{ijk}$, where μ = overall mean; D_i = fixed effect of diet; b_j = random effect of block; e_{ij} = random error A; T_k = fixed effect of the time; $(DT)_{ik}$ = fixed effect of diet × time interaction; $(bT)_{jk}$ = random effect of block × time interaction, and e_{ijk} = random error B. All data was evaluated in form of orthogonal counter: Negative control vs additives; positive control vs oil doses; linear effect and quadratic effect.

All data that were analyzed as repeated for day measures were placed on covariance matrices and tested for 'compound symmetry, heterogeneous compound symmetry, autoregressive (AR), autoregressive heterogeneous, unstructured, banded, variance components, toeplitz and heterogeneous toeplitz' and defined according to the lowest value obtained for Akaike's Information Criterion. The means were obtained by the LSMEANS command. The effect of diet, week and interaction of diet x week were defined by the test F of analysis of variance and was evaluated using the inclusion of SLICE methods. The diet effect was defined by Tukey test and significance was defined as $p < 0.05$ and tendency as $0.05 < p < 0.10$.

Results

Ruminal parameters

The effect of three doses of essential oil of lemon grass (*Cymbopogon flexuosus*) compared with negative control treatment (no additives) and positive control treatment (monensin) on the ruminal fermentation parameters at seven, fourteen, twenty-one and everyday of offering the experimental diets are summarized in Tables 3, 4, 5 and 6, respectively.

In the 7th day (Table 3), the acetate concentration was lower for MON treatment ($p= 0.05$) compared to the three doses of essential oils of lemon grass. Quadratic effect of essential oils ($p = 0.01$) was observed for methane concentration.

Table 3. Ruminal parameters in lambs feeding with monensin and doses of essential oil of lemon grass (*Cymbopogon flexuosus*) compared to a control treatment in the 7th day of offering the experimental diets.

Item	Treatment[1]					EPM[2]	p value[4]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
Ruminal pH	6.01	5.98	5.30	5.85	5.81	0.22	0.47	0.10	0.95	0.11
Total SCFA, mM[3]	108.33	95.30	127.22	91.61	110.17	12.15	0.91	0.17	0.82	0.52
Acetate, mM	57.49	46.56	66.77	45.26	54.16	5.08	0.80	0.05	0.93	0.21
Propionate, mM	29.81	34.19	45.23	34.47	42.21	10.20	0.60	0.46	0.69	0.85
Butyrate, mM	14.56	8.70	13.77	7.68	8.47	2.76	0.32	0.58	0.46	0.37
Isobutyrate, mM	1.08	0.92	0.79	0.87	1.00	0.12	0.40	0.71	0.46	0.23
Isovalerate, mM	2.58	1.55	1.65	1.17	1.88	0.37	0.13	0.95	0.67	0.34
Valerate, mM	0.58	2.69	1.76	2.36	2.29	0.75	0.21	0.38	0.80	0.50
C_2/C_3	2.11	1.42	1.75	1.37	1.48	0.47	0.46	0.78	0.89	0.79
$N\text{-}NH_3$, mg dL^{-1}	16.17	17.42	17.00	16.76	17.33	1.26	0.50	0.79	0.92	0.70
Methane, mmol	23.77	20.32	27.00	24.73	20.20	2.20	0.77	0.17	0.80	0.01

[1] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2] EPM = Standard error. [3]SCFA = short-chain fatty acids. [4]p value = means differ significantly ($p < 0.05$). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

In the 14th day (Table 4), there was a trend of linear effect ($p = 0.07$) of essential oils of lemon grass for pH and linear effect ($p = 0.03$) for isobutyrate concentration. There was a trend of lower methane concentration ($p = 0.09$) for MON treatment in relation of the three essential oil doses. There was a trend of linear effect for valerate concentration ($p = 0.08$) and a trend ($p = 0.06$) of quadratic effect for methane concentration.

Table 4. Ruminal parameters in lambs feeding with monensin and doses of essential oil of lemon grass (*Cymbopogon flexuosus*) compared with a control treatment at the 14th day of offering the experimental diets.

Item	Treatment[1]					EPM[2]	*P* value[4]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
Ruminal pH	6.01	5.62	5.34	5.85	5.83	0.19	0.30	0.79	0.07	0.44
Total SCFA, mM	93.62	97.53	126.91	76.18	103.68	13.05	0.73	0.69	0.41	0.93
Acetate, mM	44.57	46.52	58.51	38.07	55.10	3.24	0.37	0.18	0.58	0.37
Propionate, mM	35.21	37.39	48.76	27.87	32.09	10.65	0.94	0.90	0.25	0.69
Butyrate, mM	10.16	10.30	13.72	7.02	11.61	2.05	0.88	0.79	0.64	0.73
Isobutyrate, mM	0.70	0.76	0.76	0.91	0.98	0.12	0.46	0.27	0.03	0.72
Isovalerate, mM	1.61	1.32	1.70	0.93	2.09	0.37	0.87	0.45	0.17	0.23
Valerate, mM	1.17	2.93	3.28	1.82	1.56	1.03	0.48	0.45	0.08	0.72
C_2/C_3	1.50	1.33	1.41	1.49	2.21	0.54	0.90	0.46	0.11	0.49
$N\text{-}NH_3$, mg dL^{-1}	17.62	17.16	19.62	20.44	18.89	1.49	0.37	0.16	0.36	0.16
Methane, mmol	25.62	21.63	28.14	26.90	24.52	2.27	0.89	0.09	0.49	0.06

[1] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2] EPM = Standard error. [3]SCFA = short-chain fatty acids. [4] pvalue = means differ significantly ($p < 0.05$). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

In the 21st day (Table 5), a trend ($p = 0.08$) was observed for higher concentration of methane for the three oil doses in relation to the MON treatment and quadratic effect ($p = 0.02$) for methane concentration, increasing as the dosage of essential oils increased.

In all days combined (Table 6), a trend of quadratic effect of essential oil doses ($p = 0.09$) was observed for total short-chain fatty acids (SCFA).

There was an effect $p = 0.05$) of higher isovalerate concentration for the MON treatment in relation to the three doses of essential oil of lemon grass together with a trend of linear effect ($p = 0.07$) for isovalerate concentration. Higher valerate concentration ($p = 0.01$) was observed for CN treatment in relation to the additives (MON and essential oil doses). Trend of linear effect ($p = 0.08$) for $N\text{-}NH_3$ concentration was observed. Lower methane concentration was observed ($p = 0.008$) for the MON treatment in relation to the three doses of essential oil of lemon grass, and quadratic effect ($p = 0.004$) was observed for methane concentration.

Intake and digestibility of nutrients

The digestibility of dry matter ($p = 0.05$), organic matter ($p = 0.03$) and ether extract ($p = 0.05$) was lower for MON treatment in relation to the three doses of essential oil (Table 7). A trend of quadratic effect of

essential oils of lemon grass was observed for the digestibility of dry matter (p = 0.07) and digestibility of organic matter (p = 0.08). There was no effect for any of the variables related to the intake.

Table 5. Ruminal parameters in lambs feeding with monensin and doses of essential oil of lemon grass (*Cymbopogon flexuosus*) compared with a control treatment in the 21st day of offering the experimental diets.

Item	Treatment[1]					EPM[2]	p value[4]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
Ruminal pH	6.20	5.74	5.41	5.69	5.75	0.25	0.24	0.57	0.70	0.35
Total SCFA, mM[3]	86.91	96.39	115.16	88.45	100.61	11.15	0.51	0.61	0.68	0.71
Acetate, mM	39.83	47.21	53.44	43.62	51.77	3.59	0.17	0.45	0.72	0.74
Propionate, mM	36.32	35.27	46.49	30.08	35.11	8.84	0.97	0.80	0.53	0.66
Butyrate, mM	6.76	8.91	10.20	9.51	9.14	1.37	0.28	0.56	0.99	0.46
Isobutyrate, mM	0.74	0.83	0.78	1.01	0.94	0.11	0.45	0.44	0.12	0.93
Isovalerate, mM	1.94	1.58	1.55	1.68	1.73	0.36	0.64	0.82	0.61	0.89
Valerate, mM	1.30	2.57	2.68	2.53	2.91	0.85	0.47	0.79	0.42	0.59
C_2/C_3	1.33	1.39	1.38	1.54	1.74	0.35	0.77	0.59	0.27	0.71
$N\text{-}NH_3$, mg dL^{-1}	18.53	18.30	19.95	21.42	19.46	1.24	0.36	0.17	0.36	0.14
Methane, mmol	22.11	18.72	24.05	23.63	20.36	1.94	0.83	0.08	0.59	0.02

[1] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2] EPM = Standard error. [3]SCFA = short-chain fatty acids. [4] p value = means differ significantly (p < 0.05). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

Table 6. Ruminal parameters in lamb feeding with monensin and doses of essential oil of lemon grass (*Cymbopogon flexuosus*) compared with a control treatment throughout days (0, 7, 14 and 21) of offering the experimental diets.

Item	Treatment[1]					EPM[2]	p value[3]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
Ruminal pH	5.75	5.56	5.74	5.82	5.96	0.210	0.88	0.16	0.12	0.88
Total SCFA, mM	101.46	102.03	114.92	106.73	96.80	6.449	0.47	0.66	0.54	0.09
Acetate, mM	51.73	53.66	57.35	56.95	52.60	3.761	0.26	0.72	0.87	0.30
Propionate, mM	32.70	31.96	35.80	31.79	27.60	3.461	0.74	0.96	0.41	0.26
Butyrate, mM	11.55	11.19	16.58	12.88	12.73	2.200	0.34	0.40	0.94	0.25
Isobutyrate, mM	0.93	0.95	1.09	1.09	0.91	0.096	0.32	0.57	0.81	0.11
Isovalerate, mM	1.29	2.70	1.60	2.46	1.17	0.309	0.82	0.05	0.07	0.77
Valerate, mM	2.59	1.54	2.29	1.53	1.67	0.312	0.01	0.64	0.88	0.49
C_2/C_3	1.70	1.85	1.71	1.99	2.09	0.153	0.21	0.78	0.41	0.57
$N\text{-}NH_3$, mg dL^{-1}	17.74	17.59	18.95	19.55	19.95	0.94	0.24	0.10	0.08	0.62
Methane, mmol	24.11	20.42	27.34	24.91	23.07	1.50	0.91	0.008	0.41	0.004

[1]CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2]EPM = Standard error. [3]SCFA = short-chain fatty acids. [4] p value = means differ significantly (p < 0.05). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

Table 7. Nutrient intake and digestibility on total tract in lambs receiving monensin and levels of essential oils of lemon grass (*Cymbopogon flexuosus)* compared with a control treatment.

Item[1]	Treatment[2]					EPM[3]	*P* value[4]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
DMI, kg d^{-1}	1.68	1.63	1.55	1.48	1.62	0.06	0.15	0.35	0.75	0.13
DMI, %BW	2.82	2.80	2.66	2.58	2.83	0.12	0.47	0.50	0.96	0.15
$DMI^{0,75}$, g	78.38	77.19	73.29	70.83	77.84	3.7	0.35	0.46	0.97	0.14
OMI, kg d^{-1}	1.45	1.58	1.42	1.43	1.49	0.10	0.77	0.33	0.62	0.32
CPI, kg d-1	0.25	0.25	0.23	0.22	0.24	0.01	0.15	0.35	0.75	0.14
EEI, kg d-1	0.042	0.041	0.039	0.037	0.041	0.001	0.15	0.33	0.72	0.12
NDFI, kg d-1	0.34	0.33	0.31	0.30	0.33	0.01	0.15	0.26	0.58	0.13
NDFI, % BW	0.58	0.57	0.54	0.53	0.58	0.02	0.45	0.49	0.92	0.14
Digestibilities										
DM, %	83.85	82.19	85.30	84.68	84.01	1.00	0.85	0.05	0.31	0.07
OM, %	85.75	83.74	87.81	86.73	86.51	1.22	0.72	0.03	0.20	0.08
CP, %	92.41	93.42	93.06	92.53	92.15	0.49	0.46	0.16	0.06	0.97
EE, %	79.77	73.25	81.55	80.29	80.55	3.03	0.79	0.05	0.15	0.20
NDF, %	87.85	86.82	87.13	88.48	89.30	1.06	0.94	0.29	0.11	0.82

[1] DMI = dry matter intake; OMI = organic matter intake; CPI = crude protein intake; EEI = ether extract intake; NDFI = neutral detergent fiber intake. [2] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [3] EPM = Standard error. [4] p value = means differ significantly (p < 0.05). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

Nitrogen balance

There was no effect for any of the variables related to the nitrogen balance (Table 8).

Table 8. Nitrogen balance in lambs receiving monensin and levels of essential oils of lemon grass (*Cymbopogum flexuosus)* compared with a control treatment.

Item	Treatment[1]					EPM[2]	p value[3]			
	CN	MON	1.25	2.50	3.75		CN*Additives	MON*Oil	L	Q
N intake, g	41.50	40.22	38.21	36.17	39.81	0.001	0.15	0.34	0.70	0.14
N feces, g	8.66	8.07	6.38	7.50	7.98	0.0007	0.17	0.41	0.81	0.18
N urine, g	13.00	10.50	15.44	11.83	14.17	0.002	0.96	0.35	0.63	0.68
N retained, g	19.83	21.02	16.28	17.00	16.53	0.003	0.50	0.23	0.39	0.49
Apparent digestibility of N, %	78.91	79.72	83.17	79.53	79.32	1.51	0.39	0.63	0.53	0.27

[1] CN = Negative control diet (without additives); MON = positive control diet (with monensin); 1.25, 2.50 and 3.75 = Diets with addition of 1.25, 2.50 and 3.75 mL of essential oil of lemon grass (*Cymbopogon flexuosus*) per kg as feed. [2] EPM = Standard error. [3] *P* value = means differ significantly ($p < 0.05$). Contrasts of treatments CN *vs* additives; MON *vs* Oil doses; Linear effect; Quadratic effect.

Discussion

Ruminal fermentation

On day 7, the MON treatment presented acetate concentration 16.0% lower than the three doses of essential oil of lemon grass (*Cymbopogon flexuosus*) used in the present study, 30.0% lower when comparing MON with the 1.25 mL dose of essential oil of lemon grass and 14.0% less acetate when comparing to MON vs. treatment 3.75 mL of essential oil of lemon grass (Table 3).

The same concentration of acetate, propionate and butyrate were observed for the MON treatments and 2.50 mL of essential oil of lemon grass. The linear decrease on methane concentration shows that the highest dose of essential oil of lemon grass in the diet was more efficient energetically (Van Soest, 1994; Russell, 2002) that day.

The validation for this statement can be observed by the decrease in methane concentration together with the reduction of acetate concentration, butyrate, and on day seven, treatments 2.50 and 3.75 mL were identical and superior in maintaining acetate and propionate concentrations compared with MON treatment, indicating that there was no numerical difference for treatment 2.50 versus MON for acetate and propionate variables.

When comparing the treatments MON and 3.75 mL of essential oil of lemon grass, a higher concentration of propionate (20.0%) was observed for the treatment of 3.75 mL of essential oil, confirming better energy efficiency for this treatment (3.75 mL), associated with a lower concentration of methane. These data are in agreement, in which evaluating doses of lemon grass in an in vitro fermentation system, found a reduction in the concentration of methane, acetate and even propionate, confirming that the response of this essential oil is dose dependent. Also, corroborating the data of the present study, observed an increase in the concentrations of acetate, propionate and methane, validating the data of the present study which had the same behavior on day seven of evaluation.

The data are also in agreement with Demitras et al. (2019), when evaluating three doses of commercial aldehyde (187.5, 375 or 750 mg) it verified the same behavior mentioned above. The linear increase in isobutyrate concentration (Table 4), observed in the presence of additives (MON and the three doses of essential oil) used in the present study was 18.0% higher than in the CN treatment. Thus, it can be said that the additives, fourteen days offered in the experimental diets, acted positively on the ruminal digestibility of the branched valine amino acid, the main responsible for the increase in the concentration of isobutyrate in the rumen (Van Soest, 1994).

MON treatment reduced methane concentration by 19.0% in relation to the three doses of essential oil used in the present study and by 16.0% compared with the CN treatment, which shows one of the main effects of monensin in manipulating the microbial rumen population, by reducing acetate concentration (Russell, 2002).

The strong trend of quadratic effect shows that as dose of essential oil of lemon grass increased, there was a decrease of 28.14, 26.90 and 24.52 mmol in methane concentration at 1.25, 2.50 and 3.75 mL of essential oil of lemon grass, respectively. Furthermore, this observation can be verified in the 21st day of offer of experimental diets (Table 5), reinforcing that over time, the doses of essential oil of lemon grass used in the present study were efficient in reducing the methane concentration as it increased its inclusion in the diet.

Another interesting aspect to remark is that the total concentration of SCFA for the 2.50 mL treatment remained lower in relation to the other treatments in all day's evaluation, indicating that this dosage caused

an effect by decreasing both gram-negative and gram-positive activity, which is in agreement with several authors (Van Soest, 1994; Russell, 2002; Busquet et al., 2006; Calsamiglia et al., 2007).

Unlike Demitras et al. (2019) and Lin et al. (2013), which verified effects contrary to that of the present study, mainly on the acetate/propionate ratio and ammoniacal nitrogen concentration in which the essential oils used in their studies reduced these variables, such effect was not observed in the present study with the three doses of essential oil of lemon grass.

In all days combined (Table 6), MON was more efficient in reducing methane than the other treatments, and this difference reached 25.0 and 18.0% less mmol methane/day for MON compared with treatments 1.25 and 2.50 mL of essential oil used in the present study, respectively.

There is also a tendency of quadratic effect in the decrease of the concentration of total SCFA as the inclusion of essential oil of lemon grass increased, which may explain the reduction in methane production. In recent years, several studies have evaluated the effect of the inclusion of essential oils on the reduction of methane production (Cobellis, et al., 2016).

The doses 1.25 and 2.50 mL of essential oil used in the present study showed methane concentration of 25.0 and 18.0% higher in relation to the MON treatment, 27.34 and 24.91 mmol versus 20.42 mmol, respectively. These data show that for the three dosages of essential oil of lemon grass used in the present study, there was a strong tendency of quadratic effect in the decrease in the concentration of total SCFA, i.e., as the essential oil dose increased by 1.25, 2.50 and 3.75 mL, respectively, there was a numerical reduction in the concentration of total SCFA mainly reducing the concentration of acetate and propionate. That indicates that the oil at the dosage of 3.75 mL was effective in reducing the activity of both gram-positive and gram-negative bacteria, reinforcing that the lowest concentration of methane occurred with the highest dosage (3.75 mL) of essential oil of lemon grass. Even with the lowest concentration of total SCFA for this treatment, it caused numerical decrease (Cobellis, et al., 2016) in acetate and propionate concentrations.

The treatment 3.75 mL was efficient in reducing methane concentration by 16.0% compared with the treatment 1.25 mL, 8.0% compared with the treatment 2.50 mL and 4.5% in relation to CN treatment, respectively. In addition, 12.0% more methane is observed when comparing MON versus 3.75 mL of essential oil of lemon grass.

The data of the present study are in accordance with Machebouef et al. (2008) that verified 12.0% reduction of methane with oregano or cinnamon essential oil in in vitro fermentation system. In addition, these same authors verified that carvacrol and cinnamaldehyde at a dosage of 5 mM showed slight efficiency in mitigating methane when compared to essential oil of oregano.

Several essential oil compounds have been evaluated for the inhibitory effects of methanogenesis. When evaluating the inclusion of thymol in 0.4 g L^{-1} of ruminal fluid, Evans and Martin (2000) observed that this component of essential oil was a potent inhibitor of methane in vitro and also decreased acetate and propionate concentrations.

These data reinforce the hypothesis that the 2.50 mL dose of essential oil of lemon grass in the present study strongly influenced acetate and propionate concentrations, and consequently decreased the production of total SCFA (Machebouef et al., 2008; Chaves et al., 2008). In the inclusion of 0.25 g $^{-1}$ $animal^{-1}$ day^{-1} of a mixture of oregano essential oil in the diet of sheep for a period of 15 days, Wang et al. (2009) showed a decrease in methane production. These data corroborate the data of the present study, confirming that on the 21st day of evaluation, the treatment 3.75 mL was more efficient in reducing methane production in sheep.

Intake and digestibility

The treatments did not influence dry matter intake (kg d^{-1}), consequently did not influence the intake of the other variables studied (Table 7). There are several studies on food consumption, and these factors depend on the type and dose of essential oil (Patra et al., 2011). For example, Chaves et al. (2008), by evaluating the effect of the inclusion of 200 mg of carvacrol in sheep, did not find effect on dry matter intake.

The data of the present study are in accordance with Bampidis et al. (2005) that when studying the effect of doses of oregano essential oil, did not find effect on dry matter intake in sheep. Also, Benchaar et al. (2006) did not find effect on dry matter intake when evaluating the inclusion of 2 or 4 g day^{-1} in the diet of beef cattle composed of a mixture of essential oil based on timol, vanillin, limonen and eugenol.

The trends of quadratic effect for dry matter and organic matter intake show that as the dose of essential oil in the diet increased, there was a reduction in dry matter intake, which implies that this essential oil, depending on its inclusion, may reduce consumption and thus compromise the performance of the animals.

A 5.0% reduction in dry matter intake was observed for the dosages of essential oil of lemon grass in relation to CN and MON treatments, respectively. Thus, the doses of essential oil of lemon grass were potential modulators of dry matter intake, which is usually verified for monensin, especially in high concentrate diets as is the case of the present study. The oils increased the digestibility of dry matter and organic matter by 3.0 and 5.0% in relation to the MON treatment.

Nitrogen Balance

There was no effect of treatments on nitrogen consumption (Table 8), however, the additives presented nitrogen intake 7.0% lower than the CN treatment, which confirms one of its main effects in high concentrate diets, by decreasing consumption. The additives excreted on average 14.0% less nitrogen in feces compared to CN.

Treatments 2.15 and 3.75 mL of essential oil of lemon grass had 16.0 and 8.0% more nitrogen excreted in urine compared with the CN treatment, and 32.0 and 26.0% compared with MON treatment, respectively. Thus, it is clear that treatments 1.25 and 3.75 were less efficient in using nitrogen in the rumen, a fact that can be confirmed by the higher concentration of N-NH_3 on the 21st day (day referring to the evaluations of intake and digestibility; Table 5), as the destination of ammonia that is not used by microorganisms in the rumen is the absorption by the wall of this compartment and then removed by the bloodstream and goes to the liver where it will be excreted via urine mainly in the form of urea.

On average, the three doses of essential oil used in the present study had a concentration of N-NH_3 in the rumen 10.0% higher than the treatments CN and MON. This confirms the lowest efficiency of nitrogen utilization in the treatments that received the three essential oil dosages which had 17.0 and 21.0% less nitrogen retained in relation to CN and MON treatments, respectively (Oliveira et al., 2001). Consequently, these dosages evaluated for the essential oil may cause more economic, productive and reproductive losses and still harm the environment (Broderick & Clayton, 1997).

Conclusion

Thus, it is concluded that the best dose was 1.25 mL in terms of total SCFA, propionate and decreased acetate/propionate ratio, however, these same treatments can be evaluated in sheep performance and thus ensure the best dose of this essential oil so that it can have a better feed efficiency in sheep.

Data availability

The data used in the research has been made publicly available and can be accessed via the link https://teses.usp.br/teses/disponiveis/10/10135/tde-14092015-105231/pt-br.html

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