Effect of essential oils on the production of methane in the *in vitro* fermentation of Koronivia grass

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Abstract:

The objective was to evaluate the increasing use of garlic, sesame, and cinnamon oil in the production of CH_4 in 60 d *in vitro* regrowth of Koronivia grass. The addition of 0, 2.5, 5.0, 5.0, 7.5, and 10 % garlic, cinnamon, or sesame oil was evaluated in an *in vitro* fermentation using a 60-d regrowth of Koronivia grass as substrate. The variables evaluated were cumulative CH_4 production at 12, 24, 36, 36, 48, and 72 h; dry matter degradation (DMD), and CH_4 production kinetics estimators ($A=$ CH_4 production potential, $b=$ CH_4 production rate constant, and $k=$ lag time). The CH_4 production and the DMD were analyzed with a completely randomized experimental design and orthogonal contrast. The estimators were subjected to a descriptive analysis. An increase of garlic oil and cinnamon linearly reduced CH_4 production at 12, 24, 36, 48, and 72 h. The DMD decreased linearly with the use of any of the three oils ($P<0.05$). The highest value of A was obtained with 2.5 % garlic oil, and the highest value of k and b, with 10 % cinnamon oil. In conclusion, the use of garlic and

cinnamon oils resulted in a linear decrease of Koronivia grass CH_4 and Koronivia grass DMD under in vitro conditions.

Introduction

Livestock production generates 37 % of global greenhouse gas (GHG) emissions and is expected to increase to 40 % by 2050; 80.7 % of these GHGs come from the enteric fermentation of ruminants[1,2]. The main GHGs produced by ruminants are enteric and manure methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O)[3]. CH_4 is a GHG that contributes to the formation of tropospheric ozone; it also has a short life span (9 to 12 yr), but is 25 times more harmful than CO_2, so its reduction helps mitigate the adverse effects of climate change[1,2].

GHG mitigation strategies are aimed at not affecting animal performance, reducing environmental impact, and enhancing the productivity and profitability of production systems[1]. Because of this, additives should be able to modify rumen fermentation to improve energy use efficiency while decreasing rumen methanogenesis[4]. Today, approximately 200,000 plant secondary metabolites have been identified as potential modulators of the rumen microbiota, specifically in the reduction of energy loss via CH_4 synthesis[1].

Essential oil (EO) is a complex mixture of lipophilic, plant-specific volatile or aromatic chemicals; its constituents are terpenoids, phenylpropanoids, monoterpenes, sesquiterpenes, and alcohols, aldehydes, ethers, esters, ketones, and phenols[1,5]. These oils possess antioxidant properties and induce changes in the ruminal microbiome, resulting in a reduction of CH_4, increase in propionate or ruminal by-pass protein[1,4,6], antimicrobial effect of bacteria, fungi and protozoa, as well as decreased dry matter (DM) digestibility and ruminal fermentation[4], inhibition of amino acid deamination, ammonia nitrogen and acetate reduction[5], and they influence electron pathways affecting the integrity of cell membranes[1,2]. The decrease in enteric CH_4 production with the use of EO is due to the reduction of hydrogen production (alternative sinks), direct inhibition of archaea, and interruption of the symbiosis between protozoa and archaea[1].

Garlic (*Allium sativa*) oil has a broad spectrum of antibacterial activity against gram-negative and gram-positive bacteria[7]; its bioactive compounds are organic sulfides, saponins, phenolic compounds, and polysaccharides, allicin, S-allyl cysteine, diallyl disulfide, diallyl trisulfide, diallyl sulfide, and ajoene[8]. Cinnamon (*Cinnamomum verum*) oil has an antimicrobial effect due to its transcinnamaldehyde content and antioxidant activity derived from its phenolic and polyphenolic compounds[9]. For its part, sesame (*Sesamum indicum*) oil contains oleic and linoleic acid, tocopherol, sesamin, sesamolin, polyphenols, phytosterols, flavonoids, and lignans, which have anti-inflammatory and antimutagenic effects[10].

The efficiency of rumen fermentation is leading to the search for natural alternatives to mitigate GHG emissions without compromising livestock productivity; the concentration of atmospheric CH_4 continues to increase, so strategies are needed to help reduce its production. The hypothesis was that the addition of garlic, sesame, and cinnamon oils decreases CH_4 production during *in-vitro* ruminal fermentation of Koronivia grass substrate with 60 d of regrowth. Thus, the objective of this research was to evaluate the use of increasing doses of garlic, sesame, and cinnamon oil in the *in vitro* ruminal fermentation of 60-d-old Koronivia grass as a substrate for methane production and dry matter degradation.

Material and methods

The study was conducted in the animal nutrition laboratory of the Faculty of Veterinary Medicine and Animal Husbandry No. 2, located in the municipal seat of Cuajinicuilapa, Guerrero, Mexico.

Oils and substrate

The essential oils utilized were garlic (Yerbatex), sesame (Yerbatex), and cinnamon (Yerbatex). The proportions of oil evaluated were 0, 2.5, 5.0, 7.5, and 10.0 % of oil. The Koronivia grass (*Brachiaria dictyoneura*) was harvested 60 d after regrowth. The grass was dehydrated at 60 °C for 48 h in an oven (FELISA® FE-293A, Mexico) and ground to 1 mm size in a Thomas-Wiley Mill (Thomas Scientific®, Swedesboro, NJ, USA). The bromatological composition of the grass was 22.4 % dry matter (DM), 3.4 % crude protein (CP), 71.1 % neutral detergent fiber (NDF), 42.1 % acid detergent fiber (ADF), and 8.7 % ash (Ash).

In vitro gas production technique

The culture medium for the *in vitro* tests consisted of two-thirds reduced buffer-mineral solution and one-third fresh rumen fluid[11]. The reduced buffer-mineral solution contained: 150 mL of mineral solution I [6 g K_2HPO_4 (Sigma) in 1,000 mL of distilled H_2O], 150 mL of mineral solution II [6 g K_2HPO_4 (Sigma) in 1,000 mL of distilled H2O], 150 mL of mineral solution II [6 g KH_2PO_4 (Sigma) + 6 g $(NH_4)_2SO_4$ (Merck) + 12 g NaCl (Sigma-Aldrich) + 2.45 g $MgSO_4$ (Sigma) + 1.6 g $CaCl-2H_2O$ (Sigma) in 1 000 ml of distilled H_2O], 100 mL of 8 % solution of Na_2CO_3 (Merck), 100 mL of reducing solution [0.1 g L-cysteine (Sigma) + 0.1 g Na_2S-9H_2O (Meyer) + 2 mL NaOH (2N; Meyer) in 100 mL distilled H_2O] and 2 mL resazurin at 0.1% (Sigma-Aldrich). Fresh rumen fluid was obtained from a bovine with a rumen cannula grazing on pasture with pangola grass and filtered with a sky blanket to remove macroparticles of organic matter. The cattle were handled by the internal bioethics and welfare regulations of the Autonomous University of Guerrero, based on the official norms NOM-062-ZOO-1999 and NOM-051-ZOO-1995.

Subsequently, the following ratios of oil and ground plainer grass were each placed directly in a (120 mL) serological vial: 0 % (1 g grass), 2.5 % (0.025 g oil and 0.975 g grass), 5 % (0.05 g oil and 0.95 g grass), 7.5 % (0.075 g oil and 0.925 g grass), and 10 % (0.1 g oil and 0.9 g grass). 50 mL of culture medium was added to each vial, under a continuous flow of CO_2, to maintain anaerobic conditions. The vials were closed with a neoprene cap and aluminum ring with a removable center and were considered biodigesters. The biodigesters were incubated in a double boiler at 39 °C for 72 h.

Methane production

Methane (CH_4) production was determined using a Taygon® hose (2.38 mm inner Ø and 45 cm length) with hypodermic needles (20 G x 32 mm) at the ends. The needles were used to couple a biodigester with a vial trap containing NaOH (2N); this was placed inversely in a modified test tube that was used to collect the NaOH solution (2N) displaced by the CH_4 produced during incubation through the hypodermic needle placed as an outlet valve. CH_4 production was measured at 0, 12, 24, 36, 48, and 72 h[12,13]. The main reason for using this technique is that the main gases produced by the final products of *in vitro* microbial fermentation are CO_2 and CH_4, given that the rest of the gases produced in *in vitro* techniques are trace gases[14]. Likewise, NaOH can capture CO_2, as its reaction generates HCO_3[15], so the CH_4 production is measured as the displaced milliliters of NaOH solution.

Fermentation characteristics

The pH, ammonia nitrogen (N-NH₃), and dry matter degradation (DMD) were determined after 72 h of incubation. A potentiometer (Hanna® HI2211, Italy; calibration pH 7 and 4) was utilized to estimate the pH of the culture medium. To measure The ammonia nitrogen (N-NH₃) was measured by taking 1 mL of the medium contained in the biodigester and mixing it with 0.25 mL of 25 % metaphosphoric acid (Meyer®; 4:1 ratio) in a 2 mL Eppendorf tube (Neptune®, Mexico). The tube sample was centrifuged for 25 min at 3,500 xg, and the supernatant was recovered in 2 mL vials. A volume of 20 μL of this supernatant was mixed in a volumetric vial with 1 ml of phenol solution [10 mg of $Na_2(NO)\ Fe(CN)_5.H_2O$ (Meyer®) + 10 g of phenol crystals (Meyer®) in 1,000 mL of distilled water] and 1 mL of hypochlorite solution [7.5 g of NaOH (Reasol®) + 21.3 g of Na_2HPO_4 (Meyer®) + 15 mL of hypochlorite (5 %; Reasol®) in 1,000 ml of distilled water]. The mixture was incubated for 30 min at 37 °C in a (Shel Lab® 1227, USA) double boiler. Subsequently, 5 mL of distilled water was added for dilution and vortexed (Genie 2 G-560, USA). The absorbance was measured at 630 nm in a UV-VIS spectrophotometer (Jenway® 6850, USA), calibrating with a nitrogen concentration method (r^2= 0.9994)[16]. The DMD was quantified by filtering the residual solid sample from the biodigester using ANKOM® bags previously dried to constant weight. The sample bags were dried at 60 °C for 24 h in an oven. *In vitro* dry matter degradation (DMD) was calculated using the formula DMD %= (initial sample - residual sample / initial sample) * 100[17].

Methane production kinetics

The cumulative CH₄ production values were used to estimate the kinetics of CH₄ production using the Gompertz model[18]. The estimators *A*, *b* and *k* were estimated by nonlinear regression analysis, using the PROC NLMIXED procedure of the SAS statistical package[19]. The model used was:

$$\mathbf{Y} = A \exp[-b][\exp(-k\,t)];$$

Where:
$\mathbf{Y}$= CH₄ volume at time t (ml g⁻¹ of DM);
A= total CH₄ production potential when t = ∞ (ml g⁻¹ of DM);
b= constant CH₄ production rate of the potentially degradable material (ml h⁻¹);
k= time lag (h), microbial efficiency constant factor, defined as the intercept of the time axis of the tangent line at the point of inflection;
$\mathbf{t}$= incubation time.

Statistical analysis

The cumulative methane production at 12, 24, 36, 48 and 72 h, as well as the *in vitro* fermentative characteristics of each oil (garlic, sesame, and cinnamon) were analyzed using a completely randomized design with the GLM procedure of SAS[19]. Mean values were compared with Tukey's test ($P<0.05$). The response to the growing increase in oil was calculated using linear and quadratic orthogonal contrasts. It should be noted that a descriptive analysis of the CH_4 production kinetics estimators was performed.

Results

CH_4 production decreased linearly at 12, 24, 36, 36, 48, and 72 h of Koronivia grass fermentation ($P<0.05$) as the amaount of added garlic (Table 1) and cinnamon oil increased (Table 2). Sesame oil did not exhibit a linear or quadratic contrast in CH_4 production after 12, 24, 36, 48, and 72 h of grass fermentation ($P>0.05$) as more of it was added (Table 3). This indicates that garlic and cinnamon oil reduce methane production in *in vitro* tests. However, in the case of garlic oil, the trend in the decrease and difference between inclusion levels became evident only with 7.5 % or more (Table 1). While, in the case of cinnamon oil, the effect on the decrease could be observed even with as little as 2.5 % (Table 2).

Table 1: Effect of garlic oil level on CH_4 production and *in vitro* fermentative characteristics of Koronivia grass at 60 days of regrowth

Variable	Inclusion of garlic oil					MSE	Tukey test	Linear	Square
	0 %	2.5 %	5.0 %	7.5 %	10 %				
Me12	11.49[a]	11.61[a]	11.15[a]	8.64[b]	9.62[ab]	0.35	0.0027	0.0013	0.8488
Me24	24.99[ab]	28.01[a]	25.43[ab]	21.96[b]	23.31[b]	0.62	0.0027	0.0058	0.1887
Me36	34.48[ab]	37.24[a]	33.73[ab]	28.81[c]	31.08[bc]	0.84	0.0007	0.0007	0.5140
Me48	38.95[b]	43.73[a]	37.11[bc]	34.21[c]	36.63[bc]	0.88	<0.0001	<0.0001	0.7042
Me72	43.98[b]	48.86[a]	41.53[bc]	39.61[c]	40.33[c]	0.92	<0.0001	<0.0001	0.2223
pH	6.01[c]	6.10[b]	6.11[ab]	6.14[ab]	6.17[a]	0.02	<0.0001	<0.0001	0.0912
DMD	59.48	59.37	58.31	55.31	53.74	0.93	0.1815	0.0396	0.9975
N-NH$_3$	9.75	9.75	14.12	11.83	11.00	0.92	0.5416	0.5451	0.3324

Me12= methane production at 12 h of fermentation (mL g^{-1} DM), Me24= methane production at 24 h of fermentation, Me36= methane production at 36 h of fermentation, Me48= methane production after 48 h of fermentation, Me72= methane production after 72 h of fermentation, pH= hydrogen ion potential, DMD= dry matter degradation percentage, N-NH$_3$= mg dL^{-1} of ammonia nitrogen, MSE= mean standard error.
[a,b,c] Average values with different letters in the same row are different ($P<0.05$).

Table 2: Effect of cinnamon oil level on CH$_4$ production and *in vitro* fermentative characteristics of Koronivia grass at 60 days of regrowth

Variable	Inclusion of cinnamon oil					MSE	Tukey test	Linear	Square
	0 %	2.5 %	5.0 %	7.5 %	10 %				
Me12	11.66[a]	10.25[ab]	9.11[bc]	8.28[c]	8.14[c]	0.37	<0.0001	<0.0001	0.0374
Me24	25.32[a]	23.91[ab]	22.79[bc]	20.88[c]	23.68[ab]	0.42	0.0004	0.0010	0.0009
Me36	34.65[a]	31.77[b]	30.15[bc]	27.73[c]	31.45[b]	0.64	<0.0001	0.0001	0.0001
Me48	38.64[a]	36.56[ab]	34.71[bc]	33.13[c]	36.27[ab]	0.54	0.0004	0.0009	0.0004
Me72	43.31[a]	39.29[b]	38.92[b]	38.53[b]	40.71[b]	0.51	0.0007	0.0066	0.0001
pH	6.01[c]	6.11[b]	6.20[a]	6.16[ab]	6.22[a]	0.02	<0.0001	<0.0001	0.0035
DMD	59.48[a]	58.36[a]	56.88[ab]	54.58[b]	55.49[b]	0.53	0.0007	<0.0001	0.1621
N-NH$_3$	9.75[a]	7.25[a]	8.50[a]	8.50[a]	8.91[a]	0.33	0.2040	0.8484	0.0983

Me12= methane production at 12 h of fermentation (mL g^{-1} DM), Me24= methane production at 24 h of fermentation, Me36= methane production at 36 h of fermentation, Me48= methane production after 48 h of fermentation, Me72= methane production after 72 h of fermentation, pH= hydrogen ion potential, DMD= dry matter degradation percentage, N-NH$_3$= mg dL^{-1} of ammonia nitrogen, MSE= mean standard error.
[a,b,c] Average values with different letters in the same row are different (*P*<0.05).

Table 3: Effect of sesame oil level on CH$_4$ production and *in vitro* fermentative characteristics of Koronivia grass at 60 days of regrowth

Variable	Inclusion of sesame oil					EEM	Tukey test	Lineal	Cuadratic
	0 %	2.5 %	5.0 %	7.5 %	10 %				
Me12	11.66	10.59	10.51	10.80	9.99	0.22	0.1939	0.0524	0.6139
Me24	25.32	25.28	23.84	24.48	24.42	0.30	0.5145	0.2516	0.4369
Me36	34.65	32.11	32.60	33.12	32.93	0.31	0.0737	0.1977	0.0461
Me48	38.64	37.57	37.86	38.52	37.74	0.21	0.4146	0.5729	0.5973
Me72	43.31	42.70	43.12	42.49	43.29	0.24	0.8175	0.9001	0.4485
pH	6.01[c]	6.13[b]	6.14[b]	6.17[ab]	6.22[a]	0.02	<0.0001	<0.0001	0.0085
DMS	59.48[a]	57.88[ab]	56.12[b]	53.33[c]	53.29[c]	0.68	<0.0001	<0.0001	0.2876
N-NH$_3$	9.75	9.75	8.91	9.75	8.91	0.35	0.8964	0.5684	1.000

Me12= methane production at 12 h of fermentation (mL g^{-1} DM), Me24= methane production at 24 h of fermentation, Me36= methane production at 36 h of fermentation, Me48= methane production after 48 h of fermentation, Me72= methane production after 72 h of fermentation, pH= hydrogen ion potential, DMD= dry matter degradation percentage, N-NH$_3$= mg dL^{-1} of ammonia nitrogen, MSE= mean standard error.
[a,b,c] Average values with different letters in the same row are different (*P*<0.05).

Dry matter degradation (DMD) decreased linearly (*P*<0.05) as the inclusion of garlic (Table 1), cinnamon (Table 2), or sesame (Table 3) oil increased. This decrease was reflected in the pH value of the culture media; the pH augmented linearly (*P*<0.05) as the inclusion of garlic (Table 1), cinnamon (Table 2), and sesame (Table 3) oil increased.

The ammonia nitrogen content (N-NH$_3$) did not exhibit ($P>0.05$) linear or quadratic effects, or differences between levels of inclusion of garlic oil (Table 1), cinnamon oil (Table 2), or sesame oil (Table 3), as their inclusion in the fermentation of Koronivia grass increased.

The kinetics of CH$_4$ fermentation using garlic oil showed similar values in *A* and *k* when 5 and 7.5 %, respectively, were added, while in *b* they were lower with the addition of 7.5 and 10 %, compared to the values obtained without any added oil (control). Also, in relation to the control, the inclusion of 2.5 % of sesame oil resulted in lower values in the estimators *A, k*, and *b*. In contrast, in estimator *A*, all cinnamon oil inclusion levels exhibited lower values than the control; while in estimator *b*, the values decreased with the addition of 7.5 %, and in estimator *k*, with 5 and 7.5 % (Table 4).

Table 4: Average estimators of *in vitro* CH$_4$ production kinetics of plainer grass with 60 days of regrowth supplemented with increasing levels of garlic, sesame or cinnamon oil

Oil	% of inclusion	*A* (ml g^{-1} of DM)	*k* (h)	*b* (ml h^{-1})
Control	0.0	42.54	3.55	0.077
Garlic oil	2.5	47.50	3.52	0.075
	5.0	42.53	3.47	0.075
	7.5	42.55	3.37	0.068
	10.0	43.77	3.35	0.067
Sesame oil	2.5	35.62	3.23	0.071
	5.0	44.72	3.91	0.076
	7.5	41.16	3.52	0.078
	10.0	43.45	3.42	0.071
Cinnamon oil	2.5	36.61	3.69	0.077
	5.0	39.66	3.49	0.074
	7.5	34.75	3.34	0.064
	10.0	38.98	4.17	0.082

A= total methane production potential, *b*= constant methane production rate, *k*= time lag.

Discussion

The goal of ruminant microbiologists and nutritionists is to manipulate rumen microbial ecosystems to improve feed intake efficiency[7]. Additives used as CH$_4$ inhibitors act directly in the methanogenesis pathway, interrupting the process. Oils in the rumen environment exhibit toxic characteristics on methanogens and protozoa, hydrogenation of unsaturated

fatty acids (alternative sink for hydrogen), and changes in propionic production leading to reduced CH_4 production[20].

The decrease in accumulated CH_4 production at different times measured by garlic and cinnamon oils are assumed to contain terpenoids and phenylpropanoids that interact in the cell membrane, as the hydrophobic nature of their cyclic hydrocarbons allows them to accumulate in the lipid bilayer, causing conformational changes in the membrane structure that result in loss of cell membrane stability[21].

Delgadillo-Ruiz *et al*[5] utilized nonlinear models for their estimates and reported yields of 183, 99, and 141 mM L^{-1} of CH_4 when 0.1, 0.3, and 0.6 mL of cinnamon oil were added using 41.5 % alfalfa, 41.5 % wheat straw, and 17 % of a corn grain-based concentrate as substrate; these values differ from those obtained in the present study (Table 2) because they do not show a tendency to decrease CH_4 as the addition of cinnamon oil increased. Cobellis *et al*[3] reported 3.67 mL CH_4 g^{-1} of DM in a 24-h *in vitro* fermentation using alfalfa hay as substrate and adding 1.125 mL L^{-1} of cinnamon oil culture medium; these values are lower than those of the present study, even concerning the control treatment (Table 2). This is a consequence of the methodology used for measuring the CH_4, substrate, inoculum source, etc.[13], all of which influence CH_4 production.

Concentrations of up to 10 % were estimated in the present *in vitro* study; however, NRC[22] mentions that the added oils should not exceed 7 % of the dry matter of the diet, because higher contents of oil may hinder an adequate dry matter intake. For this reason, *in vivo* tests should be carried out to assess the effect of cinnamon oil on the reduction of greenhouse gases, as reductions were observed with the addition of at least 2.5 % of this oil, as well as with a minimum addition of 5 % of garlic oil.

The addition of garlic oil did not exhibit differences in dry matter degradation (DMD) between oil inclusion levels; its tendency to reduce DMD value was observed with the addition of 5 % or more. In the case of sesame and cinnamon oil, differences ($P<0.05$) and a tendency to reduce DMD were observed with the addition of as little as 5 %. The decrease in DMD can be assumed to be a consequence of hydrogen accumulation that affected fiber degradation[20], and it may be inferred that the oils reduced protein and starch degradation in response to inhibition of the bacteria used in the inoculum[23]. Also, unsaturated fatty acids are toxic to fiber-hydrolyzing bacteria; these acids adhere to the cell wall[24], thereby reducing the ability of the bacteria to attach to the grass and hydrolyze it.

In vitro fermentation of bermudagrass (*Cynodon dactylon*) produced a linear decrease in dry matter degradation with the increasing addition of thyme oil (50, 250 and 500 mL g^{-1})[25], a similar situation to that reported in the present study with the three oils. Likewise, Cobellis

et al[3] reported lower values of DMD than the present study, as they assessed 55 % of DMD in an *in vitro* fermentation where they used alfalfa hay as substrate and added 1.125 mL L^{-1} of cinnamon oil culture medium.

The pH values in the present study are ascribed to the DMD, since volatile fatty acids, which decrease the pH, are a product of its degradation; however, when the DMD decreased as the concentration of the different oils evaluated increased, the production of volatile fatty acids diminished and did not affect the pH value. Busquet *et al*[7] reported a quadratic effect on the pH value of the culture media, with a tendency to increase as more garlic oil was added to a diet containing 50:50 forage:concentrate, a behavior similar to that reported in the present study with the three oils.

The concentration of N-NH$_3$ in the present study is because oils do not inhibit the metabolism of ammonia nitrogen-producing bacteria[23]. Researchers[3] reported 13.5 mg dL^{-1} of ammonia nitrogen in an *in vitro* fermentation of alfalfa with 1.125 mL L^{-1} of cinnamon oil; these values are higher than those obtained in the present study because alfalfa has a higher protein content than the Koronivia grass. Another study reported[7] that the N-NH$_3$ content exhibited no differences concerning the treatment that did not contain garlic oil, a situation similar to that of the present study with the three oils.

The effects of these oils tend to be influenced by their components, which make it difficult to analyze their effect on ruminant nutrition. Therefore, further studies are required to identify the metabolites contained in each oil to establish their real effect on the fermentation of forages as the main producers of methane, given the fermentation stoichiometry.

The modified Gompertz equation is a common model for CH$_4$ production by degradation of a simple organic substrate[26]. The literature includes several studies that utilized this model to estimate CH$_4$ production. He *et al*[27] applied a modified Gompertz model and a first-order kinetic model to evaluate CH$_4$ production during the *in vitro* fermentation of wheat straw using bull and heifer fluid as inoculum; their results showed lower values in *A* (22 ml CH$_4$ g^{-1}) and *k* (0.945 h), as well as higher values in *b* (0.105 mL h^{-1}). Another study[28] evaluated the CH$_4$ potential and the CH$_4$ production rate of stalk bark, stalk pith, and corn stubble leaves from batch anaerobic digestion, reporting higher values than those estimated in the present study in *A* (204.8 ml CH$_4$ g^{-1}) and lower values in *k* (0.1553 h). Zhang *et al*[26] reported higher values than the present study, as they published values of 94.38 mL CH$_4$ g^{-1} for *A*, 12.38 h for *k*, and 2.46 ml h^{-1} for *b* in the fermentation of cow manure with corn stubble. With the differences in the estimators reported by other authors[26,27,28] used for comparison and those of the present study, it is assumed that whether essential oils with anti-metagenomic properties are added or not depends on the conditions under which the experiments were

performed and the substrates used, since they directly influence the kinetics of methane production.

Therefore, the modeling of CH_4 production under the conditions of the present study was important, because these serve for the design, construction and application of chemical or biochemical processes. Furthermore, they describe the characteristics of the process and allow its subsequent optimization[29].

Conclusions and implications

The addition of garlic or cinnamon oil to the *in vitro* fermentation of Koronivia grass reduces methane production and dry matter degradation. Sesame oil does not exhibit anti-methanogenic activity under the conditions of the present study, but it reduces the *in vitro* degradation of dry matter.

Acknowledgments

Conflict of interest

The authors declare that there is no conflict of interest in this manuscript.

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