Research Article

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Effects of natural blends of garlic and eucalypt essential oils on biogas production of four fibrous feeds at short-term of incubation in the ruminal anaerobic biosystem

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Abstract

BACKGROUND: The present study explored the effect of garlic and/or eucalypt oils on biogas production during *in vitro* ruminal fermentation of four agro industry byproducts. For this, 0–180 mg oil L^{-1} incubation medium was added and gas volumes were recorded from 2 to 48 h of incubation. Dry matter substrate degradability and neutral as well as acid detergent fibre were determined after 72 h.

RESULTS: Gas production and nutrient degradability was oil type dependent. The oils enhanced ($P < 0.05$) biogas and asymptotic biogas production for corn stalks and oat straw, although no effect was observed on asymptotic biogas production for sorghum straw and sugarcane bagasse. Addition of both oils decreased ($P < 0.05$) fermentation pH for corn stalks, sorghum straw and oat straw and also increased ($P < 0.05$) dry matter degradability for all four byproducts. Neutral detergent fibre degradability for all byproducts was higher ($P < 0.05$) with garlic oil. Eucalypt oil, however, decreased ($P < 0.05$) neutral detergent fibre degradability for sugarcane bagasse and corn stalks, although only weak effects were observed for sorghum and oat straws.

CONCLUSION: With respect to ruminal biogas production, the addition of garlic oil showed better environmental effects than the addition of eucalypt oil and increasing oil concentrations resulted in enhanced fermentation characteristics.

Keywords: biogases; eucalypt oil; agro byproducts; garlic oil

INTRODUCTION

The patronage of crop residues has been increased in recent years because the livestock production industry is suffering from grains and forages shortages. The large quantities of crop residues may have an important economic and environmental impact as ruminants feed.[1,2] Furthermore, some agricultural byproducts have been reported to have a low nutritional value for animals as a result of low nutrient digestibility and crude protein content, poor palatability, and high fibre content.[1,3] Therefore, the efficacy of digestive utilization is reduced in the presence of those byprducts.[4] For a better utilization of these agricultural byproducts in ruminant nutrition, an improvement of their nutritive value before feeding to an animal is necessary. Different strategies could be applied. Among those strategies, the use of feed additives, including essential and crude oils, comprises one of the most effective and safe approaches.[5]

Some crude extracts and essential oils have been reported to exhibit anti-bacterial and antioxidant activities.[6] Furthermore, an improvement of the utilization of agricultural byproducts in the presence of crude extracts and essential oils by affecting rumen fermentation was observed.[7] It was already known that animal performance was improved as a result of a better feed utilization and a higher antimicrobial activity when feed was supplemented with garlic (*Allium sativum*).[8] Extracts of garlic cloves contain several bioactive compounds such as organosulfur compounds, phenols, flavonoids and steroids.[9] Feeding of garlic oil (GO) to ruminants was shown to affect methane production. Some studies reported an inhibition of methane production accompanied by lower concentrations of acetate and short chain fatty acids and higher concentrations of butyrate and propionate.[10,11]

Eucalyptus robusta is a common plant that grows in different areas worldwide and the extracted oil has a broad-spectrum potential for medicinal and pharmaceutical applications.[12,13] The compounds identified in the essential oil of *E. robusta* leaves are mainly α-pinene, 1,8-cineole, spathulenol, globulol and viridiflorol

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Table 1. Chemical composition of the fibrous feeds (g kg^{-1} DM)

Feed ingredient	Corn stalk	Oat straw	Sorghum straw	Sugarcane bagasse
Dry matter	960	896	943	925
Organic matter	956	924	930	982
Crude protein	63	39	43	27
Ether extract	13	15	12	7
Non-structural carbohydrates	403	332	261	489
Neutral detergent fibre	477	538	614	459
Acid detergent fibre	281	380	386	324
Lignin	48	66	71	121
Cellulose	233	314	315	203
Hemicellulose	196	158	228	135

from the dried leaves of *E. robusta* from China;[12] α-pinene from fresh leaves of *E. robusta* grown in Brazil;[13] and 1,8-cineole, cryptone, α-pinene, ρ-cymene and α-terpineol from the tree grown in Tunisia.[16]

Previously, it was reported that 1,8-cineole, linalool, α-pinene spathulenol and α-terpineol were the main components in essential oil of different species of *Eucalyptus* leaves.[14,15] Moreover, eucalyptus oil (EO) exhibits substantial antibacterial properties because it contains significant amounts of tannins, phenolics, flavonoids and volatile oils.[16] Thao *et al.*[17] discussed the biological activities of the extracted oils, including their bacteriostatic, fungistatic and anti-inflammatory activity, as well as their capability to modify ruminal biogases fermentation by reducing CH_4 production and their anti-protozoal activity. For example, significant growth inhibition of *Staphylococcus aureus*, *Candida albicans* and *Escherichia coli* was reported by applying EO.[13]

The present study investigated the potential of garlic and eucalyptus oils to improve *in vitro* ruminal fibre degradation, as well as increase gas production (GP) during fermentation, resulting in higher nutritive value and feed utilization of the studied agro-industry byproducts. Hence, the present study explored the inclusion of GO and EO on biogas production and nutrient digestibility of four agricultural residues.

MATERIALS AND METHODS

Agro-industry byproducts as substrates

Four agro byproducts (corn stalk, oat straw, sorghum straw and sugarcane bagasse) were used as incubation substrates. Four batches of each feed were selected randomly. The agricultural products were manually harvested from various locations in Mexico during 2017. Forced air oven (65 °C for 72) was used for drying the samples after grinding and sieving (1 mm). Thereafter, the samples were kept in plastic bags for further treatment. Table 1 presents the chemical compositions of the studied four agriculture byproducts.

Oil extraction and gad chromatography/mass spectromety (GC/MS) identification of the essential oils

A sample of 200 g of fresh garlic clove was soaked in 200 mL of *n*-hexane for 24 h to extract the oil. The soaked material was filtered using filter paper and the marc was extracted as described above. Fresh leaves of *E. robusta* were collected, sliced into smaller pieces (100 g) and then extracted for 3 h by hydro-distillation with 500 mL of distilled water in a Clevenger type apparatus.[18] The oils obtained were dried using anhydrous Na_2SO_4. Sealed Eppendorf tubes with aluminium sheets were used to store the oils in a refrigerator at 4 °C before further use. Analysis of both oils was performed by GC/MS in accordance with methods described previously (GO,[19] EO[20]).

The chemical compositions of GO and EO were recognized by comparing their mass spectra and retention times with those deposited in the NIST 11 mass spectral database.[21]

In vitro incubations and substrates analysis

Collection of rumen inoculum from two Brown Swiss cows and subsequent laboratory procedures was carried out in accordamce with our previously reported work[22–24] and recommendations from Goering and Van Soest.[25] Collected rumen liquor was flushed with carbon dioxide (CO_2), mixed and strained through four layers of cheesecloth into a flask with oxygen (O_2)-free headspace.

Feed samples [0.5 g dry matter (DM)] were weighed into 120-mL serum bottles with the appropriate addition of oils. Garlic (GO) and *E. robusta* (EO) essential oils were included at: 30, 60, 90 and 180 mg L^{-1} incubation medium (equal to 0, 1.2, 3.6 and 7.2 mg g^{-1} DM substrate). Consequently, 10 mL of particle free rumen fluid was added to each bottle followed by 40 mL of the buffer solution[25] with continuous flashing with CO_2 to keep the media anaerobic condition. Furthermore, blanks (rumen fluid only, with no substrate) were included. Four independent samples per forage feed were used and three independent replicates for each treatment were performed. The three independent replicates were run in three different weeks. After filling all bottles, they were immediately closed with rubber stoppers, shaken and placed in a water bath at 39 °C. The volume of gas produced was recorded at 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation using a pressure transducer (Extech Instruments, Waltham, MA, USA) in accordance with the technique of Theodorou *et al.*[26]

At the end of the incubation period (48 h), the pH was recorded. Then, the content of the incubator was filtered under vacuum using sintered glass crucibles (pore size 100–160 µm, coarse porosity no. 1; Pyrex, Stone, UK). The incubation residues were dried overnight at 105 °C to obtain the apparent DM degradability. The acid (ADF) and neutral detergent fibre (NDF) were determined in the dried residues to evaluate the NDF degradability and ADF degradability. The blanks were utilized for correction of substrate contamination by the ruminal fluid.

The feed samples, ash, nitrogen, and ether extract, were analyzed using methods #934.01, #942.05, #954.01, and #920.39 as described in AOAC.[27] The NDF and ADF were measured using the methods of Van Soest *et al.*[28]

Calculations and statistical analysis

The kinetic parameters of GP were estimated according to the procedure described by France *et al.*[29] and fitted using NLIN procedure of SAS[30] using the model as:

$$y = A \times \left[1 - e^{-c(t-\text{Lag})}\right]$$

where y is the volume of GP at time t (h); A is the asymptotic GP (mL g^{-1} DM); c is the fractional rate of fermentation (h^{-1}) and Lag (h) is the discrete lag time prior to any gas is released.

Data regarding *in vitro* ruminal GP and nutrient degradability parameters (three samples of each) were analyzed in a completely randomized design, considering the fixed factors (feed type, oil type, oil dose) in the linear model.[31] The interaction (P value) between the three fixed factors was non-significant for all of the studied parameters and difficult to present in the tables. For each feed type, data for each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each feed (four samples of each) were used as the experimental unit[32] according to the model:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ijk}$$

where Y_{ijk} represents result of the ith oil type (T_i) with jth level of oil (D_j); μ is the general mean; $(T \times D)_{ij}$ is the interaction between oil type and oil dose; and e_{ijk} the experimental error. Polynomial contrasts of linear and quadratic regressions were used to show responses of feeds to increasing dietary inclusion levels of the oils (of each oil type within each feed type). Significance levels were chosen at $P < 0.05$ was considered statistically significant with $P \leq 0.10$ indicating a trend.

RESULTS

Chemical composition of agro byproducts and oils

The four byproducts roughages differed in their chemical composition. The NDF concentrations ranged from 459 g (sugarcane bagasse) to 614 g (sorghum straw) and the ADF concentrations from 281 g (corn stalk) to 386 g (sorghum straw). The CP concentrations were found to be between 27 g (sugarcane bagasse) and 63 g (corn stalk). Sugarcane bagasse contained the highest NSC concentration (489 g), whereas sorghum straw contained the lowest (261 g) (Table 1).

Thirty compounds in GO were identified by GC/MS (Table 2) and the most appendant compounds were phenol, 2-6-bis(1,1-dimethylethyl)-4-methyl (177 mg g^{-1}), carboethox ymethyl disulphide (31 mg g^{-1}), butylboronic acid (28 mg g^{-1}) and 2-propylheptanol (21 mg g^{-1}). In EO, 18 compounds could be identified. GC/MS analysis showed that the principal compounds were eucalyptol or 1,8-cineole (423.2 mg g^{-1}), α-pinene (236 mg g^{-1}), spathulenol (87.7 mg g^{-1}), terpinen-4-ol (42.4 mg g^{-1}) and 4-terpineol (26.8 mg g^{-1}) (Table 3).

GP and degradability

The asymptotic GP ($P = 0.025$) and the lag time of biogas formation ($P = 0.002$) differed between EO and GO with corn stalks. Increasing the dose of EO and GO resulted in higher (linear effect, $P = 0.019$; quadratic effect, $P < 0.001$) asymptotic GP and GP ($P < 0.05$). By contrast, at different incubation times, neither the rate of GP, nor the lag time of GP were affected ($P > 0.05$) by the addition of EO and GO. Fermentation pH ($P < 0.001$), as well as degradability of DM, NDF and ADF ($P < 0.004$), differed between EO and GO. Greater DM (linear effect, $P = 0.008$; the quadratic effect, $P = 0.002$), NDF (linear effect, $P = 0.004$; quadratic effect, $P < 0.001$) and linearly lower ADF degradability ($P = 0.048$) were observed with GO at all doses. The NDF (linear effect, $P = 0.004$; quadratic effect, $P < 0.001$) and ADF degradability (linear effect, $P = 0.048$) were decreased upon the addition of EO (Figs 1–3 and Table 4).

For oat straw, the GP rate, lag time of gas formation, GP at different incubation time, fermentation pH and degradability of ADF, NDF and DM differed ($P < 0.05$) between EO and GO. Linearly greater asymptotic GP ($P = 0.049$), rate of GP (linear effect, $P < 0.001$) and GP at diverse incubation time (quadratic and linear effects, $P < 0.05$) were observed upon inclusion of EO and GO. At different concentrations, linearly lower fermentation pH ($P < 0.001$) and greater DM degradability (linear effect, $P = 0.015$)

Table 2. Oil composition of garlic cloves (*Allium sativu* L.)

Constituent	RT[a]	Concentration[b]
Butylboronic acid	5.10	28.4
3,3′-Methylenbis (1,5,8,11-tetraoxacyclotridecane)	5.51	0.5
Triethylantimony	5.86	1.9
Methyl-thiirane	6.22	1.3
Ethanamine	6.45	0.6
Coumarin-6-ol, 3,4-dihydro-4,4-dimethyl-5,7-dinitro-	6.85	0.7
(2*E*)-2-hexenyl benzoate	6.94	1.2
2-Chloro-*N*-(3-cyano-4,6-dihydro-4,4,6, 6-tetramethylthieno[2,3-*c*]furan-2-yl)-acetamide	7.02	1.0
5-Bromo-4-nitroimidazole-2-[2-thioacetic acid]	7.14	0.8
6,7-Dimethoxy-isoflavone	7.20	1.0
1-(-Propynyl)-1-cyclohexene	7.69	0.3
7-Hexyl-eicosane	7.93	1.8
Ethyl isobutyl-ether	9.97	1.2
4H-1-Benzopyran-8-carboxylic acid-3-methyl-4-oxo-2-phenyl-, 2-(1-piperidinyl)ethyl ester	8.00	2.0
4-[*N*-Methylpiperazino]-5-nitro veratrole	8.05	2.2
3-(Prop-2-enoyloxy)tetradecane	8.69	1.8
Carbomethoxy disulphide	8.87	31.2
1,2,2,5,5-pentamethyl-3,8,9-trioxabicyclo (4.2.1) nonane	11.45	13.0
2-Propylheptanol	15.02	20.5
Ethyl nonyl ketone	15.50	7.0
2,6-Dimethyldecane	17.56	8.3
2-(3-isopropylphenyl)-1-propanol	18.29	1.2
2,6,11-Trimethyldodecane	18.53	12.1
2-Hexadecanol	21.10	1.9
2,6,10-Trimethyltetradecane	21.90	5.0
Tetradecanoic acid	23.87	1.6
Ingol-12-acetate	24.16	0.8
Phenol,2-6-bis(1,1-dimethylethyl)-4-methyl	25.43	176.6
(*Z*)-9-Octadecenoic acid	29.53	0.6
Adipic acid, dioctyl ester	38.54	5.1

[a] RT, retention time (min).
[b] Concentration (mg g^{-1} DM) based on the total areas of the total FID area obtained on an HP-5 capillary column (Hewlett-Packard, Palo Alto, CA, USA).

Table 3. Essential oil composition of eucalypt oil (*Eucalyptus robusta*)

Compound name	RT[a]	Concentration[b]
α-Pinene	6.88	236
β-Pinene	8.22	6.4
Myrcene	8.83	5.2
α-Phellandrene	9.21	5.4
p-Cymene	9.92	6.2
Limonene	10.05	5.4
Eucalyptol (1,8-cineole)	10.41	423.2
Linalool	11.26	2.1
α-Terpinene	12.24	10.1
trans-Pinocarveol	14.02	7.7
Terpinen-4-ol	15.51	42.4
Spathulenol	15.80	87.7
4-Terpineol	16.00	26.8
α-Terpineol	16.08	3.5
Cyclohexanol	17.28	1.7
Citronellol	17.36	2.7
Carvacrol	19.43	2
Pyrogallol	24.48	5.2

[a] RT, retention time (min).
[b] Concentration (mg g^{-1} DM) based on the total areas of the total FID area obtained on HP-5 capillary column (Hewlett-Packard).

and NDF (linear effect, $P = 0.004$) were found in the presence of GO (Figs 1–3 and Table 5).

The rate of GP ($P = 0.028$), GP at different incubation time, fermentation pH and degradability of DM, NDF and ADF of sorghum straw differed ($P < 0.05$) between EO and GO. With different doses, greater rates of GP ($P = 0.031$) and GP at different incubation times (quadratic and linear effects, $P < 0.05$) were found in the presence of EO and GO. Lowered pH values (linear and quadratic effect, $P < 0.001$ and $P = 0.006$) and linearly increased degradability of DM and NDF ($P < 0.05$) were noted upon inclusion of GO (Figs 1–3 and Table 6).

For sugarcane bagasse, the rate of lag time, GP of gas formation, GP at different incubation hours and degradability of NDF and DM differed ($P < 0.05$) between EO and GO. Without affecting gas formation (asymptotic GP and the lag time), linearly greater rates of GP ($P = 0.004$) and GP at diverse incubation times were observed upon inclusion of EO and GO. At various doses, greater DM degradability was observed (linear effect, $P = 0.005$) upon the addition of EO and GO (Figs 1–3 and Table 7).

DISCUSSION

Agro-industry byproducts and oil type effects

EO and GO showed different phenomena in the values reported with respect to GP parameters and nutrient degradability. Additionally, crude extracts and essential oils can modify microbial populations, digestion and fermentation of diets.[7] In this experiment, two oils (EO and GO) (Fig. 2) were evaluated for their efficacy to affect fermentation and GP of four fibrous substrates using the technique of GP *in vitro*.[7]

It was previously reported that chemical compounds such as dithin, allicin, diallyl trisulphide and sallylcysteine; minerals such as Mg, Zn, Se and germanium; and amino acids, saponins and flavonoids are found in different extracts of garlic.[33] The extracts of garlic were shown to have various bioactivities, such as bactericidal, fungicidal, antithrombotic, anti-tumour and anti-inflammatory properties.[34] In addition, EO was reported to exhibit strong antibacterial, antifungal and antioxidant activities.[13,35] α-Pinene, β-pinene and limonene were suggested to be responsible for its activity against microbial growth[36] and α-terpineol and its isomers (terpinen-4-ol and 4-terpineol) were identified as the major contributors with respect to its bioactive properties.[37]

The chemical composition of the different roughages used differed, although all compositions were in accordance with those reported previously.[1] Furthermore, the different chemical composition of substrates greatly affects the fermentation characteristics.[1,38] Generally, fibre content in the substrates had good effects on the fermentation characteristics.[24,38] Fermentation is responsible for yielding short-chain fatty acids and CO_2, H_2 and

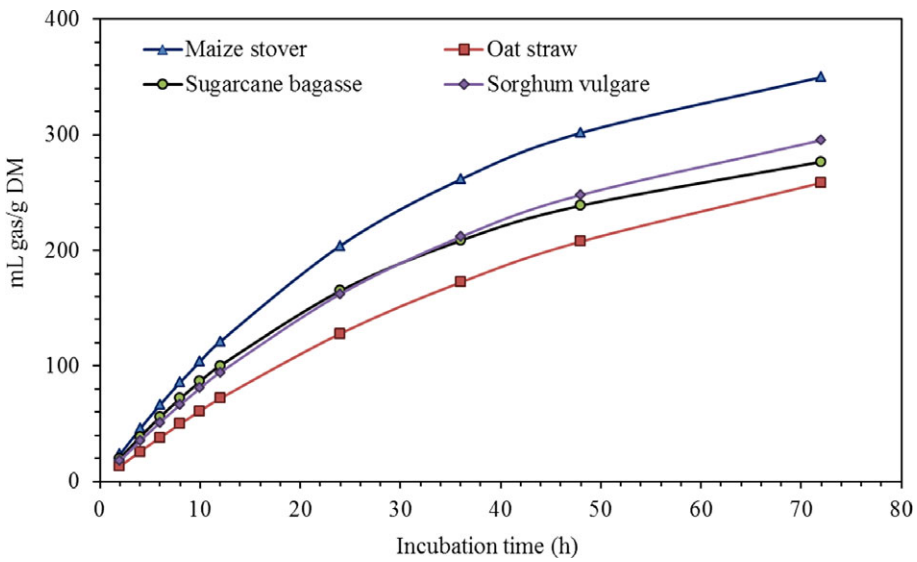

Figure 1. *In vitro* rumen gas production (mL g^{-1} DM) of the four forage substrates incubated with different levels of garlic and *Eucalyptus robusta* oils. Forage effect: SEM = 8.92, $P = 0.0001$.

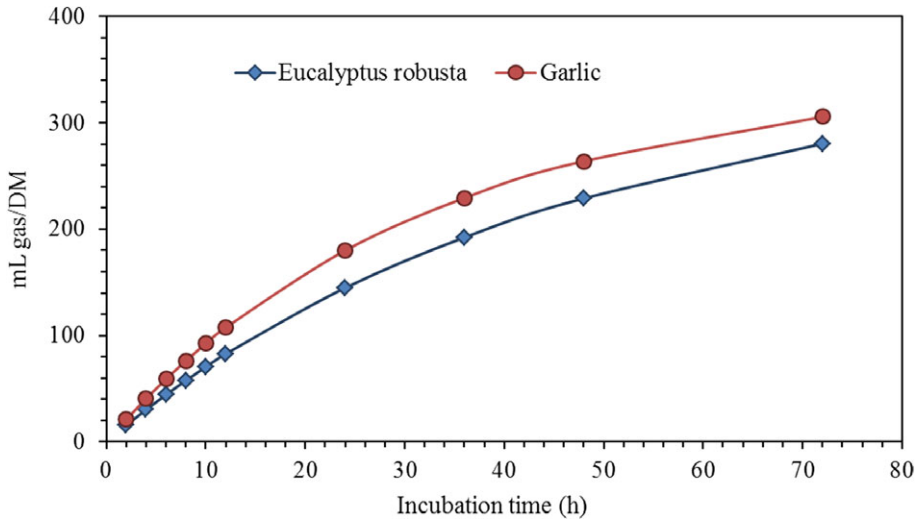

Figure 2. *In vitro* rumen gas production (mL g^{-1} DM) of the four forage substrates incubated with different levels of garlic and *Eucalyptus robusta* oils. Oil effect: SEM = 6.37, $P = 0.0038$.

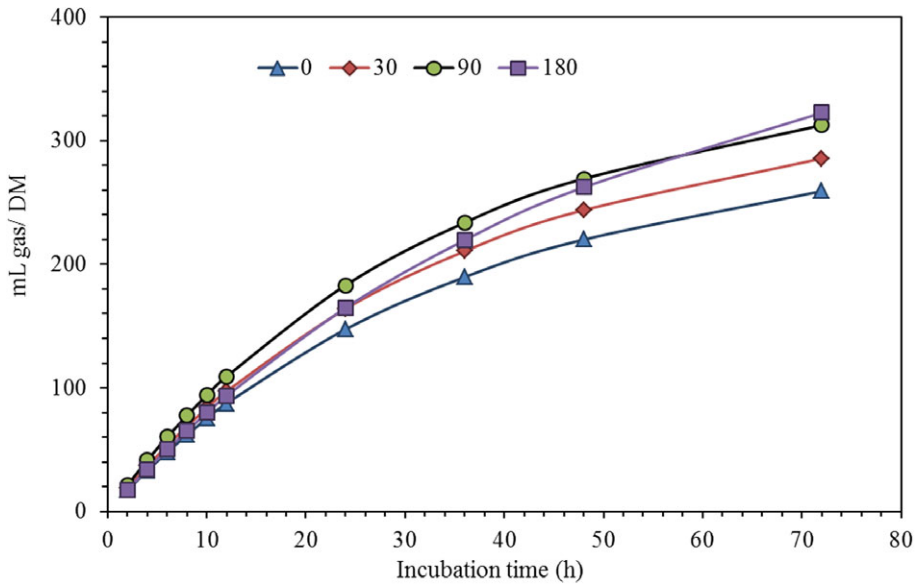

Figure 3. *In vitro* rumen gas production (mL g^{-1} DM) of the four forage substrates incubated with different levels of garlic and *Eucalyptus robusta* oils. Oil level effect: SEM = 8.92, $P < 0.001$.

CH_4.[5] Increasing the level of structural carbohydrates in the fibre causes a decrease in fermentation efficiency and GP[1,38] (Fig. 1).

Biogas production

EO and GO increased GP of corn stalks and oat straw without affecting the asymptotic GP of sorghum straw and sugarcane bagasse (Fig. 1). It was expected that increasing the doses of GO would result in detrimental effects on ruminal fermentation and nutrients digestibility because of the antimicrobial activity attributed to allicin, the sulfur compound.[39] This reveals that the tested levels of garlic oils were within the acceptable range for modifying the fermentation characteristics without any negative effects. Additionally, a reduction in acetate production and NDF digestibility was observed when adding GO at 312 mg L^{-1} culture fluid.[11] Fermentation and GP have been improved by increasing the GO doses (Fig. 3). The presence of GO might have a stimulating effect on ruminal microorganisms[40] resulting in the higher production of H_2 without increasing CH_4 emission.[41]

The increased GP in the presence of EO reveals that the levels of EO were within the acceptable range and tolerant for ruminal microbial activity and growth. The inclusion of EO increased GP[42]; whereas inclusion of eucalyptus leaf powder at 6% decreased GP *in vitro*.[43] The observed difference might be a result of the much higher EO levels in the studies carried out by Pierre[42] and Manh *et al.*[43] However, at low and moderate levels of phenolic compounds, the microbial activity was improved,[44] which might be a result of rumen microorganisms being capable of accepting those compounds as a source of energy.[45]

Oils inclusion increased the rates of GP from sorghum straw and sugarcane bagasse without any significant change in the rate GP of corn stalks and oat straw (Fig. 1). The greater rate of GP observed upon the addition of the oils may be related to the antioxidant activity of GO and EO, which are able to remove free

Table 4. *In vitro* rumen gas kinetics of corn stalk as affected by the addition of garlic and eucalypt essential oils (mg L^{-a} incubation medium)

	Gas production parameters[a]			Gas production (mL g^{-1} DM) at										pH and degradability (mg degraded g^{-1} incubated)[b]			
Oil	*A*	*c*	Lag	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	319	0.033	3.48	20	38	55	72	87	101	169	216	248	284	6.79	620	483	347
30	373	0.032	3.70	24	45	66	85	103	119	199	252	288	330	6.76	677	429	316
90	461	0.027	4.78	23	45	66	86	104	122	209	272	319	379	6.76	676	435	322
180	527	0.021	3.30	21	40	59	77	95	111	198	266	319	395	6.76	654	412	305
30	380	0.035	4.52	25	49	71	92	111	129	213	269	306	347	6.61	716	724	318
90	412	0.034	5.62	27	51	75	96	117	136	227	288	328	374	6.62	707	643	274
180	413	0.032	5.47	25	49	71	92	111	130	217	276	317	364	6.41	702	660	310
SEM	21.9	0.0038	0.360	1.7	3.2	4.5	5.5	6.3	7.0	8.5	7.9	7.1	9.1	0.041	25.0	24.6	13.8
P value																	
Oil type	0.025	0.095	0.002	0.068	0.068	0.066	0.064	0.062	0.061	0.060	0.087	0.261	0.499	< 0.001	0.004	< 0.001	0.003
Oil dose																	
Linear	0.019	0.689	0.108	0.016	0.013	0.011	0.008	0.006	0.005	0.005	< 0.001	< 0.001	< 0.001	0.027	0.008	0.004	0.048
Quadratic	< 0.001	0.061	0.091	0.586	0.520	0.442	0.375	0.302	0.241	0.021	0.002	< 0.001	< 0.001	0.003	0.002	< 0.001	0.060
Oil type × oil dose	0.054	0.438	0.062	0.591	0.603	0.606	0.611	0.617	0.626	0.688	0.722	0.530	0.141	0.002	0.005	< 0.001	0.009

[a] *A* is the asymptotic gas production (mL g^{-1} DM); *c* is the rate of gas production (h^{-1}); Lag is the initial delay before gas production begins (h).
[b] ADFD, acid detergent fibre; degradability DMD, dry matter degradability; NDFD, neutral detergent fibre degradability.

Table 5. *In vitro* rumen gas kinetics of oat straw as affected by the addition of garlic and eucalypt essential oils (mg L^{-a} incubation medium)

Oil (mg L^{-1} incubation medium)	Gas production parameters[a]			Gas production (mL g^{-1} DM) at										pH and degradability (mg degraded g^{-1} incubated)[b]			
	A	*c*	Lag	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	317	0.011	2.24	8	16	23	31	38	45	84	118	148	198	6.81	503	597	393
30	343	0.016	2.18	11	21	31	40	49	58	106	146	179	229	6.71	490	627	418
90	411	0.014	1.79	11	21	31	41	51	60	111	155	192	250	6.76	522	612	439
180	382	0.010	2.50	15	29	43	57	69	82	146	196	236	292	6.78	527	593	435
30	340	0.026	3.71	17	34	49	64	78	91	158	206	242	287	6.68	650	694	463
90	333	0.022	5.31	20	39	57	74	89	104	175	224	258	297	6.70	639	745	423
180	383	0.023	1.97	12	24	35	46	57	67	124	173	215	282	6.68	593	732	455
SEM	35.0	0.0019	0.561	1.0	1.9	2.8	3.6	4.4	5.2	8.6	11.0	12.5	3.9	0.012	24.1	18.9	19.6
P value																	
Oil type	0.860	0.008	< 0.001	0.003	0.003	0.004	0.004	0.005	0.006	0.002	0.004	0.009	0.034	< 0.001	0.001	< 0.001	0.018
Oil dose																	
Linear	0.049	< 0.001	0.242	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001	0.015	0.004	0.908
Quadratic	0.114	0.756	0.495	0.011	0.011	0.009	0.008	0.007	0.006	0.003	0.001	0.007	0.002	0.734	0.306	0.067	0.869
Oil type × oil dose	0.166	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	0.006	0.043	< 0.001	0.026	0.006	0.221

[a] *A* is the asymptotic gas production (mL g^{-1} DM); *c* is the rate of gas production (h^{-1}); Lag is the initial delay before gas production begins (h).
[b] ADF, acid detergent fibre; degradability DM, dry matter degradability; NDF, neutral detergent fibre degradability.

radicals from the fermentation medium, resulting in conditions more appropriate for microbial activity.[16,46]

Fermentation pH and nutrient degradability

EO and GO decreased the fermentation pH of corn stalks, oat straw and sorghum straw. The values for all roughages ranged from 6.41 to 6.85, which are within the acceptable range for fibre digestion.[47] Adding essential oils to animal diets was already reported to decrease the pH in the rumen as a result of increasing dietary energy density[5] and altered total ruminal volatile fatty acids concentrations.[11] With the results of the present study, the linear decrease in the pH with the inclusion of GO was expected because DM degradation increased with GO inclusion at different levels.

In ruminant diets, the observed decrease in pH during fermentation may be responsible for the inconsistency between the results

Table 6. *In vitro* rumen gas kinetics of sorghum straw as affected by the addition of garlic and eucalypt essential oils (mg L^{-a} incubation medium)

Oil (mg L^{-1} incubation medium	Gas production parameters[a]			Gas production (mL g^{-1} DM) at										pH and degradability (mg degraded g^{-1} incubated)[b]			
	A	*c*	Lag	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	320	0.019	3.45	11	22	32	42	51	60	108	146	176	220	6.81	493	604	397
30	322	0.021	3.19	13	25	36	48	58	68	121	162	194	240	6.79	489	633	432
90	340	0.030	4.27	20	39	56	73	88	103	173	222	256	297	6.78	520	605	435
180	379	0.028	4.39	16	32	47	62	76	90	163	223	271	342	6.79	549	582	425
30	359	0.032	5.04	23	44	63	82	99	115	193	246	282	324	6.68	783	687	449
90	353	0.029	4.92	27	51	74	95	114	132	215	267	299	332	6.67	601	750	380
180	442	0.020	7.44	17	33	49	64	79	93	166	223	269	333	6.67	640	742	318
SEM	21.1	0.0032	1.481	1.5	2.8	4.0	5.0	5.9	6.6	9.3	9.7	9.2	7.8	0.011	37.6	18.8	12.5
P value																	
Oil type	0.843	0.028	0.226	0.009	0.009	0.008	0.008	0.007	0.007	0.004	0.002	0.001	0.001	< 0.001	0.005	< 0.001	< 0.001
Oil dose																	
Linear	0.365	0.031	0.669	0.005	0.004	0.003	0.003	0.002	0.002	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	0.010	0.630
Quadratic	< 0.001	0.180	0.134	0.104	0.077	0.058	0.042	0.030	0.021	0.001	< 0.001	< 0.001	< 0.001	0.006	0.397	0.100	0.061
Oil type × oil dose	0.442	0.317	0.773	0.013	0.012	0.011	0.010	0.009	0.008	0.003	0.001	0.004	< 0.001	< 0.001	0.009	0.001	0.003

[a] *A* is the asymptotic gas production (mL g^{-1} DM); *c* is the rate of gas production (h^{-1}); Lag is the initial delay before gas production begins (h).
[b] ADF, acid detergent fibre; degradability DM, dry matter degradability; NDF, neutral detergent fibre degradability.

Table 7. *In vitro* rumen gas kinetics of sugarcane bagasse as affected by the addition of garlic and eucalypt essential oils (mg L^{-a} incubation medium)

Oil (mg L^{-1} incubation medium	Gas production parameters[a]			Gas production (mL g^{-1} DM) at										pH and degradability (mg degraded g^{-1} incubated)[b]			
	A	*c*	Lag	2 h	4 h	6 h	8 h	10 h	12 h	24 h	36 h	48 h	72 h	pH	DM	NDF	ADF
Control	383	0.015	3.53	11	22	32	42	51	60	110	151	185	237	6.76	477	652	468
Eucalyptus oil																	
30	396	0.024	4.29	16	30	44	58	70	82	143	189	223	271	6.71	523	437	389
90	398	0.027	2.93	20	39	56	72	86	100	162	202	229	262	6.85	524	457	389
180	453	0.013	4.24	11	22	32	42	52	62	115	161	200	263	6.85	522	435	391
Garlic oil																	
30	290	0.053	1.52	29	55	79	100	119	136	208	246	267	283	6.81	580	606	407
90	350	0.041	0.86	28	53	77	98	118	136	219	270	301	332	6.73	593	633	420
180	333	0.039	0.59	25	48	70	90	108	125	202	250	281	312	6.63	593	602	409
SEM	26.5	0.0052	0.639	2.7	5.0	7.0	8.7	10.2	11.5	16.5	18.8	19.9	20.7	0.0	22.5	21.3	13.6
P value																	
Oil type	0.108	0.009	0.002	0.003	0.003	0.002	0.002	0.002	0.002	0.004	0.001	0.004	0.047	0.864	0.008	< 0.001	0.113
Oil dose																	
Linear	0.055	0.004	0.361	0.006	0.005	0.005	0.005	0.005	0.006	0.001	0.003	0.009	0.047	0.924	0.005	0.993	0.064
Quadratic	0.116	0.845	0.190	0.566	0.540	0.511	0.485	0.457	0.435	0.312	0.232	0.182	0.125	0.587	0.050	0.845	0.515
Oil type × oil dose	0.034	0.028	0.066	0.062	0.062	0.064	0.065	0.067	0.069	0.097	0.150	0.228	0.373	0.003	0.383	< 0.001	0.742

[a] *A* is the asymptotic gas production (mL g^{-1} DM); *c* is the rate of gas production (h^{-1}); *Lag* is the initial delay before gas production begins (h).
[b] ADF, acid detergent fiber; degradability DM, dry matter degradability; NDF, neutral detergent fiber degradability.

of the present study and those of previous studies. The inclusion of oil was shown to have a positive effect on fermentation characteristics, whereas other studies reported negative or weak effects.[44] At low pH, microorganisms are more susceptible to the presence of essential oils in ruminant diets.[10]

Biogas is an indicator of DM digestibility. Increased DM and NDF digestibility with GO supplementation suggests a higher availability of energy to the microorganisms present in the rumen.[8] This also results in an increase in GP. Supplementation with GO resulted in higher ruminal DM and OM digestibility.[48] Moreover, enhanced nutrient digestibility with feeding garlic to dairy cows at 1% of feed intake was observed.[49] Conversely, Busquet *et al.*[11] reported a missing effect of GO on DM, OM, NDF and ADF digestibility, suggesting that the presence of GO did not result in a modification of the fermentability of the overall diet. They included GO at 31.2 mg L^{-1} incubation medium, which is very small amount compared to doses used in the present experiment.

The addition of EO decreased NDF degradability of corn stalks and sugarcane bagasse, with weak effects on NDF degradability of sorghum straw and oat straw, indicating the effect of the chemical composition of the incubated substrates. Both corn stalks and sugarcane bagasse have lower NDF and greater NSC concentrations compared to sorghum straw and oat straw. A reduction in cellulolytic bacteria[50] and bacterial adhesion to the substrate and fibrolytic activity of rumen microbes[51] resulted in lower degradability. A decreased *in vitro* degradability with the inclusion of eucalyptus leaf powder was observed at increasing levels up to 6%.[44] The inconsistent results may be related to the level and type of eucalyptus sources and incubated substrates. However, EO decreased NDF degradability with increased GP, revealing that the observed increase in GP is a result of the fermentation of another feed nutrient such as non-structural carbohydrates.

CONCLUSIONS

The inclusion of garlic and eucalypt oils improved the environmental effects on ruminal biogases and the fermentation of the four evaluated agro byproducts in a different manner depending on the chemical composition of each feed. Garlic oil had a better effect compared to eucalyptus oil as an environmentally friendly additive to agro byproducts in ruminant feeding. Increasing the dose of garlic and eucalyptus oils enhanced the fermentation parameters, with greater effect being observed with the dose 180 mg oil L^{-1} incubation medium. However, further research is necessary to establish the efficiency of garlic and eucalyptus oils *in vivo* trials.

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