



Effect of Supplying Rosemary (*Rosmarinus officinalis* L.) and Garlic (*Allium sativum* L.) Essential Oils to Feedlot Lambs on *in vitro* Ruminal Fermentation

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The aim of this trial was to evaluate the ruminal degradation kinetics of carbohydrates in diets with different roughage:concentrate ratios and dosages of garlic and rosemary essential oils, in order to find the most suitable dosage to supply feedlot lambs. Three roughage:concentrate ratios (50:50, 40:60, and 20:80) and six dosages of garlic and rosemary essential oils (0.0, 0.10, 0.25, 1.0, 1.50, and 2.0 g L⁻¹) were tested. Kinetic parameters for carbohydrate breakdown were estimated using a semi-automated *in vitro* gas production technique. Ruminal degradation parameters were subjected to variance analysis and then regression analysis at a 5% significance level. There was no interaction between the roughage:concentrate ratios and the dosage of rosemary essential oil. The roughage:concentrate ratios in diets with rosemary oil affected the non-fiber carbohydrate degradation rate (Kdnfc), colonization time (L), gas volume and breakdown rate from the degradation of fiber carbohydrates (Vfc and Kdfc, respectively), and final gas volume of both fiber and non-fiber carbohydrates (Vfinal). Rosemary dosages affected Vnfc and Vfc, which presented a quadratic response with a peak at 0.71 g L⁻¹ and a nip at 1.17 g L⁻¹, respectively. Bacterial colonization time was quadratic, reaching a maximum value at 1.18 g L⁻¹. Vfinal showed a decreasing linear trend, such that each gram of rosemary essential oil added to the diet could reduce gas production by 30.312 mL. Therefore, rosemary essential oil has an effect on carbohydrate degradation kinetics. There was no interaction between roughage:concentrate ratios and different garlic oil dosages, except for colonization time. Roughage:concentrate ratios with garlic oil had affected the Vnfc, Kdnfc, and L. Garlic oil dosages affected Vfc and Vfinal in a quadratic manner, with the lowest values of gas production at 1.35 and 1.54 g L⁻¹, respectively. L was affected by the garlic oil dosage and roughage:concentrate ratios in a decreasing linear trend for a 50:50 ratio and quadratic response for a 40:60 ratio, peaking at 0.14 g L⁻¹. Based on these *in vitro* results, a ruminal content of 1.0 g L⁻¹ is recommended for both rosemary and garlic essential oils.

Keywords: garlic, natural additives, rosemary, ruminant nutrition, sheep

INTRODUCTION

Finishing in sheep production has been widely discussed in recent years due to its importance in providing quality to meet consumer demand (Bettencourt et al., 2020). In order to intensify this production phase, there are alternatives to modulate ruminal fermentation, leading to a reduction of metabolic disorders and gas production that causes energy loss through eructation. These strategies can be performed by reducing metabolic H⁺ available to methanogenesis with alternative reducers to eliminate H⁺ (Bodas et al., 2012).

Since 2006 the European Union, through regulation 1831/2003/EC (Comissão Europeia, 2003) banned antibiotics and other synthetic additives that promote growth in animal nutrition, and prohibited meat from animals that had been fed with such additives.

This scenario prompted research for finding natural replacements to antibiotics. Therefore, secondary plant metabolites, such as essential oils, have been presenting great potential due to their selective antibacterial activity, as well as antioxidative and anti-free radical properties (Matkowski et al., 2008).

Rosemary (*Rosmarinus officinalis* L.) is a perennial plant belonging to the Lamiaceae family. It is commonly used as a spice and renowned for its antibacterial properties. The main components of rosemary essential oil are monoterpenoids, 1,8-cineole, α -pinene, β -pinene, camphor, caryophyllene, and D-limonene (Oualdi et al., 2021), which have antibacterial effects against both gram-positive and gram-negative bacteria (Jiang et al., 2011; Tavassoli et al., 2011).

Several researchers have reported the significant effects of essential oils in modulating ruminal fermentation. Pinski et al. (2015) tested different essential oils, and only cinnamon and rosemary oils were capable of reducing methane production *in vitro*. In a similar trial, Roy et al. (2014) tested several oils (such as rosemary) as ruminal fermentation conditioners in 50:50 roughage:concentrate diets, and observed that at a dosage of 600 mg L⁻¹, both methane and ammonia concentrations were reduced in ruminal content, with no changes in propionate amount and dry matter digestibility *in vitro*.

While investigating how rosemary interacts with ruminal microbiota in ewes, Cobellis et al. (2016) included essential oil (7 g day⁻¹ animal⁻¹ adsorbed on inert support), grounded and dried leaves (10 g day⁻¹ animal⁻¹), and grounded and dried leaves in pellets added to the concentrate (10 g day⁻¹ animal⁻¹). There was no difference in bacterial populations, protozoa, and *Ruminococcus flavefaciens* among the control and treatments. Rosemary leaves, both grounded or in pellets, promoted a decrease in the populations of *Archaea* and *Prevotella* bacteria. Rosemary (grounded leaves) also reduced the populations of *Ruminococcus albus* and *Clostridium aminophilum*, while its essential oil formulation increased *Fibrobacter succinogenes* populations.

Garlic (*Allium sativum* L.) essential oil, especially its sulfur components, inhibits *in vitro* methanogenesis (Blanch et al., 2016) and changes the acetate:propionate ratios and butyrate

concentration in the rumen (Yang et al., 2007; Klevenhusen et al., 2011). Busquet et al. (2005) suggested that the antimethagenic activity of garlic may be related to the direct inhibition of *Archaea* and ruminal bacteria through the suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) and its organosulfur compounds.

Eslam et al. (2021) tested a natural combination of garlic and citrus in sheep, and observed that 5 and 10 g kg⁻¹ of dry matter (DM) decreased the CH₄ emission yield per digestible DM intake up to 7 and 12.8% when compared to control. When testing garlic essential oil on performance, ruminal fermentation and blood parameters of Kivircik lambs, Canbolat et al. (2021) reported that garlic oil had reduced the levels of glucose, urea, protein, triglyceride, insulin and cholesterol. Therefore, the supplementation up to 0.8 g/kg⁻¹ DM can be recommended for growing lambs to manipulate rumen and blood parameters without compromising important growth parameters.

Besides their positive effects, the method of supply and the ideal dosage of these essential oils are not well-established. Therefore, the aim of this trial was to evaluate the ruminal degradation kinetics of carbohydrates in diets with different roughage:concentrate ratios and dosages of garlic and rosemary essential oils.

MATERIALS AND METHODS

All the procedures that were performed on animals followed the legal guidelines of scientific management of experimental herds. They were approved by the Ethics Committee in Animal Use (CEUA) of the State University of Londrina (UEL; Protocol No. 9571.2018.80).

This study was conducted at the Animal Nutrition Laboratory of the State University of Londrina. Three diets with roughage:concentrate ratios of 50:50, 40:60, and 20:80 were combined with six dosages of rosemary and garlic essential oils: 0.0 (control group), 0.10, 0.25, 1.0, 1.50, and 2.0 g L⁻¹.

Diets were formulated to meet the lamb requirements for a daily average gain of 250 g (National Research Council, 2007) with 11% crude protein and containing Coast Cross (*Cynodon dactylon*) hay, ground corn (*Zea mays*), and soybean (*Glycine max*) meal (Table 1). Rosemary and garlic essential oils were purchased from Ferquima and Gran Oil, respectively. Rosemary essential oil was obtained through stem distillation of the leaves, and its content was 40% of 1,8-cineol, 15% of camphor, 13% of alpha-pinene, 7% of beta-pinene, and 3% of limonene. Garlic essential oil was 100% pure and naturally extracted by cold crushing and filtration of guaranteed origin bulbs.

The feed was subjected to chemical analyses in order to determine dry matter (DM, method 930,15), ash (ash method 923,03), crude protein (CP, method 990,03), ether extract (EE, method 920,39), hemicellulose (HEM) according to AOAC (2000), organic matter (OM, method 942,05) following AOAC (2006), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG), as reported by Detmann et al. (2012). The chemical compositions of both the feed and diets are presented in Table 2.

TABLE 1 | Centesimal composition of diets with different roughage : concentrate ratios.

Feed (% DM)	Roughage:concentrate ratios		
	50:50	40:60	20:80
Coast Cross Hay (% DM)	50.00	40.00	20.00
Grounded Corn (% DM)	36.15	47.07	68.95
Soybean Meal (% DM)	13.85	12.93	11.05

DM, dry matter.

TABLE 2 | Chemical composition and carbohydrate fractionation of feed and diets containing rosemary and garlic essential oils, as basis dry matter (%).

Parameter	Feed			Diet (R:C ratios)		
	Grounded Corn	Soybean Meal	Coast Cross Hay	50:50	40:60	20:80
Dry matter	89.17	89.73	87.56	88.44	88.60	88.91
Organic matter	98.56	93.38	92.75	94.94	95.57	96.83
Crude protein	8.12	44.21	5.76	11.94	11.84	11.64
Ether extract	4.62	1.81	1.41	2.63	2.97	3.67
Neutral detergent fiber	12.8	13.48	74.7	43.84	37.65	25.26
Acid detergent fiber	1.56	5.09	30.41	16.47	13.56	7.72
Hemicellulose	11.24	8.39	47.12	28.79	25.22	18.1
Lignin	1.06	0.82	1.08	1.04	1.04	1.04
Total carbohydrates (CHOT)	85.82	47.36	85.58	80.37	80.75	81.52
Non-fiber carbohydrates	79.59	42.35	23.01	46.14	52.14	64.16
A + B1 (% CHOT)	93.77	94.99	37.43	65.77	71.39	82.64
B2 (% CHOT)	3.68	3.04	59.97	31.74	26.12	14.87
C (% CHOT)	2.54	1.97	2.59	2.49	2.49	2.49

A + B1, soluble and fast degradable fraction; B2, potential degradable fraction; C, non-degradable fraction.

Carbohydrate fractions were estimated according to equations proposed by Sniffen et al. (1992), and total carbohydrates (TC) were estimated using the formula: $TC = 100 - (CP + EE + MM)$. Non-fiber carbohydrates (NFC) were calculated using the equation: $NFC = 100 - (CP + NDF_{ap} + EE + MM)$, in which NDF_{ap} stands for NDF adjusted for ash and protein. The B2 fraction, which was slowly degradable in the rumen, was estimated using the formula: $B2 = NDF_{cp} - C_{fraction}$. The C fraction, which represents indigestible material from the cell wall, was calculated by multiplying the lignin percentage by 2.4. The A + B1 fraction, corresponding to fast and intermediate ruminal breakdown, was estimated using the equation $A + B1 = 100 - (C + B2)$ (Table 2).

Ruminal fluid for the *in vitro* gas production technique was manually collected through a rumen cannula of one castrated lamb with 75 kg of body weight (BW), fed for 7 days with 50:50

roughage:concentrate diet (Table 1). Ruminal fluid collection was performed before the morning meal (07h30m), filtered with a thin mesh cotton fabric and stored in a thermos bottle previously heated to 39°C. Subsequently, samples were incubated in the laboratory. The time between collection and incubation was 60 min.

Kinetic parameters of carbohydrate degradation were estimated using a semi-automated *in vitro* cumulative gas production technique described by Schofield et al. (1994). Therefore, samples were dried in a force-air dryer at $55 \pm 5^\circ\text{C}$ and then grounded at 1 mm. Later, 300 mg were placed into 50-mL glass bottles. Each essential oil dosage was previously diluted in a buffer solution before placement in incubation bottles. Considering that buffering solution added represented 80% of incubated volume, essential oil dosages after dilution were, 0.0 g L^{-1} of buffering solution, 0.125 g L^{-1} of buffering solution (0.1 divided by 80%), 0.3125 g L^{-1} of buffering solution (0.25 divided by 80%), 1.25 g L^{-1} of buffering solution (1.0 divided by 80%), 1.87 g L^{-1} of buffering solution (1.5 divided by 80%), and 2.5 g L^{-1} of buffering solution (2.0 divided by 80%). After dilution, the buffering solution and adjusted dosages of garlic and rosemary were homogenized for 5 min with Turrax (Marconi, model MA102/A).

All bottles were filled with 24 mL of Mcdougal (1949) buffering solution and essential oils, which were previously reduced with CO_2 to reach a pH of 6.9. Subsequently, 6 mL of inoculum from the cannulated lamb was added to each bottle, with CO_2 sprinkling.

There were 95 incubated bottles: 5 bottles for essential oil dosages and 6 for each roughage:concentrate ratios. In order to settle adjustments, five bottles without substrate were incubated to discount the gas volume from the ruminal fluid and buffering solution.

Glass bottles were tightly sealed with a rubber stopper and immediately placed in a refrigerated orbital incubator (Tecnal®, TE 421) set at 39°C and 80 rpm. Before starting the incubation countdown, bottles were depressurized with needles so that every bottle could be at the same initial pressure condition. From that moment, gas pressure from substrate fermentation was measured with a manometer model MPD-79, from Instrutherm, at 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, and 144 h. Depressurization was performed after each measurement.

Pressure values in psi were converted to volume (mL) in line with the equation developed in the Animal Nutrition Laboratory of UEL and established for local conditions: $V = 0.5702 + 3.2399 \times Psi + 0.1074 \times Psi^2$ where V = gas volume in mL; Psi = gas pressure in psi, ($R^2 = 0.99$), adjusted for one gram of DM and discounted values from bottles without substrate.

The kinetic parameters for gas production were estimated using a bicompartamental logistic model as stated by Schofield et al. (1994), $V(t) = \frac{V_{nfc}}{1 + \exp(2 - 4 \times K_{dnc} \times (T - L))} + \frac{V_{fc}}{1 + \exp(2 - 4 \times K_{dnc} \times (T - L))}$ where V(t) = volume accumulated over time t, V_{nfc} (mL) = gas volume from non-fiber carbohydrate degradation, K_{dnc} (% h^{-1}) = rate of non-fiber carbohydrate degradation, L (h) = colonization time, V_{fc} (% h^{-1}) = gas volume from fiber carbohydrate degradation,

Kd_{fc} (% h⁻¹) = rate of fiber carbohydrate degradation, and V_{final} (mL) = final gas volume from both non-fiber and fiber carbohydrate degradation. Incubation for each oil was done separately, so it was taken turns for rosemary and garlic analyses.

Subsequently, parameter values of ruminal degradation observed were obtained using R statistics (2013), with the Gauss-Newton algorithm, and were subjected to variance analysis in a 3 × 6 factorial design comprising three roughage:concentrate ratios and six essential oil dosages (R Development Core Team, 2013). When the effect of roughage:concentrate ratio was significant, mean values were compared using Tukey's test; when the effect of essential oil dosage was significant, regression analysis was performed up to second order (quadratic effect) and then equations were derived to establish minimum and maximum points. All analyses were performed at a significance level of 5%.

RESULTS

There was no interaction between rosemary oil dosage in experimental diets and roughage:concentrate ratios ($P > 0.05$), but there was an effect ($P < 0.05$) of roughage:concentrate ratios (Table 3) and rosemary oil dosage (Figure 1) on the degradation kinetics of carbohydrates.

Garlic oil did not present any interaction between roughage:concentrate ratios and dosage fed to animals ($P > 0.05$), except for the time of colonization (Figure 3). However, there was an effect ($P < 0.05$) of roughage:concentrate ratios (Table 3) and garlic oil dosage (Figure 2) on the degradation kinetics of carbohydrates.

With the addition of rosemary essential oils to diets, the roughage:concentrate ratios affected ($P < 0.05$) degradation rate of non-fiber carbohydrates, time of colonization, fiber degradation rate, volume, and final quantity of gases from total carbohydrate breakdown (Table 3).

The roughage:concentrate ratios of diets containing rosemary essential oil did not affect ($P > 0.05$) the volume of gases from the degradation of non-fiber carbohydrates, with an average value of 155.88 mL g⁻¹ of DM (Table 3). Diets containing 80% concentrate presented higher values when compared to others in terms of non-fiber carbohydrate degradation rate, gas volume, fiber carbohydrate degradation rate, and final volume. Diets containing 60% concentrate had a longer colonization time (3.72 h) than those with 50% (3.19 h) and 80% concentrate (3.20 h), which did not differ from each other (Table 3).

With garlic essential oil addition, roughage:concentrate ratios of diets did not affect ($P < 0.05$) the gas volume of non-fiber degradation and its rate (V_{nfc} and K_{d_{nfc}}, respectively), colonization time (L), volume of fiber carbohydrate degradation (V_{fc}), and final gas volume from both fiber and non-fiber carbohydrate degradation (V_{final}) (Table 3).

Diet with 60% concentrate in garlic essential oil trial presented higher values of non-fiber carbohydrate breakdown and colonization time, averaging 0.0953% h⁻¹ and 5.15 h, respectively ($P < 0.05$; Table 3). Gas volume from non-fiber and fiber carbohydrate degradation and final gas volume from both

TABLE 3 | Carbohydrate degradation kinetics in lambs fed diets with different roughage:concentrate ratios containing garlic and rosemary essential oils.

Parameter	Roughage:concentrate ratio			P	VC
	50:50	40:60	20:80		
Rosemary essential oil					
V _{nfc} (mL g ⁻¹ of DM)	155.68	158.94	153.02	0.5153	12.82
K _{d_{nfc}} (% h ⁻¹)	0.0940b	0.1015b	0.1346a	0.0001	22.61
L (h)	3.19b	3.72a	3.20b	0.0001	13.48
V _{fc} (mL g ⁻¹ of DM)	161.45b	173.59b	206.25a	0.0046	15.80
K _{d_{fc}} (% h ⁻¹)	0.0214c	0.0249b	0.0300a	0.0091	12.59
V _{final} (mL g ⁻¹ of DM)	317.10b	330.23b	359.29a	0.0001	8.21
Garlic essential oil					
V _{nfc} (mL g ⁻¹ of DM)	135.99c	147.31b	186.52a	0.0001	9.04
K _{d_{nfc}} (% h ⁻¹)	0.0829b	0.0953a	0.0919ab	0.0332	18.74
L (h)	4.75ab	5.15a	4.59b	0.0043	12.42
V _{fc} (mL g ⁻¹ of DM)	142.90b	149.15ab	163.47a	0.0046	14.83
K _{d_{fc}} (% h ⁻¹)	0.0239	0.0271	0.0270	0.1651	24.63
V _{final} (mL g ⁻¹ of DM)	278.87c	296.47b	350.51a	0.0001	7.07

V_{nfc}, Gas volume from non-fiber carbohydrate degradation; K_{d_{nfc}}, Rate of non-fiber carbohydrate degradation; L, Colonization time; V_{fc}, Gas volume from fiber carbohydrate degradation; K_{d_{fc}}, Rate of fiber carbohydrate degradation; V_{final}, Final gas volume from both non-fiber and fiber carbohydrate degradation. Mean values in the same row followed by the same letters do not differ in Tukey's test ($P > 0.05$); P, Probability; VC, variation coefficient.

processes of the 80% concentrate diet showed greater values of gas production. They had mean values of 186.52, 163.47, and 350.51 mL g⁻¹ of DM, respectively (Table 3).

There was no interaction between rosemary and garlic essential oils and roughage:concentrate ratios, so both oils were analyzed together, regardless of the roughage:concentrate ratios. However, when garlic oil was added, the bacterial colonization time interacted with the concentrate content. The mean value from the three diets was 63.33% of concentrate, meaning that dosages were analyzed within a high-concentrate diet, similar to the roughage:concentrate ratio (40:60) used in feedlot sheep production.

Rosemary essential oil dosages affected V_{nfc} in a quadratic manner, with a peak of gas production at a dose of 0.71 g L⁻¹ ($P < 0.05$, Figure 1A). The non-fiber carbohydrate degradation rate was also affected by rosemary oil in a quadratic response ($P < 0.05$, Figure 1B), with the lowest value at 0.89 g L⁻¹. The quadratic effect was again presented by the gas volume from fiber carbohydrate breakdown, with the minimum point at 1.17 mg L⁻¹ ($P < 0.05$; Figure 1C). The same quadratic response was observed for fiber carbohydrate degradation, with a dip at 1.07 g L⁻¹ of rosemary essential oil ($p < 0.05$; Figure 1D).

The experimental diets in this trial presented 43.84, 37.65, and 25.26% of NDF for diets with 50, 60, and 80% concentrate, respectively (Table 2). Therefore, diets had low quantities of fiber carbohydrates and high values of fast degradable carbohydrates: 65.77, 71.39, and 82.64% for diets with 50, 60, and 80% concentrate, respectively. They also presented low indigestible fraction, and C fraction average of 2.49%. These data indicate that diets with high potential digestibility.

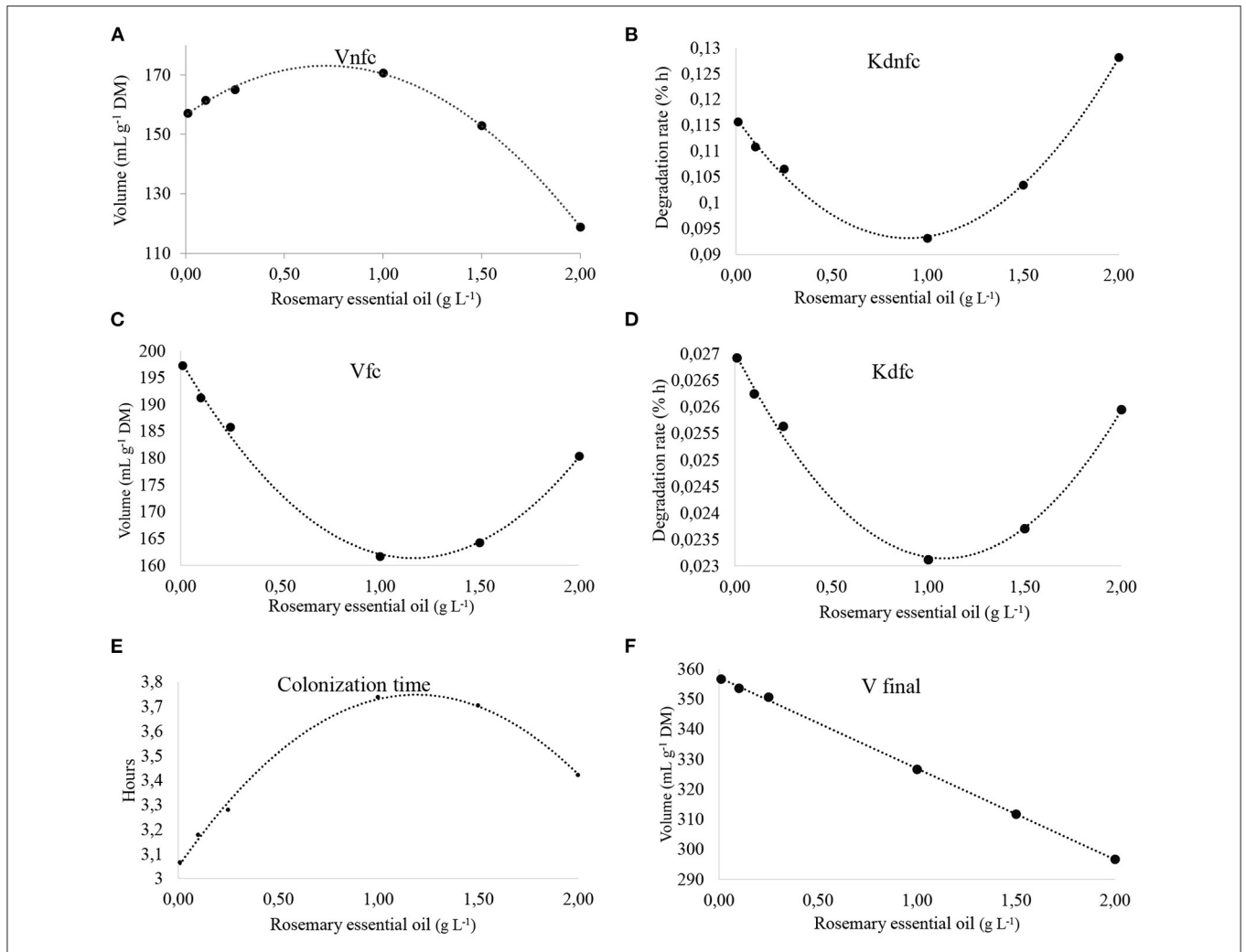


FIGURE 1 | Gas volume from non-fiber (Vnfc) and fiber (Vfc) carbohydrate degradation in mL g⁻¹ of DM, rate of non-fiber (Kdnfc) and fiber (Kdfc) carbohydrate degradation in % of DM h⁻¹, and colonization time (L) in hours and final gas volume from both non-fiber and fiber carbohydrate degradation (Vfinal) for diets containing different doses of rosemary essential oil. **(A)** (Vnfc): $y = -32.67630x^2 + 46.20715x + 157.11194$; Maximum point: 0.71; P : 0.0001; R^2 : 0.35; **(B)** (Kdnfc): $y = 0.02874x^2 - 0.05126x + 0.11567$; Minimum point: 0.89; P : 0.0123; R^2 : 0.11; **(C)** (Vfc): $y = 29.03914x^2 - 67.75866x + 197.84891$; Minimum point: 1.17; P : 0.0006; R^2 : 0.18; **(D)** (Kdfc): $y = 0.00332x^2 - 0.00713x + 0.02693$; Minimum point: 1.07; P : 0.0341; R^2 : 0.08; **(E)** (L): $y = -0.49358x^2 + 1.16382x + 3.06580$; Maximum point: 1.18; P : 0.0075; R^2 : 0.12; **(F)** (Vfinal): $y = -30.31229x + 356.63060$; P : 0.0001; R^2 : 0.31; P , Probability; R^2 , Determination coefficient.

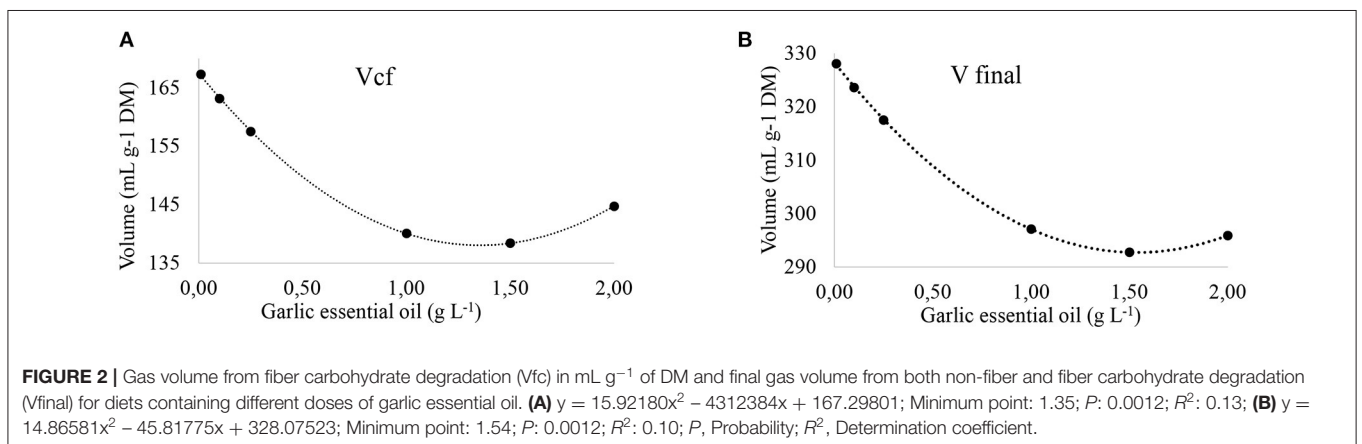
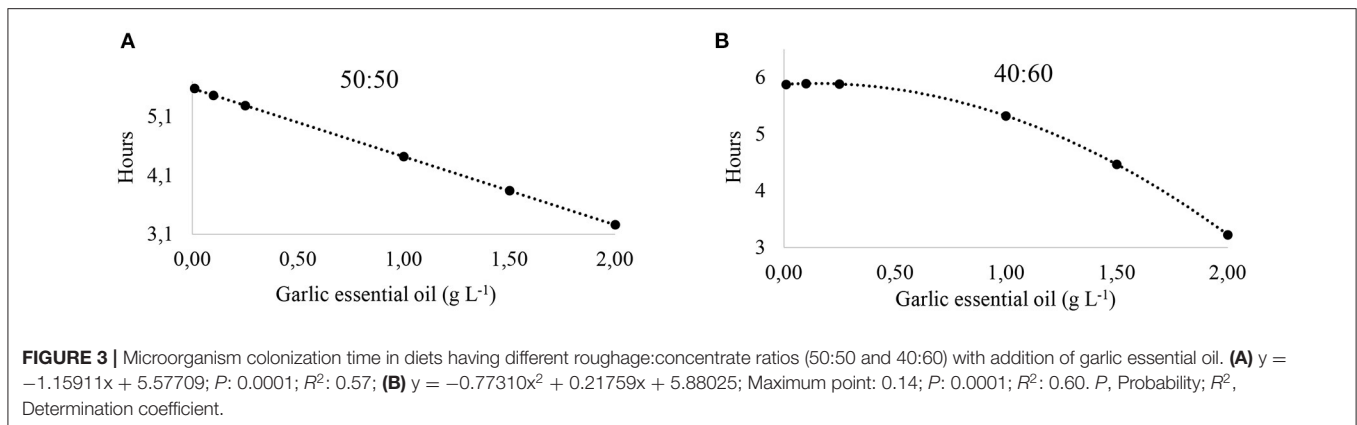


FIGURE 2 | Gas volume from fiber carbohydrate degradation (Vfc) in mL g⁻¹ of DM and final gas volume from both non-fiber and fiber carbohydrate degradation (Vfinal) for diets containing different doses of garlic essential oil. **(A)** $y = 15.92180x^2 - 4312384x + 167.29801$; Minimum point: 1.35; P : 0.0012; R^2 : 0.13; **(B)** $y = 14.86581x^2 - 45.81775x + 328.07523$; Minimum point: 1.54; P : 0.0012; R^2 : 0.10; P , Probability; R^2 , Determination coefficient.



Colonization time by ruminal microbiota was affected in a quadratic manner with rosemary essential oil dosages, with a maximum point at 1.18 g L^{-1} ($P < 0.05$, **Figure 1E**). In high-concentrate diets, it is desirable that substrates stay more time in the rumen to be degraded in order to avoid metabolic peaks and substrate desynchronization.

Rosemary oil dosages also affected the final volume gas from carbohydrate breakdown as a result of the data presented previously, but in a decreasing linear way ($P < 0.05$; **Figure 1F**). In other words, for each gram of rosemary essential oil added to the diet, there was a decrease of 30.312 mL of gas production.

Garlic essential oil dosages did not affect parameters of gas volume from non-fiber carbohydrates degradation (V_{nfc} , averaging 157.43 mL g^{-1} of DM) or fiber and non-fiber carbohydrates degradation rate (K_{dfc} and K_{dnfc} , averaging 0.0261 and $0.0902\% \text{ h}^{-1}$, respectively).

Gas volume from fiber carbohydrate degradation (V_{fc}) and final gas volume from both non-fiber and fiber carbohydrate degradation (V_{final}) presented a quadratic effect with garlic oil dosages, with a dip at 1.35 and 1.54 g L^{-1} , respectively (**Figure 2**).

Colonization time of ruminal microbiota showed interaction within dosage and roughage:concentrate ratio in a decreasing linear manner with a 50:50 ratio ($P < 0.05$; **Figure 3A**) and quadratic with a 40:60 ratio, with a peak at 0.14 g L^{-1} ($P < 0.05$; **Figure 3B**).

DISCUSSION

Non-fiber carbohydrates are expected to be fermented in concentrate diets once they are present in higher amounts. In this trial, non-fiber carbohydrate levels were 46.14 , 52.14 , and 67.16% in diets with roughage:concentrate ratios of 50:50, 40:60, and 20:80, respectively (**Table 2**). However, such fermentation should be slow to avoid substrate peaks that could lead to short-chain fatty acid accumulation, which cannot be absorbed by some animals and ruminal microbiota (Oliveira et al., 2016).

Rosemary essential oil has antimicrobial activity (Jiang et al., 2011; Tavassoli et al., 2011), so that some active substances in lower amounts probably select bacteria that break NFC; however, high levels of rosemary essential oil no longer promote selective and bacteriostatic effects, but act as bactericides, drastically

reducing the gas volume from NCF fermentation, as observed for the highest dosages used in this experiment.

When studying the effects of rosemary essential oil on *in vitro* ruminal, Castillejos et al. (2008) performed a trial with three dosages (5 , 50 , and 500 mg L^{-1}) in a diet with a 10:90 roughage:concentrate ratios and observed an increase in propionate and valerate proportions. They also reported a reduction in the proportion of acetate and butyrate without reducing the short-chain fatty acid concentration. If such results could be verified in future trials, rosemary essential oil would have potential for application in ruminant nutrition.

Once rosemary essential oil has influence in ruminal fermentation by reducing gas production from NFC and FC degradation, an intermediate dosage of 1.0 g L^{-1} , the average of optimal points, could present most of the desirable characteristics of a high concentrate diet: gas production from NFC fermentation (173.44 mL g^{-1} at dosage 0.71 g L^{-1}), low NFC degradation rate ($0.0928\% \text{ h}$ at a dosage of 0.89 g L^{-1}), low gas production from FC (161.01 mL g^{-1} at dosage 1.17 g L^{-1}), low FC degradation rate ($0.0231\% \text{ h}$ at a dosage of 1.07 g L^{-1}), and greater colonization time (3.75 h at dosage 1.18 g L^{-1}).

Gas volume from fiber carbohydrate degradation (V_{fc}) and final gas volume from both non-fiber and fiber carbohydrate degradation (V_{final}) were affected in a quadratic manner with garlic oil dosages, with minimum points at 1.35 and 1.54 g L^{-1} , respectively. As the garlic essential oil dosage was increased, cellulolytic microbiota activity was reduced to a minimum level. Experimental diets presented FC amounts varying from 43.84 to 25.25% , with an average of 35.58% , which indicates a diet with adequate fiber content to maintain animal health. Despite the possible reduction of cellulolytic microbiota, the colonization time of ruminal microbiota decreased linearly with a 50:50 ratio ($P < 0.05$) and in a quadratic manner with a 40:60 ratio, peaking at 0.14 g L^{-1} ($P < 0.05$). It is likely that garlic essential oil can select microorganisms that break non-fiber carbohydrates, reducing cellulolytic microbiota activity, and also *Archaeas*, receptors of metabolites (H^{+2} form) of fiber degradation and methanogenic.

Eom et al. (2020) performed a trial to determine the effects of *A. fistulosum* L. supplementation (once allicin was used as a secondary compound) on *in vitro* ruminal fermentation but

did not find any significant response in ruminal fermentation parameters or dry matter degradability. However, the authors noticed a reduction in methane emissions and an abundance of *Archaea*.

Nonetheless, Sahli et al. (2018) found different results from this present trial. By analyzing the effects of increasing dosages of grounded garlic on *in vitro* ruminal fermentation, they reported an increase in gas production when 32 and 64 mg of garlic were added. However, it must be stated that grounded garlic might contain water-soluble substances that are not present in garlic essential oil.

In an experiment to study the isolated chemical compounds of garlic oil, or propylpropane thiosulfonate (PTSO), Foskolos et al. (2015) evaluated ruminal fermentation parameters at several dosages and concluded that a high dose of PTSO (300 mg L⁻¹) drastically reduced ruminal fermentation, suggesting that PTSO has severe antimicrobial activity. Our results were similar to the result of this study, with respect to gas production from both non-fiber and fiber carbohydrate degradation (V_{final}), with a minimum level at garlic oil dosage of 1.54 g L⁻¹.

Results from Foskolos et al. (2015) suggested that the most effective PTSO dosage is between 50 and 100 mg L⁻¹ when ruminal fermentation produces a greater molar proportion of propionate. The difference from the results of this trial can be explained by the composition of each oil, as it can change according to the extraction process and interaction among several active substances.

Regarding colonization time, the ideal would be that digesta remained in the rumen for a longer period of time, especially because diets are highly concentrated. This was also observed for the diet with 40:60 roughage:concentrate ratio at a garlic oil dosage of 0.14 g L⁻¹. In addition to major swings in the microbiota population and differences in feed intake, rumen proportions of short-chain fatty acids remained stable, with levels generally close to 65:25:10 mols of acetate:propionate:butyrate in forage-based diets, and 50:40:10 in high-concentrate diets. Some diseases, such as acidosis, ketosis, and tympanism, are caused by ruminal fermentation disorders (Nussio et al., 2011). In order to minimize these disorders in concentrate diets, the substrate should stay in the rumen longer to be degraded more slowly, avoiding metabolic peaks and desynchronization of substrate breakdown.

Therefore, the average of minimum points (1.35 and 1.54 g L⁻¹) and maximum points (0.14 g L⁻¹ of 1.01 g L⁻¹) of garlic essential oil may promote good fermentation characteristics in diets with high concentrate amount fed to lambs.

Guided by the results of this study regarding the kinetics of carbohydrate degradation in concentrate diets fed to lambs, 1.0 g

L⁻¹ of both rosemary and garlic essential oils are recommended. Considering that sheep presents around 10.1% of BW in ruminal content (Goopy et al., 2014), the recommendation can be done as 0.01% BW for the two essential oils in this experiment. With an average daily intake of 1.44 kg DM day⁻¹ for finishing lambs weighing about 31.13 kg at feedlot (Grandis et al., 2015), the recommendation of rosemary and garlic essential oils is 0.216 g kg DM⁻¹.

CONCLUSION

Adding rosemary and garlic essential oils to diets supplied to feedlot lambs affected carbohydrate degradation kinetics. Oil dosages did not depend on the roughage:concentrate ratios. Therefore, a ruminal 1.0 g L⁻¹ is recommended for both rosemary and garlic essential oils.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee in Animal Use (CEUA) of the State University of Londrina (UEL; Protocol No. 9571.2018.80).

AUTHOR CONTRIBUTIONS

EC: wrote the manuscript and conduction of the trial. CS, AP, and GG: support in chemical analyses and incubation. ER: support in data collection and translation to English. JC and TS: support in data collection and animal care. SS: support in statistical analyses. IM: text revision and scientific consultation. OP: project coordinator, dissertation advisor, text revision, and responsible for funding provided by CNPq. All authors contributed to the article and approved the submitted version.

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