Original Article

Use of Essential Oils for Manipulation of Rumen Microbial Fermentation Using Batch Culture

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Abstract

The objective of this study was to evaluate the effects of various levels of essential oils on feed digestibility, gas production and rumen fermentation. Two batch cultures were designed for screening various doses of each essential oil (EO). Treatments were control (CON), cinnamon oil (CIN), clove oil (CLO), garlic oil (GAR), ginger oil (GIN), and lemongrass oil (LEM). Dosages were 0, 200, 400, 800, and 1600 mg/kg DM in experiment I (1st batch) and 50, 100, 150, and 200 mg/kg DM in experiment II (2nd batch). Digestibility of DM (DMD), neutral detergent fiber and acid detergent fiber was measured at 24 h and 48 h post incubation, while gas production (GP) was read at 3, 6, 12, 24, 36, and 48 h post incubation. Experimental diet used was a dairy type ration consisting of 50% forage (35% grass hay and 15% alfalfa hay) and 50% concentrate (20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplements). All essential oils could improve DM disappearance with consistent results in both experiment I and experiment II. Meanwhile, the essential oils had no effect on NDF and ADF digestibility. Total VFA concentration and individual VFA proportion in experiment I were not affected by the essential oils. However, 200 mg/kg DM of each EO increased total VFA concentration without any effect on individual VFA proportion in experiment II. Ammonia N concentration was reduced by the essential oils in both experiments I and II, confirming the effect of essential oils on deamination. However, the effect of EOs on methane production was apparently negligible. These results suggest that the EOs used in the present study could be potentially developed as rumen modifiers to improve feed digestibility in the rumen.

Keywords: ammonia N, essential oil, feed digestion, gas production, rumen fermentation

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Introduction

Plant essential oils (EOs) from a variety of sources have been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several reviewed papers (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011). In rumen, protein is hydrolyzed to oligo-peptides by proteolytic bacteria. Afterwards *prevotella* degrades oligopeptides to dipeptides. Then, various species of bacteria produce dipeptidases and metaloproteases for degrading dipeptides to amino acids. Afterwards deamination takes place, changing amino acids to ammonia by Hyper Ammonia-Producing Bacteria (HAPB) including *Clostridium sticklandii* and *Peptostreptococcus anaerobius*. Cinnamon, clove, garlic, ginger, and lemongrass are herbs of interest. These herbs are widely used in tropical countries as one of human food composition. Cinnamon oil (cinnamaldehyde) and clove oil (eugenol) were tested in several studies (Cardozo et al., 2005; Busquet et al., 2006; Cardozo et al., 2006; Fraser et al., 2007). Garlic oil and garlic oil compounds were explored as alternatives to antibiotics to manipulate rumen fermentation due to their well-known antimicrobial effects (Ramos-Morales et al., 2013). Garlic oil and garlic derived compounds were demonstrated to have antimethanogenic property with mixed effects on rumen fermentation (Busquet et al., 2005a; Chaves et al., 2008c). In addition, ginger oil can decrease ammonia N without affecting VFAs (Busquet et al., 2006). Ginger oil was also detected to have inhibitory effects on 10 different micro-organisms (Hammer et al., 1999), but limited studies showed no effect on rumen VFA concentration in a continuous culture (Busquet et al., 2005b). Lemongrass showed its antibacterial (Valero and Salmeroìn, 2003), antioxidant (Cheel et al., 2005), and antihyper-NH_3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as its effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). However, lemongrass EO has been evaluated insufficiently on its effects on ruminal fermentation. Lemongrass powder can decrease ammonia N without affecting VFAs. Moreover, lemongrass powder can decrease protozoal population (Wanapat et al., 2008). The objective of this study was to determine the effect of EO supplementation on nutrient degradability, gas production (GP) and fermentation characteristics using batch culture.

Materials and Methods

Experimental design and treatments: Both experimental designs were complete randomized designs with three replicates per treatment. Treatments were control (CON), cinnamon oil (CIN), clove oil (CLO), garlic oil (GAR), ginger oil (GIN), and lemongrass oil (LEM). The EOs were purchased commercially (purity >99%; Phodé S.A., Albi, France). In experimental I (Exp. I) five different doses were used for each EO; 0, 200, 400, 800, and 1600 mg/kg substrate DM. In experiment II (Exp. II) treatments were the same as in Exp. I; the highest dose (200 mg/kg DM) for each EO was selected based on the results from Exp. I; *i.e.* digestibility of DM and ammonia N. The dosages of EO were 0, 50, 100, 150, and 200 mg/kg substrate DM in Exp. II. Nutrient degradability, gas production (GP) and fermentation characteristics were evaluated in batch culture. Diet used was a dairy type ration consisting of 50% forage (35% grass hay and 15% alfalfa hay) and 50% concentrate (20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplements) (Table 1). The substrates were ground through 1 mm screen (standard model 4 Wiley Mill; Arthur Thomas Co., Philadelphia, PA, USA) and mixed with the EOs before weighing into a test bag. Zero point five gram (DM basis) of substrate was weighed into a ANKOM F57 filter bag (pore size of 50 µm, Ankom Technology Corp., Macedon, NY, USA) and sealed for *in vitro* incubation.

Table 1 Ingredient and chemical composition of the diet

Ingredient composition (%)	
Grass hay	35.0
Alfalfa hay	15.0
Barley grain	20.0
Corn DDGS	10.0
Wheat DDGS	10.0
Canola meal	5.0
Vitamin and mineral supplements	5.0
Chemical composition (%)	
Dry matter	93.2
Neutral detergent fiber	41.8
Acid detergent fiber	20.5
Crude protein	16.1

Source of rumen fluid for in vitro incubation: Inoculum for the batch culture was obtained from three ruminally fistulated beef heifers (Spayed beef heifer) fed a diet consisting of 64% barley silage, 6% grass hay, 27% dry-rolled barley grain, and 3% vitamin and mineral supplements. Rumen fluid was collected from different sites within the rumen, pooled, and squeezed through PeCAP® polyester screen (pore size 355 µm; B & S Thompson, Ville Mont-Royal, QC, Canada) into an insulated thermos, and transported immediately to the laboratory. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care (1993).

In vitro incubation was in 100 ml glass bottles (with 3 replicates/run) fitted with rubber stoppers to prevent escape of fermentation gases. Sufficient anaerobic media was prepared the day before the incubation according to the method of Hall et al. (1998) except that cysteine HCl was not substituted for Na_2S. Forty-five milliliters of prewarmed media and 15 ml of inoculum were added anaerobically to the 100 ml bottles by flushing with oxygen free CO_2. The bottles were sealed immediately with a 14 mm butyl rubber stopper plus aluminum crimp cap and incubated at 39°C for 24 or 48 h. The incubation was repeated with two runs.

Sample collection and processing: At pre-determined time points, headspace gas production (GP) was measured at 3, 6, 12, 24, 36 and 48 h post incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI, Omega

Engineering, Inc., Laval, QC., Canada) connected to a visual display (Data Track, Christchurch, UK). A volume of 15 ml gas was sampled using a syringe and transferred into 6.8 ml Exetainer vials (Labco Ltd., Wycombe, Bucks, UK) for immediate measurement of CH_4. Methane concentration was determined using a gas chromatography (Varian 4900 GC; Agilent Technologies Canada Inc., Mississauga, ON, Canada). Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio et al. (1999) as follows:

$$\text{Gas volume} = 0.18 + (3.697 \times \text{gas pressure}) + (0.082 \times \text{gas pressure}^2)$$

Kinetic parameters of GP were calculated using the equation of France et al. (2000) as follows:

$$A = b \times (1 - e^{-c(t-L)})$$

where A is the volume of GP at time t, b is the asymptotic GP (ml/g DM), c is the rate of GP (/h), and L (h) is the discrete lag time prior to gas production.

After 24 h and 48 h of incubation, the bags were removed from the vials and washed under stream of cold water until the water ran clear. The bags were dried in an oven at 55°C for 48 h to determine DM degradability. NDF concentration in the residue was determined as described by Van Soest et al. (1991) using heat-stable α-amylase (Termamyl 120 L, Novo Nordisk Biochem, Franklinton, NC, USA) and sodium sulfite. Procedures to analyze NDF and ADF were adapted for use in an ANKOM^{200} fiber analyzer (Ankom Technology Corp., Macedon, NY). The NDF and ADF values were expressed inclusive of residual ash. Total N using flash combustion (Carlo Erba Instruments, Milan, Italy).

At the end of incubation, the vials were removed from the incubator. Gas pressure and gas samples were then taken into the vials and placed in ice to stop fermentation. The vials were opened as soon as possible for measurement of ending fermentation pH and taking of supernatant aliquots for VFA and NH_3-N analyses.

The volatile fatty acid (VFA) and NH_3-N analysis were measured for the 24 and 48 h incubation after measuring gas and pH. Two 5 ml samples were taken from the bottle directly at the end of time point, placed in screw-capped vials preserved with 1 ml of 25% (wt wt^{-1}) metaphosphoric solution, or with 1 ml of 1% H_2SO_4, and immediately frozen at -20°C for VFA and NH_3-N analysis, respectively. Concentration of VFA was quantified using gas chromatograph (model 5890, Hewkett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mmi.d., 1 µm phase thickness, Zeborn ZB-FAAP, Phenomenex, Torrance, CA) and flame ionization detection, and crotonic acid (trans-2-butenoic acid) was used as the internal standard. The NH_3-N was determined as described by Rhine et al. (1998). Five milliliter of samples from the vials was added to 1 ml of 1.07N sulfuric acid.

Statistical analysis: Data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) to account for the fixed effect of EO source, EO dosage, interaction between EO and dosage, and the run was the random effect (experimental unit). The effect of increasing levels of EO from 0, 200, 400, 800 to 1600 mg/kg DM or 0, 50, 100, 150 to 200 mg/kg DM in the substrate was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. Differences were declared significant at $p \leq 0.05$. Trends were discussed at $0.05 < p \leq 0.10$ unless otherwise stated.

Results

Feed digestibility: In experiment I, DM digestibility (DMD) increased in all treatments both at 24 and 48 h when compared with the control group (Table 2). The 200 and 400 mg/kg DM CIN linearly increased, but the 1600 mg/kg DM decreased DMD ($p<0.01$) at 24 h incubation. Only the 800 mg/kg DM CIN quadratically increased DMD at 48 h incubation ($p<0.01$). All CLO doses linearly increased DMD at either 24 or 48 h post incubation ($p<0.01$). The 200 and 400 mg/kg DM GAR linearly increased, but the 1600 mg/kg DM linearly decreased DMD both at 24 and 48 h ($p<0.01$). The 200 to 800 mg/kg DM GIN and LEM quadratically improved DMD either at 24 or 48 h ($p<0.05$), but the dose of 1600 mg/kg DM did not affect DMD. NDF and ADF digestibility was unaffected by all EO treatments (Table 2).

In experiment II, the DM digestibility also increased in all treatments at both 24 and 48 h when compared with the control group (Table 6). In addition, at the highest dose of 200 mg/kg DM the EOs linearly improved DMD in all treatments at either 24 or 48 h incubation ($p<0.05$) when compared with the control group. The EOs at the dose of below 200 mg/kg DM also linearly improved DMD, including the 150 mg/kg DM CIN at 24 h ($p<0.01$), 150 mg/kg DM CLO at 48 h ($p<0.01$), and 150 mg/kg DM GAR at 24 h ($p<0.05$). However, disappearances of NDF and ADF were unaffected by the treatments (Table 6).

Rumen fermentation: In experiment I, the cumulative gas production was not different in most treatments, but was quadratically increased by the 200, 400, and 800 mg/kg DM CIN, GAR and GIN at 24 h (Table 3). The total VFA, individual VFA, and methane production were similar in all treatments (Table 4 and Table 5). The ammonia N concentration linearly reduced in all treatments ($p<0.05$) (Table 5). The methane production was quadratically reduced by the 200 ($p<0.05$), 400 ($p<0.05$), and 800 ($p<0.05$) mg/kg DM CIN and GAR at 24 h, whereas it was increased by the 200 ($p<0.05$) and 800 ($p<0.05$) mg/kg DM GIN. In contrast, CLO and LEM did not affect the methane production. At 48 h of incubation, the VFA and methane production were not significantly different among the treatments (Table 4 and Table 5, respectively). In experiment II, at the dose of 200 mg/kg DM, all EOs linearly increased the cumulative gas production at 24 h and 48 h (Table 7). However, the 50 mg/kg DM of all EOs had no effect, but the 100 and 150 mg/kg DM CLO, GAR, and LEM linearly

increased the cumulative GP (p<0.01). Table 8 shows that at 200 mg/kg DM all EOs linearly increased the total VFA at 48 h of incubation, however, only the 200 mg/kg DM GAR and LEM improved the total VFA at 24 h (p<0.05 and p<0.05, respectively). All of the treatments did not affect individual VFA. The ammonia N concentration was linearly decreased by the 200 mg/kg DM of all EOs (Table 9). The dose of 200 mg/kg DM CLO, GIN, and LEM linearly increased the methane production (p<0.01) at 24 h, while the 200 mg/kg DM CIN and LEM increased the methane production at 48 h (p<0.01 and p<0.05, respectively) (Table 9).

Table 2 Effects of essential oils on degradability of DM, NDF and ADF in batch culture (Experiment 1)

	EO[a]	Dose (mg/kg DM)						*P*-value	
		0	200	400	800	1600	SEM[b]	Linear	Quadratic
					DM degradability (%)				
24 h	CIN	54.9[b]	56.9[ab]	57.1[a]	54.4[b]	48.3[c]	0.75	0.01	0.01
	CLO	54.9[b]	58.0[a]	59.4[a]	59.0[a]	54.8[b]	0.53	0.01	0.01
	GAR	54.9[b]	58.5[a]	55.9[b]	52.1[c]	49.8[d]	0.33	0.01	0.14
	GIN	54.9[c]	60.4[a]	59.7[ab]	58.1[b]	54.2[c]	0.69	0.76	0.01
	LEM	54.9[c]	56.2[b]	56.7[b]	58.8[a]	54.3[c]	0.47	0.59	0.01
48 h	CIN	62.9[b]	62.6[b]	63.2[b]	66.3[a]	62.6[b]	0.98	0.68	0.01
	CLO	62.9[c]	64.7[b]	67.2[a]	67.0[a]	62.2[c]	0.55	0.01	0.01
	GAR	62.9[bc]	65.5[a]	63.8[b]	61.9[c]	59.8[d]	0.44	0.01	0.06
	GIN	62.9[c]	65.2[b]	67.4[a]	66.5[ab]	62.3[c]	0.71	0.01	0.05
	LEM	62.9[c]	65.6[b]	65.7[b]	67.6[a]	64.1[c]	0.65	0.41	0.01
					NDF degradability (%)				
24 h	CIN	34.4	36.3	37.8	34.2	34.0	3.00	0.77	0.77
	CLO	34.4	35.5	36.2	40.9	33.5	2.03	0.23	0.22
	GAR	34.4	36.3	34.6	31.3	32.6	3.32	0.34	0.60
	GIN	34.4	32.3	36.9	39.4	31.3	3.57	0.61	0.33
	LEM	34.4	34.2	30.3	36.5	31.7	3.70	0.70	0.23
48 h	CIN	44.1	42.9	45.3	47.5	45.8	3.39	0.45	0.52
	CLO	44.1	46.9	47.9	48.8	43.5	2.65	0.22	0.65
	GAR	44.1	49.2	46.3	45.8	45.8	2.50	0.86	0.24
	GIN	44.1	43.4	49.5	50.8	43.6	1.83	0.28	0.42
	LEM	44.1	50.0	43.9	51.7	40.7	2.60	0.92	0.36
					ADF degradability (%)				
24 h	CIN	27.4	29.3	30.1	27.5	25.4	4.66	0.48	0.64
	CLO	27.4	27.4	28.1	34.3	27.1	2.89	0.32	0.12
	GAR	27.4	30.7	27.4	23.7	24.6	3.11	0.16	0.56
	GIN	27.4	25.5	28.5	32.9	25.1	4.98	0.89	0.23
	LEM	27.4	27.5	23.9	30.1	25.2	5.18	0.84	0.71
48 h	CIN	37.0	35.1	38.4	40.3	36.8	4.40	0.50	0.56
	CLO	37.0	41.0	41.5	41.1	36.2	3.09	0.27	0.42
	GAR	37.0	42.9	41.1	38.7	36.5	2.93	0.63	0.46
	GIN	37.0	36.2	43.3	44.2	36.4	2.71	0.29	0.37
	LEM	37.0	44.2	41.5	46.8	36.9	3.65	0.93	0.29

[(a, b, c, d)] within a row means without a common superscript letter differ.

[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil

[b]SEM = standard error of the mean

Discussion

CIN: Decreases in disappearance of DM, NDF, CP and starch with 300 mg/l of cinnamaldehyde were observed by Li et al. (2012) in continuous culture using a high-grain diet (90%). In contrast, according to the findings of the present study, supplementing 400 and 800 mg/kg DM CIN improved DMD at 24 h and 48 h incubation. However, the 1600 mg/kg DM CIN decreased DMD at 24 h incubation, suggesting that at this dose CIN might cause a negative effect on feed digestion. In addition, the NDF and ADF digestibility was unaffected by CIN in Exp. I. The result of DMD was confirmed by the 200 mg/kg DM CIN at either 24 or 48 h in Exp. II. However, DM and NDF digestibility was not affected by cinnamaldehyde at the doses of 31.2 or 312 mg/l as reported by Busquet et al. (2005c). Supplementing CIN had no effect on the GP kinetics, however, there was great similarity between the cumulative GP and DMD. The cumulative GP of the 200 to 800 mg/kg DM CIN was higher than that of the control group at early hours, 3 to 24 h incubation in Exp. I (3 to 48 h with 200 mg/kg DM in Exp. II). The similar effect of CIN on DMD between the experimental measurements suggested that CIN effectively improved feed digestion. Although CIN improved DMD, there was no difference between the treatments in the total VFA and individual VFA. Similarly, Fraser et al. (2007) reported that cinnamon leaf oil had no effect on total VFA in continuous culture. The concentrations of VFA and individual VFA were unaffected by 200 mg/kg cinnamaldehyde in lamb (Chaves et al., 2008a). The methane production increased with the 200 to 800 mg/kg DM of CIN in Exp. I only at 24 h, consistent with the DMD and cumulative GP. Fraser et al. (2007) observed that CIN had no effect on methane production. In contrast, cinnamaldehyde decreased methane production using continuous culture (Li et al., 2012). Cinnamaldehyde did not

change total protozoa number in lactating dairy cows (Benchaar et al., 2008). The effects of EO on rumen methane production are actually inconsistent (Benchaar and Greathead, 2011), depending on the number of factors such as EO source, dose, substrates used, etc. In the present study, the ammonia N concentration was consistently reduced with CIN added either at 24 or 48 h post incubation in both experiments I and II, suggesting that these additives reduced deamination of AA and could be used as alternative additives for reducing ammonia N loss in the rumen. This result was in agreement with previous reports that cinnamaldehyde or cinnamon oil reduced ammonia N concentration (Busquet et al., 2005c; Cardozo et al., 2005). However, several reports suggested that cinnamaldehyde or cinnamon oil had no effect on ammonia N concentration in animals (Chaves et al., 2008a; Chaves et al., 2008b).

Table 3 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 1)

EO[b]	Dose	Gas production parameters[a]			*In vitro* gas production (ml/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
CIN	0	177	0.043	0.127	20.9[b]	34.8[c]	61.1[b]	94.6[b]	117.2	133.7
	200	156	0.059	0.117	29.4[a]	46.7[a]	75.8[a]	112.2[a]	131.3	147.4
	400	152	0.060	0.129	28.5[ab]	46.1[ab]	76.4[a]	113.6[a]	129.5	145.8
	800	149	0.052	0.051	27.4[ab]	44.9[ab]	73.8[a]	112.3[a]	125.8	140.5
	1600	147	0.042	0.180	22.9[ab]	37.5[bc]	65.5[ab]	100.9[ab]	118.1	133.6
	SEM[c]	14.4	0.005	0.090	2.91	3.15	4.21	5.33	8.78	9.08
	Linear	0.20	0.574	0.680	0.55	0.47	0.62	0.85	0.52	0.46
	Quadratic	0.32	0.007	0.389	0.05	0.02	0.02	0.02	0.23	0.28
CLO	0	177	0.043	0.127	20.9[c]	34.8[c]	61.1	94.6	117.2	133.7
	200	167	0.050	0.081	25.9[ab]	43.8[ab]	73.9	111.6	135.7	152.5
	400	160	0.054	0.028	28.5[a]	46.0[a]	74.5	111.0	134.3	150.1
	800	142	0.060	0.004	27.1[a]	42.6[ab]	69.3	105.0	118.3	133.8
	1600	150	0.056	0.334	21.8[bc]	38.0[bc]	67.8	108.0	120.8	135.6
	SEM[c]	14.4	0.005	0.090	2.19	2.41	4.29	5.59	7.16	7.90
	Linear	0.14	0.029	0.030	0.40	0.64	0.89	0.34	0.34	0.28
	Quadratic	0.24	0.033	0.014	0.01	0.01	0.09	0.19	0.44	0.50
GAR	0	177	0.042	0.127	20.9[c]	34.8[c]	61.1[b]	94.6[c]	117.2	133.7
	200	163	0.055	0.144	28.0[a]	46.1[a]	75.4[a]	112.7[a]	134.6	151.1
	400	141	0.063	0.300	24.9[ab]	43.2[ab]	72.2[a]	109.3[ab]	120.4	136.3
	800	152	0.056	0.028	27.4[ab]	44.6[a]	71.5[a]	107.5[ab]	126.9	143.0
	1600	148	0.052	0.042	23.7[bc]	39.5[b]	64.6[b]	101.4[bc]	118.5	134.8
	SEM[c]	14.4	0.005	0.090	2.79	1.47	1.58	3.44	7.06	8.32
	Linear	0.25	0.648	0.116	0.73	0.73	0.17	0.80	0.53	0.58
	Quadratic	0.26	0.007	0.901	0.01	0.01	0.01	0.02	0.28	0.38
GIN	0	177	0.043	0.127	20.9[b]	34.8[c]	61.1[c]	94.6[c]	117.2	133.7
	200	177	0.046	0.059	25.4[a]	43.3[a]	72.4[a]	112.2[a]	138.8	156.9
	400	151	0.055	0.078	24.5[a]	40.9[ab]	66.7[abc]	102.8[b]	120.4	136.7
	800	144	0.061	0.069	26.7[a]	42.7[a]	69.6[ab]	107.9[ab]	119.4	135.1
	1600	153	0.049	0.014	23.7[ab]	38.7[bc]	64.0[b]	101.4[bc]	121.5	136.5
	SEM[c]	14.4	0.005	0.090	2.59	1.18	2.18	2.92	5.19	5.89
	Linear	0.21	0.310	0.147	0.31	0.45	0.65	0.76	0.38	0.26
	Quadratic	0.22	0.007	0.996	0.02	0.01	0.02	0.02	0.99	0.99
LEM	0	177	0.043	0.127	20.9	34.8	61.1	94.6	117.2	133.7
	200	148	0.060	0.290	24.9	42.3	70.4	107.0	121.4	138.2
	400	135	0.062	0.269	22.7	38.1	65.6	101.8	109.4	125.5
	800	137	0.059	0.254	22.5	37.3	65.1	103.1	111.5	127.0
	1600	137	0.059	0.237	23.5	38.9	65.8	104.4	113.5	128.9
	SEM[c]	14.4	0.005	0.090	2.87	3.72	5.41	6.59	12.59	13.27
	Linear	0.12	0.181	0.752	0.73	0.79	0.87	0.43	0.69	0.63
	Quadratic	0.11	0.068	0.451	0.85	0.77	0.64	0.49	0.61	0.60

[(a, b, c)] within a column means without a common superscript letter differ.

[a]Parameters: b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[b]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil

[c]SEM = standard error of the mean

CLO: Ruminal degradability of NDF was linearly decreased and degradation of N in the rumen tended to be linearly decreased with increasing eugenol supplementation, while OM and starch degradability did not differ (Yang et al., 2010). In contrast, the present study suggested that supplementing CLO increased DMD without affecting NDF or ADF degradability. The kinetics GP was not different between the treatments, but the cumulative GP was consistently increased, together with the DMD increasing with CLO supplementation. The total VFA concentration and individual VFA were unaffected by the treatments, although the DMD was improved. Benchaar et al. (2012) reported that adding eugenol had no effect on total VFA or individual VFA in dairy cow either with low concentrate or high concentrate ratio of dairy ration. The methane production in the present study was inconsistent, although it increased with the 200

mg/kg DM CLO at 24 h in Exp. II but could not maintain until 48 h post incubation. This result might be relative to cumulative GP. In contrast, Yang et al. (2010) reported that molar proportion of propionate tended to linearly increase, thus the ratio of acetate to propionate tended to linearly decrease with increasing dose of eugenol. In fact, the reduced methane production would result in an increase in propionate as the H^+ must have a recipient. Supplementing CLO reduced ammonia N concentration in Exp. I and Exp. II at 24 and 48 h incubation. Busquet et al. (2006) demonstrated that 3000 mg/l eugenol inhibited NH_3-N concentration using 50:50 ratio of forage:concentrate. Meanwhile, NH_3-N concentration was reduced in high concentrate with 300 mg/l as reported by Cardozo et al. (2005). However, the inconsistency between the reduction in ruminal degradability of CP and the lack of effect on ruminal NH_3-N, ruminal branched-chain VFA concentration, and blood urea N concentration suggests that deamination and/or proteolytic activity in the rumen might not be inhibited by eugenol supplementation (Yang et al., 2010).

Table 4 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 1)

	EO[a]	Dose (mg/kg DM)						*P*-value	
		0	200	400	800	1600	SEM[b]	Linear	Quadratic
					Total VFA (m*M*)				
24 h	CIN	110.2	120.8	109.2	112.5	104.9	4.06	0.07	0.36
	CLO	110.2	115.0	111.3	110.3	109.1	5.43	0.55	0.87
	GAR	110.2	114.9	115.7	106.4	102.7	6.04	0.11	0.63
	GIN	110.2	114.6	107.6	112.6	105.4	4.26	0.22	0.45
	LEM	110.2	116.3	117.3	102.2	107.8	6.06	0.22	0.66
48 h	CIN	118.8	120.0	117.6	126.6	112.6	6.85	0.47	0.22
	CLO	118.8	124.6	127.9	132.9	117.8	5.64	0.66	0.06
	GAR	118.8	123.3	133.3	128.5	113.2	8.65	0.34	0.10
	GIN	118.8	118.4	119.6	119.4	118.2	5.27	0.93	0.82
	LEM	118.8	118.6	133.3	118.1	116.2	6.50	0.39	0.30
					Acetic acid (mol/100 mol)				
24 h	CIN	57.2	57.3	57.5	56.9	56.2	0.81	0.17	0.59
	CLO	57.2	57.1	57.5	56.1	57.0	1.08	0.65	0.54
	GAR	57.2	58.2	57.8	56.0	56.3	1.29	0.23	0.78
	GIN	57.2	59.3	57.2	56.2	56.8	1.20	0.27	0.57
	LEM	57.2	56.8	57.3	56.5	56.7	0.50	0.31	0.54
48 h	CIN	55.9	56.2	54.7	54.6	54.0	1.19	0.14	0.61
	CLO	55.9	55.6	55.1	53.9	53.9	1.14	0.10	0.40
	GAR	55.9	55.0	54.2	53.7	54.1	2.19	0.47	0.46
	GIN	55.9	54.9	54.9	54.1	54.1	1.11	0.19	0.40
	LEM	55.9	54.3	54.9	54.3	54.2	1.99	0.53	0.68
					Propionic acid (mol/100 mol)				
24 h	CIN	19.7	20.0	19.9	20.0	20.3	0.70	0.52	0.99
	CLO	19.7	19.9	19.5	20.4	20.2	0.58	0.34	0.73
	GAR	19.7	19.4	19.7	20.5	20.2	0.73	0.31	0.57
	GIN	19.7	19.4	19.7	20.4	20.2	0.71	0.32	0.62
	LEM	19.7	20.1	20.0	20.2	20.1	0.49	0.53	0.55
48 h	CIN	20.5	20.3	20.6	20.7	20.7	0.17	0.13	0.48
	CLO	20.5	20.5	20.7	20.8	20.8	0.25	0.21	0.52
	GAR	20.5	20.6	20.6	20.8	20.6	0.34	0.79	0.43
	GIN	20.5	20.6	20.6	20.5	20.8	0.23	0.25	0.74
	LEM	20.5	20.8	20.4	20.4	20.7	0.48	0.86	0.70
					A+B/P[c]				
24 h	CIN	3.6	3.6	3.6	3.5	3.5	0.16	0.41	0.99
	CLO	3.6	3.6	3.7	3.4	3.5	0.15	0.34	0.72
	GAR	3.6	3.7	3.6	3.4	3.5	0.19	0.28	0.65
	GIN	3.6	3.7	3.6	3.4	3.5	0.18	0.31	0.61
	LEM	3.6	3.5	3.5	3.5	3.5	0.11	0.42	0.56
48 h	CIN	3.4	3.4	3.4	3.3	3.3	0.06	0.09	0.40
	CLO	3.4	3.4	3.3	3.3	3.3	0.06	0.07	0.27
	GAR	3.4	3.4	3.3	3.3	3.3	0.12	0.61	0.41
	GIN	3.4	3.3	3.3	3.3	3.3	0.07	0.21	0.77
	LEM	3.4	3.3	3.4	3.4	3.3	0.12	0.62	0.93

[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil
[b]SEM = standard error of the mean
[c]A+B/P = acetic acid+butyric acid/propionic acid

GAR: Garlic oil is a complex mix of many different compounds presented in the plant or derived from processing. It has antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria and its potential effect on modifying rumen microbial fermentation has been studied recently (Calsamiglia et al., 2007; Chaves et al., 2008c; Kongmun et al., 2010). Garlic oil and 4 purified active components (allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan) thought to play a major role in its antimicrobial activity were tested *in vitro* to determine their effect on rumen microbial fermentation (Busquet et al., 2005a). In the

present study, supplementing garlic oil consistently improved the DMD and cumulative GP but had no effect on the digestibility of NDF and ADF, kinetics parameters. The result is similar to that of Yang et al. (2007), who observed that supplementing 5 g/d of garlic oil increased truly the digestibility of DM without any effects on NDF, ADF and starch digestibility. Nanon et al. (2014a) suggested that 200 mg/kg DM equal blend of garlic and ginger oil improved DM and NDF digestibility at 24 and 48 h post incubation for wheat DDGS, grass hay and total mixed ration except barley grain (DMD, only 24 h post incubation) using batch culture. In contrast, Klevenhusen et al. (2011) reported that although garlic oil supplementation had no effect on feed digestion, its principal organosulfur compound improved feed digestion in sheep. Regarding the DMD result, the cumulative GP and total VFA concentration were increased by GAR (*i.e.* 200 mg/kg DM) in the current study. The effect of GAR on methane production was inconsistent. The 200 or 400 mg/kg DM GAR increased the methane production at 24 h, but it could not maintain until 48 h in Exp. I. Kongmun et al. (2010) demonstrated that supplementing coconut oil and garlic powder affected total VFAs and individual VFAs production. Supplementing coconut oil and garlic powder at ratios of 8:4, 4:8 and 0:16 reduced total VFA and methane production, while at the ratio of 0:16 reduced NH_3-N and acetate proportion but increased propionate proportion. However, garlic oil had no effect on VFA concentration, NH_3-N concentration and protozoa in lactating dairy cows as reported by Yang et al. (2007).

Table 5 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 1)

	EO[a]	Dose (mg/kg of DM)						*P*-value	
		0	200	400	800	1600	SEM[b]	Linear	Quadratic
					CH_4 (ml/g DM)				
24 h	CIN	15.8^{c}	18.8^{a}	18.7^{a}	18.7^{ab}	16.0^{bc}	1.02	0.33	0.02
	CLO	15.8	18.4	18.1	17.0	17.8	1.18	0.55	0.47
	GAR	15.8^{c}	18.8^{a}	18.1^{ab}	17.5^{abc}	16.4^{bc}	0.73	0.32	0.04
	GIN	15.8^{c}	18.6^{a}	16.7^{bc}	17.9^{ab}	16.5^{bc}	0.57	0.77	0.03
	LEM	15.8	17.7	16.7	16.8	17.3	1.36	0.57	0.78
48 h	CIN	20.8	23.6	23.1	22.4	21.0	2.26	0.62	0.36
	CLO	20.8	24.6	23.9	21.6	21.3	1.84	0.41	0.40
	GAR	20.8	24.1	22.0	23.2	21.3	1.87	0.74	0.28
	GIN	20.8	24.6	22.0	21.6	21.7	1.69	0.68	0.74
	LEM	20.8	21.9	19.4	20.0	19.9	2.42	0.59	0.73
					Ammonia N (mg/100 ml)				
24 h	CIN	42.3^{a}	31.8^{b}	31.5^{b}	32.2^{b}	30.3^{b}	2.87	0.04	0.07
	CLO	42.3^{a}	31.9^{b}	32.4^{b}	32.6^{b}	31.2^{b}	2.55	0.04	0.06
	GAR	42.3^{a}	32.5^{b}	31.1^{b}	31.4^{b}	30.2^{b}	3.03	0.04	0.06
	GIN	42.3^{a}	31.9^{b}	34.3^{b}	33.0^{b}	25.2^{b}	3.28	0.01	0.57
	LEM	42.3^{a}	31.8^{b}	34.0^{b}	31.9^{b}	29.9^{b}	2.53	0.02	0.08
48 h	CIN	52.8^{a}	46.3^{b}	45.2^{b}	45.1^{b}	41.6^{b}	2.01	0.01	0.11
	CLO	52.8^{a}	45.9^{b}	44.1^{b}	44.8^{b}	44.4^{b}	1.92	0.03	0.03
	GAR	52.8^{a}	45.6^{b}	44.9^{b}	46.6^{b}	43.9^{b}	1.34	0.01	0.04
	GIN	52.8^{a}	44.2^{b}	45.0^{b}	45.4^{b}	43.8^{b}	2.00	0.04	0.07
	LEM	52.8^{a}	42.6^{b}	44.6^{b}	43.9^{b}	43.5^{b}	2.08	0.04	0.04

(a, b, c) within a row means without a common superscript letter differ.
[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil
[b]SEM = standard error of the mean

GIN: Lacking the effect of GIN on rumen fermentation, there was only one *in vitro* study reporting that ginger oil had no effect on total VFA concentration, individual VFA proportion, large peptide, small peptide plus amino acid, and ammonia concentration using continuous culture (Busquet et al., 2006). In contrast, the degradability of DM and cumulative GP were improved with GIN supplementation, resulting in the increased methane production at 24 h. The total VFA concentration also increased with the 150 and 200 mg/kg DM GIN at 48 h. The result is similar to that of Nanon et al. (2014a), who reported that the 200 mg/kg DM equal blend of garlic and ginger oil improved feed digestion for wheat DDGS, barley grain, grass hay, and TMR in *in vitro* experiment. Nanon et al. (2014a) also reported that an equal blend of garlic and ginger oil increased DMD at 4 and 24 h for wheat DDGS and barley grain, and 24 and 48 h for grass hay and TMR *in situ*. In addition, microbial attachment on the residues of grass hay increased with the 200 mg/kg DM equal blend of garlic and ginger oil at 6 h post incubation (Nanon et al., 2014a). NH_3-N was consistently reduced when the 200 mg/kg DM GIN was supplemented at either 24 or 48 h incubation. The results suggested that GIN had potential to improve DMD while reduced NH_3-N by inhibited deamination.

LEM: There are few studies that reported the effects of lemongrass supplementation on rumen fermentation and feed digestion. Nanon et al. (2014a) suggested that LEM improved feed digestion such as DM and NDF for concentrate feed (wheat DDGS and barley grain) and fibrous feed both *in vitro* and *in situ*. Meanwhile, microbial attachment of grass hay increased with the 200 mg/kg DM LEM. Wanapat et al. (2008) reported that supplementing 100 g/d lemongrass powder increased DM digestibility, quadratic change DM and NDF digestibility without affecting digestibility of CP

and ADF in steer fed on high forage (73% diet DM) diets. The present study demonstrated that adding LEM at 200 mg/kg DM improved DMD, resulting in the higher cumulative GP, methane production and total VFA concentration at 24 and 48 h, whereas the ammonia N concentration was lowest at 200 mg/kg DM. In contrast, Nanon et al. (2014b) showed that nutrient digestibility and VFA concentration were unaffected by LEM supplementation in continuous culture. However, the lack of difference of crude protein digestibility with the accumulation of peptides and reduction in ammonia N confirms the previous suggestion of inhibition of deamination by LEM. Wanapat et al. (2008) showed that ammonia N concentration was lower at 100 or 200 g/d of lemongrass powder compared with the control group, resulting in lower plasma urea N. Urea is synthesized in the liver from ammonia absorbed from the rumen or gut, therefore a urea N concentration in blood positively correlates with the ruminal concentration of ammonia (Hosada et al., 2006). In contrast, supplementing 50 g/kg of lemongrass had no effect on rumen VFA concentration and individual VFA proportion, but it increased rumen ammonia concentration (Hosada et al., 2006). Furthermore, supplementing mixtures of thyme, oregano, cinnamon and lemon that varied in ratios inhibited rumen fermentation and reduced population of rumen microbes (Lin et al., 2012). The methane production was induced by the 200 mg/kg DM at either 24 or 48 h followed by the DMD and cumulative GP results. However, protozoa population was decreased with increasing levels of lemongrass powder from 0 to 300 g/d (Wanapat et al., 2008).

Table 6 Effects of essential oils on degradability of DM, NDF and ADF in batch culture (Experiment 2)

	EO[a]	Dose (mg/kg DM)						*P*-value	
		0	50	100	150	200	SEM[b]	Linear	Quadratic
					DM degradability (%)				
24 h	CIN	49.9[c]	51.2[bc]	51.7[bc]	53.2[ab]	54.0[a]	0.72	0.01	0.67
	CLO	51.1[b]	51.8[b]	52.3[ab]	52.7[ab]	53.6[a]	0.55	0.01	0.74
	GAR	50.4[b]	51.5[ab]	51.6[ab]	53.9[a]	53.5[a]	1.16	0.03	0.82
	GIN	50.0[b]	50.9[b]	52.8[ab]	53.0[ab]	54.5[a]	0.86	0.01	0.55
	LEM	50.4[b]	51.5[ab]	53.2[ab]	52.6[ab]	53.7[a]	1.10	0.03	0.52
48 h	CIN	65.1[c]	66.9[bc]	66.7[bc]	65.7[bc]	68.8[a]	0.58	0.01	0.32
	CLO	64.7[b]	65.5[b]	66.7[ab]	68.1[a]	67.9[a]	0.86	0.01	0.49
	GAR	64.6[b]	65.3[ab]	64.4[b]	66.0[ab]	67.6[a]	1.14	0.05	0.23
	GIN	62.8[b]	64.7[b]	64.8[ab]	64.2[b]	66.9[a]	0.78	0.01	0.74
	LEM	64.3[b]	66.1[ab]	66.0[ab]	66.0[ab]	67.3[a]	1.00	0.05	0.75
					NDF degradability (%)				
24 h	CIN	27.5	26.7	28.7	29.7	29.6	1.98	0.18	0.93
	CLO	27.3	28.7	27.3	27.9	29.3	3.15	0.20	0.67
	GAR	26.3	26.6	28.7	28.9	29.2	1.59	0.99	0.69
	GIN	26.6	26.8	29.2	27.2	29.1	1.67	0.53	0.80
	LEM	26.9	27.7	28.0	27.8	28.0	2.16	0.71	0.53
48 h	CIN	43.8	44.7	44.4	44.1	44.5	3.24	0.98	0.82
	CLO	41.8	42.5	43.5	43.9	43.5	2.26	0.38	0.66
	GAR	45.6	46.8	46.1	46.5	47.1	2.17	0.72	0.88
	GIN	41.3	44.3	43.7	44.1	45.6	2.97	0.27	0.81
	LEM	43.7	46.3	46.8	46.9	47.0	2.69	0.45	0.13
					ADF degradability (%)				
24 h	CIN	16.0	16.4	17.7	18.7	19.7	1.76	0.06	0.71
	CLO	16.3	16.4	17.0	18.7	18.3	2.76	0.39	0.32
	GAR	17.3	16.9	19.3	19.6	18.9	1.65	0.89	0.86
	GIN	17.9	17.9	17.9	18.2	18.1	2.10	0.51	0.77
	LEM	16.7	18.9	18.9	19.6	19.0	1.80	0.35	0.24
48 h	CIN	33.4	33.9	33.3	33.0	35.2	3.72	0.89	0.71
	CLO	36.1	33.3	34.8	35.2	33.1	3.05	0.57	0.99
	GAR	36.5	38.3	37.2	36.5	38.4	2.32	0.73	0.93
	GIN	31.3	35.4	34.5	34.9	36.5	3.70	0.30	0.73
	LEM	34.2	38.0	37.5	38.5	37.4	3.35	0.41	0.11

[(a, b, c)] within a row means without a common superscript letter differ.
[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil
[b]SEM = standard error of the mean

The present study demonstrated that the EOs consistently improved the DMD at either 24 or 48 h incubation, resulting in the higher cumulative GP. The degradability of DM may be relative to microbial attachment. The results confirm that GP is a reliable indicator of feed fermentation in the batch culture. The ammonia N concentration was consistently reduced in all treatments at both 24 and 48 h of incubation in both of the experiments, suggesting that these EOs reduced the deamination of amino acids and could be alternatives for reducing ammonia N loss in the rumen.

In the present study, some of the parameters are different from previous results depending on the dose of essential oils and substrate used. Most previous studies were tested in Europe or the United States and used diet with high concentration but low in fiber.

Their ratios of concentrate to forage were around 60:40 or higher because essential oils were focused on beef cattle. However, the doses of essential oils varied and depended on researchers. That is why the results of the present study varied and sometimes they are in conflict with others. However, very high dose (1000 mg/kg DM substrate or above) of essential oils always show negative effects for nutrient degradability and rumen fermentation. Nanon et al. (2014a) suggested that low dose of essential oils (200 mg/kg DM substrate) improved DM and NDF digestibility both in *in vitro* and *in situ* experiments via microbial attachment especially for high fiber diet (grass hay or TMR). This suggested that low dose of essential oils might have more potential with high fibrous feed.

Table 7 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 2)

EO[b]	Dose	Gas production parameters[a]			*In vitro* gas production (ml/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
CIN	0	152	0.044	0.050	17.9	36.0^{b}	61.8^{b}	94.0^{b}	118.2^{b}	131.8^{c}
	50	153	0.043	0.075	18.0	36.1^{b}	62.3^{b}	94.8^{b}	119.5^{b}	133.1^{bc}
	100	153	0.043	0.056	18.2	36.2^{b}	62.4^{b}	95.1^{b}	119.7^{b}	133.7^{bc}
	150	156	0.043	0.060	18.6	37.0^{b}	63.3^{b}	96.0^{b}	121.3^{b}	135.6^{b}
	200	162	0.044	0.017	20.7	39.5^{a}	66.5^{a}	100.8^{a}	126.7^{a}	142.7^{a}
	SEM[c]	3.85	0.002	0.047	2.29	0.76	0.80	1.32	1.16	1.19
	Linear	0.02	0.973	0.368	0.28	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.26	0.897	0.283	0.52	0.05	0.03	0.07	0.03	0.01
CLO	0	157	0.042	0.040	17.9	35.9^{b}	62.1^{b}	94.2^{c}	120.1^{b}	133.7^{c}
	50	155	0.042	0.056	17.7	35.8^{b}	62.1^{b}	94.6^{bc}	120.3^{ab}	133.8^{bc}
	100	155	0.043	0.034	18.4	36.7^{ab}	63.2^{ab}	95.8^{b}	120.9^{ab}	134.6^{bc}
	150	158	0.042	0.039	18.4	36.8^{ab}	63.2^{ab}	95.8^{b}	122.3^{ab}	136.0^{ab}
	200	154	0.046	0.208	20.1	37.6^{a}	64.7^{a}	98.5^{a}	122.7^{a}	136.9^{a}
	SEM[c]	3.85	0.002	0.047	3.13	0.67	0.75	0.56	1.08	0.85
	Linear	0.79	0.621	0.276	0.50	0.04	0.02	0.01	0.04	0.01
	Quadratic	0.94	0.725	0.336	0.73	0.64	0.40	0.08	0.70	0.39
GAR	0	155	0.043	0.059	18.1	36.2^{b}	62.4^{b}	94.8^{b}	120.1^{b}	133.9^{b}
	50	158	0.042	0.044	18.1	36.3^{b}	62.6^{b}	95.0^{b}	121.1^{b}	135.4^{b}
	100	161	0.042	0.046	18.2	36.6^{b}	63.1^{b}	95.6^{b}	122.3^{b}	136.4^{b}
	150	159	0.042	0.037	18.4	37.0^{b}	63.7^{b}	96.7^{b}	122.7^{b}	137.2^{b}
	200	161	0.045	0.025	19.6	39.4^{a}	66.9^{a}	100.6^{a}	126.0^{a}	141.1^{a}
	SEM[c]	3.85	0.002	0.047	3.15	0.65	0.80	0.84	1.14	1.32
	Linear	0.20	0.508	0.271	0.65	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.62	0.330	0.960	0.79	0.05	0.04	0.03	0.30	0.26
GIN	0	155	0.043	0.051	18.2	36.3^{c}	62.3^{d}	94.7^{c}	120.0^{b}	133.8^{b}
	50	156	0.043	0.037	18.4	36.7^{c}	63.0^{cd}	95.4^{bc}	120.9^{b}	134.7^{b}
	100	157	0.043	0.039	18.2	36.7^{c}	63.1^{bc}	95.6^{bc}	121.2^{b}	135.4^{b}
	150	157	0.043	0.014	18.8	37.4^{b}	63.7^{b}	96.4^{b}	121.6^{b}	136.1^{b}
	200	164	0.044	0.008	20.1	39.4^{a}	66.7^{a}	100.8^{a}	126.9^{a}	141.9^{a}
	SEM[c]	3.85	0.002	0.047	3.45	0.25	0.26	0.49	1.27	1.22
	Linear	0.12	0.683	0.054	0.62	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.40	0.764	0.860	0.76	0.01	0.01	0.01	0.06	0.04
LEM	0	155	0.042	0.034	18.1	35.9^{b}	61.8^{b}	94.0^{c}	119.1^{b}	132.9^{c}
	50	153	0.043	0.037	18.4	36.2^{b}	62.4^{b}	94.9^{bc}	119.0^{b}	133.0^{c}
	100	155	0.043	0.038	18.3	36.3^{b}	62.7^{b}	95.0^{bc}	120.2^{b}	134.3^{bc}
	150	157	0.043	0.034	18.6	36.6^{b}	63.1^{b}	96.4^{b}	121.9^{b}	135.9^{b}
	200	160	0.045	0.034	20.2	38.9^{a}	66.1^{a}	100.4^{a}	125.1^{a}	139.7^{a}
	SEM[c]	3.85	0.002	0.047	3.46	0.46	0.58	0.67	1.04	0.90
	Linear	0.21	0.522	0.228	0.60	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.49	0.663	0.271	0.75	0.03	0.03	0.01	0.07	0.03

(a, b, c) within a column means without a common superscript letter differ.

[a]Parameters: b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[b]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil

[c]SEM = standard error of the mean

Table 8 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 2)

EO[a]		Dose (mg/Kg DM)					SEM[b]	*P*-value	
		0	50	100	150	200		Linear	Quadratic
					Total VFA (m*M*)				
24 h	CIN	81.8	81.3	84.9	87.9	87.3	3.89	0.10	0.94
	CLO	83.8	81.1	85.8	86.0	86.8	2.98	0.16	0.77
	GAR	81.3[b]	81.9[b]	82.6[ab]	82.5[ab]	85.0[a]	1.36	0.05	0.45
	GIN	83.1	81.1	83.0	84.5	85.2	1.98	0.16	0.38
	LEM	79.9[b]	80.6[ab]	81.3[ab]	82.8[ab]	83.5[a]	1.13	0.02	0.77
48 h	CIN	90.2[b]	90.1[b]	91.6[ab]	91.3[ab]	92.9[a]	0.89	0.02	0.55
	CLO	93.4[b]	93.1[b]	93.3[b]	96.2[a]	97.4[a]	0.86	0.01	0.07
	GAR	92.5[b]	94.6[ab]	95.3[ab]	95.1[ab]	97.2[a]	1.12	0.02	0.80
	GIN	93.2[b]	94.1[b]	95.8[ab]	96.6[a]	97.3[a]	1.01	0.01	0.70
	LEM	95.3[b]	96.3[b]	97.0[b]	96.0[b]	99.3[a]	0.69	0.01	0.21
					Acetic acid (mol/100 mol)				
24 h	CIN	55.1	55.2	55.9	55.0	54.7	0.52	0.46	0.36
	CLO	55.3	53.9	55.4	54.0	55.5	0.71	0.72	0.19
	GAR	51.0	54.9	54.5	54.8	54.9	0.84	0.43	0.69
	GIN	55.2	55.1	55.2	55.3	55.2	0.71	0.91	0.99
	LEM	54.9	54.6	54.3	54.8	55.1	0.88	0.74	0.47
48 h	CIN	54.0	53.9	53.7	53.3	53.6	1.02	0.52	0.78
	CLO	54.1	54.4	54.4	54.3	54.9	0.31	0.08	0.72
	GAR	53.4	54.3	52.9	52.4	52.6	0.77	0.06	0.76
	GIN	52.8	52.5	51.4	52.3	51.8	1.20	0.43	0.65
	LEM	52.3	51.3	54.7	55.0	53.5	1.94	0.23	0.46
					Propionic acid (mol/100 mol)				
24 h	CIN	21.0	21.0	21.3	21.2	21.1	0.22	0.46	0.35
	CLO	21.2	21.1	21.1	21.2	21.1	0.38	0.99	0.98
	GAR	21.1	21.3	20.9	21.4	21.1	0.44	0.98	0.83
	GIN	21.2	21.4	21.3	21.3	21.1	0.11	0.33	0.12
	LEM	21.3	20.9	21.2	21.1	20.4	0.43	0.18	0.43
48 h	CIN	21.6	21.7	21.4	20.9	21.2	0.39	0.27	0.75
	CLO	21.5	21.1	21.2	21.5	20.9	0.29	0.21	0.89
	GAR	21.6	21.5	21.7	21.5	21.5	0.12	0.69	0.61
	GIN	21.1	21.5	21.4	21.5	21.6	0.33	0.23	0.70
	LEM	21.3	21.3	21.7	21.8	21.6	0.33	0.25	0.55
					A+B/P				
24 h	CIN	3.3	3.3	3.2	3.2	3.2	0.05	0.45	0.34
	CLO	3.2	3.2	3.3	3.2	3.3	0.06	0.82	0.62
	GAR	3.2	3.2	3.3	3.2	3.3	0.08	0.75	0.85
	GIN	3.2	3.2	3.2	3.2	3.2	0.02	0.28	0.23
	LEM	3.2	3.3	3.2	3.2	3.4	0.11	0.24	0.40
48 h	CIN	3.1	3.1	3.1	3.2	3.2	0.03	0.06	0.93
	CLO	3.1	3.2	3.2	3.1	3.3	0.06	0.21	0.82
	GAR	3.1	3.1	3.1	3.1	3.1	0.02	0.10	0.26
	GIN	3.2	3.1	3.1	3.1	3.0	0.06	0.17	0.54
	LEM	3.1	3.1	3.1	3.1	3.1	0.03	0.59	0.84

[(a, b, c)] within a row means without a common superscript letter differ.

[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil

[b]SEM = standard error of the mean

Table 9 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 2)

EO[a]		Dose (mg/kg DM)					SEM[b]	*P*-value	
		0	50	100	150	200		Linear	Quadratic
		CH_4 (ml/g DM)							
24 h	CIN	25.2	25.1	24.8	25.0	25.9	0.57	0.32	0.09
	CLO	24.7[b]	23.9[b]	24.5[b]	25.0[b]	26.4[a]	0.55	0.01	0.01
	GAR	24.8	24.1	25.1	24.4	26.2	1.03	0.19	0.24
	GIN	24.0[b]	24.5[b]	24.3[b]	25.0[ab]	26.1[a]	0.65	0.01	0.23
	LEM	24.4[b]	25.6[b]	25.2[b]	25.1[b]	27.0[a]	0.37	0.01	0.08
48 h	CIN	32.9[b]	33.4[b]	32.3[b]	33.2[b]	34.9[a]	0.58	0.01	0.01
	CLO	33.5	33.5	33.6	33.6	34.8	1.16	0.23	0.23
	GAR	31.5	32.5	30.2	34.3	36.1	2.47	0.06	0.23
	GIN	33.6	31.4	32.8	35.2	32.8	2.28	0.13	0.10
	LEM	27.1[b]	28.9[b]	30.8[ab]	30.5[ab]	35.2[a]	3.27	0.02	0.18
		Ammonia N (mg/100 ml)							
24 h	CIN	38.7[a]	38.8[a]	37.6[ab]	37.7[ab]	36.1[b]	1.01	0.05	0.48
	CLO	39.0[a]	37.3[b]	37.4[b]	38.0[ab]	36.8[b]	0.52	0.03	0.31
	GAR	37.1[a]	36.8[a]	36.8[a]	36.2[a]	34.6[b]	0.42	0.01	0.04
	GIN	38.4[a]	38.3[a]	37.7[a]	37.8[a]	36.1[b]	0.30	0.01	0.03
	LEM	38.0[a]	37.3[ab]	37.9[a]	37.2[ab]	35.9[b]	0.59	0.03	0.20
48 h	CIN	49.7[a]	48.3[ab]	46.8[b]	47.4[b]	47.0[b]	0.58	0.01	0.06
	CLO	49.4[a]	48.7[a]	48.6[a]	48.1[a]	45.2[b]	1.00	0.02	0.14
	GAR	48.1[a]	47.2[ab]	47.3[ab]	47.7[a]	45.6[b]	0.63	0.03	0.29
	GIN	47.5[a]	46.7[ab]	47.1[ab]	46.4[bc]	45.7[c]	0.37	0.01	0.48
	LEM	48.3[a]	47.6[ab]	47.5[b]	47.2[b]	45.6[c]	0.26	0.01	0.04

[(a, b, c)] within a row means without a common superscript letter differ.
[a]EO: CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil
[b]SEM = standard error of the mean

In conclusion, supplementing EO increased DMD, but reduced NH_3-N concentration, with EO increasing from 0, 200, 400, 800, to 1600 mg/kg feed DM in Exp. I, indicating that the EOs used in the present study affected feed digestion in a dose-dependent manner. The results suggest that the dose of 200 mg/kg DM is cost-effective for each EO, according to the DMD and NH_3-N concentration in Exp. II. However, the effect of EO on methane production was apparently negligible. These results suggest that the EO used in the present study could potentially be developed as rumen modifier to improve feed digestion, especially high fiber feeds in ruminant animals.

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บทคัดย่อ

การใช้น้ำมันหอมระเหยเพื่อปรับปรุงกระบวนการหมักของจุลินทรีย์ในกระเพาะหมักโดยวิธีการ batch culture

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วัตถุประสงค์ของการทดลองนี้เพื่อประเมินผลของน้ำมันหอมระเหยต่อการย่อยได้ของอาหาร ปริมาณแก๊ส และการหมักย่อยของจุลินทรีย์ในกระเพาะหมัก ทำการทดลอง 2 การทดลองโดยใช้เทคนิค batch culture โดยในการทดลองที่ 1 ใช้น้ำมันหอมระเหยจากอบเชย กานพลู กระเทียม ขิง และตะไคร้ในปริมาณ 0 200 400 800 **และ** 1600 มิลลิกรัมต่อกิโลกรัมของอาหารแห้ง ส่วนในการทดลองที่ 2 ใช้น้ำมันหอมระเหยเหมือนกับการทดลองที่ 1 แต่ในปริมาณ 0 50 100 150 และ 200 มิลลิกรัมต่อกิโลกรัมของอาหารแห้ง ทำการวัดการย่อยได้ของวัตถุแห้ง (DM) และเยื่อใย (NDF **และ** ADF) 24 และ 48 ชั่วโมงหลังการบ่ม และอ่านค่าปริมาณแก๊ส 3 6 12 24 36 และ 48 ชั่วโมงหลังการบ่ม อาหารที่ใช้ในการทดลองนี้คือ อาหารหยาบร้อยละ 50 (หญ้าแห้งร้อยละ 35 และถั่วอัลฟัลฟ่าแห้งร้อยละ 15) และอาหารข้นร้อยละ 50 (ข้าวบาร์เลย์ร้อยละ 20, corn DDGS ร้อยละ 10 wheat DDGS ร้อยละ 10 กากคาโนลาร้อยละ 5 และวิตามินและแร่ธาตุร้อยละ 5) จากการทดลองพบว่าน้ำมันหอมระเหยทุกชนิดสามารถปรับปรุงการย่อยได้ของวัตถุแห้งทั้งในการทดลองที่ 1 และ 2 แต่น้ำมันหอมระเหยไม่มีผลต่อการย่อยได้ของเยื่อใย ไม่พบความแตกต่างของปริมาณ VFA ในการทดลองที่ 1 แต่ในการทดลองที่ 2 พบการเพิ่มขึ้นของ total VFA เมื่อใช้น้ำมันหอมระเหยในปริมาณ 200 มิลลิกรัมต่อกิโลกรัมอาหารแห้ง นอกจากนี้ยังพบการลดลงของความเข้มข้นของแอมโมเนีย-ไนโตรเจนทั้งในการทดลองที่ 1 และ 2 ซึ่งเป็นการยืนยันว่าน้ำมันหอมระเหยลดการย่อยสลายของกรดอะมิโน อย่างไรก็ตามน้ำมันหอมระเหยไม่มีผลต่อแก๊สมีเทน ผลการทดลองดังกล่าวข้างต้นแสดงให้เห็นว่าน้ำมันหอมระเหยมีความสามารถในการปรับปรุงการย่อยได้ของอาหาร

คำสำคัญ: แอมโมเนีย-ไนโตรเจน น้ำมันหอมระเหย การย่อยได้ของอาหาร ปริมาณแก๊ส การหมักย่อย

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