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The effects of different compounds in some essential oils on *in vitro* gas production

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ABSTRACT

The aim of this study was to determine the effect of essential oils (EO) of oregano, ORE (*Origanum vulgare*); black seed, BSD (*Nigella sativa*); laurel, LAU (*Laurus nobilis*); cumin, CUM (*Cumminum cyminum*); garlic, GAR (*Allium sativum*); anise, ANI (*Pimpinella anisum*), and cinnamon, CIN (*Cinnamomum verum*) on *in vitro* gas production (IVGP) and IVGP kinetics of barley, wheat straw and soyabean meal. IVGP values were determined by using rumen liquor from three dry Holstein cows. The findings of this study indicate that the effects of EO, doses, and EO × dose interactions were significant. IVGP was decreased by ANI, GAR and ORE, and only CUM increased IVGP. These EO and their different doses or combinations in diets could be used to improve the performance of ruminants. Moreover, EO may act at different levels in energy and protein metabolic pathways, thus their careful selection and combination may be a useful tool to effectively manipulate rumen fermentation.

KEY WORDS: essential oils, *in vitro* gas production, barley, wheat straw, soyabean meal

INTRODUCTION

For decades, one of the goals of ruminant microbiologists and nutritionists has been to manipulate the ruminal microbial ecosystem to improve the efficiency of converting feeds to animal products edible by humans. The use of antibiotics

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as feed additives, e.g., ionophore antibiotics, has proved to be a useful tool in reducing energy (methane) and nitrogen (ammonia) losses (McGuffey et al., 2001). The use of antibiotics as growth promoters in animal nutrition was banned after January 2006 in the European Union. For this reason in the last few years nutritonists have become increasingly more interested in bioactive plant factors that can modify rumen fermentation processes. The use of essential oils (plant extracts, phytofactors, volatile or etheral oils) appears to be one of the most natural alternatives to antibiotic use in animal nutrition. The possibility of using biologically active plant compounds for modulating changes in the rumen was first reported in 1911. In response to the requirements of animal production, the animal feed industry has marketed animal feed additives containing mixtures of secondary plant metabolites. Many researchers have demonstrated potentially favourable effects of mixtures of essential oils (Szumacher-Strabel and Cieślak, 2010).

Dong et al. (2010) reported that addition of some plant extracts to different diets reduced methane production. They reported that phytogenic products can be used as alternatives to monensin in altering *in vitro* rumen fermentation and reducing methane production in goats. The inclusion of oregano oil at different levels (0, 400, 800 and 1200 mg/l) significantly decreased *in vitro* gas production, OMD and ME values. The inclusion of 1200 mg/l ORE has been found to be the most effective dose, but a negative impact on the data was also seen. It can be said that low doses of essential oils should be used in ruminant nutrition but high doses of essential oils may decrease rumen function and feed efficiency (Canbolat et al., 2011). It is known that essential oils seem to have a detrimental effect on microbial fermentation when administered at high doses (Benchaar et al., 2007).

Many plant extracts have antimicrobial activities against a wide range of rumen microorganisms (Voda et al., 2003). Manipulation of the rumen microbial ecosystem for enhancing fibrous feed digestibility, reducing methane emission and nitrogen excretion by ruminants to improve their performance are some of the most important goals for animal nutritionists. Essential oils and their metabolites as natural feed additives are good candidates for achieving these objectives. Turkey is one of the largest aromatic plant and essential oil producers. The present study aimed to screen 7 plant extracts (essential oils) selected for rumen manipulations. Determination of the best doses of essential oils for optimization of rumen fermentation was the objective of this study.

MATERIAL AND METHODS

Material

This study was conducted from July 2008 to April 2009 at the University of Cukurova (Turkey). Seven essential oils (oregano, ORE (*Origanum vulgare*);

black seed, BSD (*Nigella sativa*); laurel, LAU (*Laurusnobilis*); cumin, CUM (*Cumminumcyminum*); garlic, GAR (*Allium sativum*); anise, ANI (*Pimpinella anisum*) and cinnamon, CIN (*Cinnamomum verum*)) were used. Essential oils (plant extracts) were obtained from Hatay province (Turkey). Wheat straw, soyabean meal and barley were used as feedstuffs (substrates for incubation). Barley (highly degradable starch), soyabean meal (highly degradable protein) and wheat straw (fibre sources) were chosen to evaluate the effects of essential oils on starch, protein and fibre degradation in *in vitro* rumen fermentation. The samples were milled in a hammer mill through a 1 mm sieve for chemical analysis and *in vitro* gas production assays.

Chemical analysis

Dry matter (DM) was determined by drying samples at 105ºC for 24 h, ash content, by ashing in a muffle furnace at 550ºC for 8 h. Nitrogen (N) content was estimated using the Kjeldahl method (AOAC, 1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) determinations were based on the method of Van Soest et al. (1991) using an ANKOM fibre analyzer. Essential oils were bought from a commercial factory in Hatay Province and their composition (after distillation using a Neo-Clevenger apparatus) was determined by gas chromatography-mass spectrometry (Perkin Elmer Clarus model 500). All chemical analyses were carried out in triplicate.

In vitro gas production technique

Three dry Holstein cows with rumen cannulas (average liveweight of 650 kg) were used in the *in vitro* gas production study. Approximately 200 mg dry weight of samples (wheat straw, soyabean meal and barley) were weighed in triplicate into 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1988). The syringes were pre-warmed at 39ºC before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39ºC. McIntosh et al. (2003) reported that essential oil levels lower than 35, 80 and 100 ppm for various bacteria could not affect rumen fermentation. Cardozo et al. (2005) also reported that they studied five different doses (0, 0.3, 3, 30 and 300 ppm) of essential oils in their experiment. To determine the effects of essential oils on *in vitro* gas production and gas production kinetics, doses of 0 (control), 50, 100 and 150 ppm were used. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Five repetitions of each sample were used in the *in vitro* gas production experiment. Rumen fluid was obtained from fistulated Holstein cows fed twice daily (08.30-16.30) with a diet containing

maize silage (60%) and concentrate (40%). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) by the NEWAY computer package programme:

$$y = a + b\ (1\text{-}exp^{-ct})$$

where: a - gas production from the immediately soluble fraction (ml), b - gas production from the insoluble fraction (ml), a + b - potential gas production (ml), c - gas production rate constant for the insoluble fraction (ml/h), t - incubation time (h), y - gas produced at time t.

Statistical analysis

A completely randomised design was used to compare gas production and gas production kinetics using the general linear model (GLM) of the SPSS (SPSS version 10.0) programme package. The significance of differences between individual means was determined using Duncan's multiple comparation test:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

Y_{ijk} - the observed value of k^{th} repetition of j^{th} dose of i^{th} plant extract; μ - the population mean; α_i - the effect of i^{th} - plant extract; β_i - the effect of j^{th} dose; $(\alpha\beta)_{ij}$ - interaction of i^{th} plant extract and j^{th} dose and e_{ijk} - error term.

RESULTS

The main compounds of the essential oils were determined to be, %: carvacrol (0.03), octadecadienoic acid methyl ester (51.04), hexadecanoic acid methyl ester (16.17), oleic acid methyl ester (23.13) in *Nigella sativa*; γ-terpinene (21.20), thymol (0.53) and carvacrol (57.01) in oregano; α-piene (10.03), β-myrcene (14.71), eugenol (0.02) and 1.8 cineol (41.52) in laurel; (69.57), acetaugenol (6.58) and cinnamyl acetate (3.83) in cinnamon; linoleic acid (34.75), diallyldisulphide (25.00), dially tetrasulphide (1.69), diallytrisulphide (19.48) and allylmethyltrisulphide (5.24) in garlic; elaidic acid methyl ester (18.08), cuminyl aldehyde (13.13), safrana (3.36) and hexadecanoic acid methyl ester (15.49) in cumin; and isoanethol (2.49), limoene (1.14), cuparene (3.37) and anethol (79.56) in anise.

The chemical composition of barley, soyabean meal, and wheat straw and of feedstuffs used for cows, from which rumen fluid was obtained, is presented in Table 1.

Table 1. Chemical compositions of the feedstuffs and forage and concentrate fed to animals, g/kg DM

Feeds	DM	CP	EE	CF	Ash	NFE	NDF	ADF
	g/kg	g/kg DM						
Forage (maize silage)	318.3	105.7	35.1	236.1	88.8	534.3	453.6	296.1
Concentrates	907.9	281.4	44.3	96.2	60.7	517.4	266.9	93.4
Wheat straw	92.93	40.28	12.85	457.60	69.75	419.5	814.9	570.1
Soyabean meal	92.08	448.40	19.98	60.59	79.95	391.1	125.2	113.3
Barley	90.30	131.68	18.36	72.90	40.57	736.5	222.5	97.9

DM - dry matter, CP - crude protein, EE - ether extract, NFE - nitrogen free ectractives, NDF - neutral detergent fibre, ADF - acid detergent fibre

The effects of different doses of essential oils on *in vitro* gas production of different feed ingredients are presented in Table 2. The findings of the present study indicate that essential oils, doses, and essential oil x dose interaction had significant effects ($P<0.01$) on gas production. Each essential oil, each incubation time and each feedstuff gave different results depending on the dose. The addition of CUM resulted in the highest *in vitro* gas production values in barley, soyabean meal and wheat straw, whereas the addition of ORE-150 gave the lowest values. BDS and LAU did not affect the *in vitro* gas production pattern of any of the three feeds ($P>0.05$).

The values obtained with barley using GAR-150, ORE-150 and ANI-100 were significantly lower than control values. On the other hand, those from CUM-100 and CUM-150 were higher than controls ($P<0.05$). The effects of BSD, LAU, CIN, CUM and ANI added to barley did not differ ($P<0.05$), however. Adding GAR-150 and GAR-50 resulted in lower gas production at 12 and 24 h of incubation ($P<0.05$), but the difference disappeared at 48 and 96 h ($P>0.05$).

In vitro gas production values of soyabean meal with the addition of ANI-50, ANI-100, ANI-150 and ORE-150 were lower than control, but were higher with CUM-50, CUM-100 and CUM-150 ($P<0.05$). Other essential oil treatments did not differ from the control. The effect of dose application of BSD, LAU, CIN and CUM to soyabean meal were not significant ($P>0.05$). Gas production at 12 h of the incubation was lower with the addition of ANI-150 and GAR-150 ($P<0.05$), but did not differ at other incubation times.

In vitro gas production of wheat straw with ANI-50, ANI-100, ANI-150, GAR-150 and ORE-150 was lower than in the control group, but higher when CUM-50, CUM-100 and CUM-150 were added ($P<0.05$). Additions of BSD were not efective. Lower gas production was obtained with the addition of ORE-150 and GAR-150 than with other doses. Gas production was also lower with CIN-150 at 12 h of incubation, with LAU-150 at 12 and 24 h, and with ANI-150 at 24 and 96 h ($P<0.05$).

The influence of different doses of essential oils on gas production parameters of feeds used in the present study is presented in Table 3. Potential gas production

Table 2. The effects of essential oils on *in vitro* gas production of wheat straw, soyabean meal and barley, ml/200 mg DM

Treatment	Barley				Soyabean meal				Wheat straw			
	12.00 h	24.00 h	48.00 h	96.00 h	12.00 h	24.00 h	48.00 h	96.00 h	12.00 h	24.00 h	48.00 h	96.00 h
Control	45.8^{c}	55.8^{cde}	64.3^{bcde}	68.2^{bcde}	29.7^{bcde}	36.6^{bc}	42.1^{bcd}	43.9^{bcd}	12.0^{bc}	25.2^{bc}	35.7^{b}	41.0^{c}
BSD-50	48.3^{bc}	57.7^{bc}	64.6^{bcde}	68.5^{bcde}	30.1^{bcd}	38.2^{b}	43.7^{bc}	46.3^{bc}	13.5^{b}	25.6^{bc}	33.5^{bcd}	39.6^{c}
BSD-100	48.3^{bc}	58.0^{bc}	65.1^{bcde}	69.7^{bc}	27.9^{bcde}	36.6^{bc}	43.4^{bc}	45.7^{bc}	13.7^{b}	25.8^{b}	35.8^{b}	40.4^{c}
BSD-150	46.1^{c}	55.6^{cdef}	63.2^{cdef}	67.7^{bcde}	28.0^{bcde}	36.1^{bc}	41.5^{bcde}	43.8^{bcd}	11.7^{bcd}	24.4^{bcd}	36.1^{b}	41.9^{bc}
ORE-50	39.2^{de}	52.7^{cdefg}	62.4^{cdefg}	67.7^{bcde}	26.6^{cdef}	35.6^{bc}	41.6^{bcde}	43.6^{bcde}	8.5^{de}	25.0^{bc}	36.5^{b}	42.0^{bc}
ORE-100	36.1^{de}	48.0^{gh}	57.4^{gh}	63.1^{ef}	25.1^{efg}	32.0^{cd}	39.1^{cdef}	42.0^{cde}	4.4^{fgh}	20.8^{def}	34.5^{bc}	39.3^{c}
ORE-150	21.5^{f}	32.0^{i}	40.6^{i}	47.1^{g}	2.1^{j}	7.4^{e}	18.0^{g}	25.5^{g}	2.1^{ghi}	6.8^{h}	12.3^{g}	20.1^{f}
LAU-50	47.2^{c}	58.0^{bc}	65.5^{bcde}	69.5^{bc}	29.8^{bcde}	39.2^{b}	44.1^{b}	46.0^{bc}	7.6^{ef}	23.3^{bcd}	36.0^{b}	42.0^{bc}
LAU-100	46.4^{c}	58.0^{bc}	66.0^{bcd}	69.9^{bc}	27.3^{bcde}	36.1^{bc}	42.0^{bcd}	44.7^{bc}	5.4^{efg}	21.8^{bcde}	36.5^{b}	41.9^{bc}
LAU-150	47.9^{c}	58.2^{bc}	66.1^{bcd}	71.2^{b}	26.8^{cdef}	37.4^{b}	43.7^{bc}	46.9^{b}	3.4^{ghi}	17.7^{f}	32.7^{bcde}	41.4^{c}
CIN-50	47.7^{c}	57.4^{bcd}	66.1^{bc}	69.1^{bc}	31.3^{bc}	38.4^{b}	43.1^{bcd}	44.6^{bc}	12.3^{b}	25.4^{bc}	35.9^{b}	40.8^{c}
CIN-100	47.5^{c}	57.3^{bcd}	65.3^{bcde}	68.7^{bcd}	31.6^{b}	39.7^{b}	45.0^{b}	46.1^{bc}	12.4^{b}	25.7^{bc}	35.7^{b}	42.2^{bc}
CIN -150	46.0^{c}	56.6^{bcd}	64.2^{bcde}	68.6^{bcd}	29.3^{bcde}	35.2^{bc}	41.8^{bcd}	43.9^{bcd}	8.7^{cde}	23.0^{bcd}	34.3^{bc}	39.0^{cd}
GAR-50	40.2^{d}	52.1^{defg}	60.5^{cdefgh}	64.6^{cdef}	27.0^{bcde}	34.9^{bc}	38.6^{def}	40.1^{def}	7.5^{ef}	20.8^{def}	32.1^{bcde}	37.5^{cd}
GAR-100	39.6^{de}	50.7^{efgh}	60.2^{defgh}	64.3^{cdef}	26.5^{def}	35.9^{bc}	41.1^{bcde}	43.5^{bcde}	7.5^{ef}	21.5^{cdef}	31.8^{bcde}	38.7^{cd}
GAR-150	34.8^{e}	45.7^{h}	55.0^{h}	62.3^{f}	19.8^{hi}	31.8^{cd}	37.2^{ef}	40.1^{def}	0.8^{i}	5.4^{h}	19.6^{f}	30.3^{e}
CUM-50	52.8^{ab}	62.0^{ab}	69.1^{ab}	72.7^{ab}	37.6^{a}	45.0^{a}	49.4^{a}	50.9^{a}	18.3^{a}	33.0^{a}	41.8^{a}	47.1^{ab}
CUM-100	56.5^{a}	66.6^{a}	74.1^{a}	77.4^{a}	38.6^{a}	45.8^{a}	50.0^{a}	51.8^{a}	19.8^{a}	34.2^{a}	43.1^{a}	49.0^{a}
CUM-150	56.8^{a}	66.9^{a}	73.1^{a}	76.9^{a}	40.6^{a}	48.0^{a}	52.6^{a}	54.4^{a}	20.6^{a}	35.4^{a}	44.0^{a}	50.2^{a}
ANI-50	40.4^{d}	50.8^{efgh}	60.0^{efgh}	63.2^{def}	22.5^{fgh}	29.2^{d}	36.0^{f}	37.6^{f}	5.2^{efgh}	17.9^{ef}	28.6^{de}	31.8^{e}
ANI-100	38.2^{de}	50.2^{fgh}	58.2^{fgh}	61.8^{f}	21.7^{ghi}	30.1^{d}	35.4^{f}	37.0^{f}	1.7^{hi}	12.2^{g}	28.2^{e}	34.0^{de}
ANI-150	37.0^{de}	50.1^{fgh}	58.3^{fgh}	63.1^{ef}	17.9^{i}	28.4^{d}	36.1^{f}	39.4^{ef}	1.8^{hi}	11.9^{g}	30.4^{cde}	38.2^{cd}
SEM	0.71	0.68	0.64	0.59	0.67	0.68	0.59	0.52	0.51	0.62	0.56	0.54
EFFECTS EO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EO x dose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

EO - essential oils, ORE - oregano EO, BSD - blackseed EO, LAU- laurel EO, CUM- cummin EO, GAR - garlic EO, ANI - anise EO; CIN - Cinnamon EO, 50- 50 ppm dose, 100 - 100 ppm dose, 150 - 150 ppm dose, SEM - stantard error of means $^{abc}P<0.05$

Table 3. The effects of essential oils on *in vitro* gas production kinetics of wheat straw, soyabean meal and barley

Treatment	Barley				Soyabean meal				Wheat straw			
	a	b	c	a+b	a	b	c	a+b	a	b	c	a+b
Control	-8.5^{abcd}	74.3^{de}	0.11^{bcde}	65.8^{bcde}	-2.9^{abcde}	45.8^{de}	0.10^{bcd}	43.0^{bcd}	-5.3^{cde}	47.9^{fgh}	0.04^{bcd}	42.5^{ef}
BSD-50	-10.9^{bcde}	76.9^{cde}	0.12^{ab}	66.0^{bcde}	2.5^{a}	42.6^{e}	0.09^{cdef}	45.1^{bc}	-4.1^{bc}	44.6^{hi}	0.04^{bc}	40.5^{f}
BSD-100	-12.6^{de}	79.4^{cde}	0.12^{abc}	66.9^{bcd}	2.3^{a}	42.8^{e}	0.07^{efg}	45.0^{bc}	-5.6^{cde}	46.7^{gh}	0.04^{ab}	41.1^{ef}
BSD-150	-13.7^{de}	78.7^{cde}	0.11^{abc}	65.0^{bcdef}	-1.6^{abcd}	44.3^{e}	0.09^{cdef}	42.7^{bcd}	-6.0^{cdef}	49.6^{cdefgh}	0.05^{bcd}	43.6^{def}
ORE-50	-10.8^{bcde}	76.6^{cde}	0.08^{fg}	65.8^{bcde}	-5.7^{cdefg}	48.4^{cde}	0.08^{cdef}	42.8^{bcd}	-7.1^{def}	51.1^{bcdefg}	0.04^{bcde}	44.0^{cdef}
ORE-100	-13.1^{de}	74.5^{de}	0.07^{g}	61.5^{defg}	-6.2^{cdefg}	47.2^{cde}	0.08^{defg}	41.0^{bcde}	-7.2^{ef}	50.7^{bcdefg}	0.03^{ef}	43.6^{def}
ORE-150	-10.3^{bcde}	56.3^{f}	0.05^{h}	46.0^{h}	-2.8^{abcde}	43.3^{e}	0.01^{h}	40.5^{cde}	-1.7^{a}	32.6^{j}	0.01^{hi}	30.8^{g}
LAU-50	-20.6^{f}	87.4^{b}	0.12^{ab}	66.9^{bcd}	-12.3^{g}	57.3^{ab}	0.11^{bc}	44.9^{bc}	-7.2^{ef}	52.3^{bcdefg}	0.03^{de}	45.1^{bcdef}
LAU-100	-20.2^{f}	87.9^{b}	0.11^{abcd}	67.7^{bc}	-10.6^{fg}	53.9^{bc}	0.10^{bcde}	43.4^{bcd}	-7.9^{f}	53.9^{bcde}	0.03^{ef}	46.0^{bcdef}
LAU-150	-27.7^{g}	95.9^{a}	0.12^{abc}	68.2^{b}	-11.6^{g}	57.1^{ab}	0.09^{cdef}	45.6^{b}	-6.4^{def}	55.1^{bc}	0.02^{fg}	48.7^{bcd}
CIN-50	5.2^{ab}	72.1^{e}	0.11^{abc}	66.9^{bcd}	-0.6^{abc}	44.2^{e}	0.10^{bcd}	43.6^{bcd}	-5.8^{cde}	47.5^{fgh}	0.04^{bcd}	41.7^{ef}
CIN-100	-8.4^{abcd}	74.6^{de}	0.11^{abc}	66.3^{bcde}	-2.7^{abcde}	47.9^{cde}	0.10^{bc}	45.2^{bc}	-5.8^{cde}	49.1^{defgh}	0.03^{bcde}	43.3^{def}
CIN -150	-11.3^{cde}	77.2^{cde}	0.11^{abc}	65.9^{bcde}	-4.6^{bcdef}	46.9^{cde}	0.10^{bcd}	42.3^{bcd}	-6.9^{def}	47.8^{fgh}	0.04^{bcd}	41.0^{ef}
GAR-50	-14.5^{e}	76.8^{cde}	0.10^{cde}	62.3^{cdefg}	-10.0^{fg}	49.4^{cde}	0.11^{bc}	39.4^{de}	-6.8^{def}	46.7^{gh}	0.03^{cde}	39.9^{f}
GAR-100	-14.6^{e}	76.9^{cde}	0.09^{def}	62.2^{cdefg}	-10.6^{fg}	53.1^{bcd}	0.09^{cdef}	42.5^{bcd}	-6.8^{def}	48.4^{efgh}	0.03^{ef}	41.6^{ef}
GAR-150	-16.1^{ef}	75.2^{de}	0.08^{fg}	59.2^{g}	-10.1^{fg}	49.8^{cde}	0.07^{fg}	39.6^{de}	-3.4^{ab}	64.7^{a}	0.01^{i}	61.4^{a}
CUM-50	-6.5^{abc}	76.9^{cde}	0.12^{abc}	70.4^{ab}	1.1^{ab}	48.7^{cde}	0.12^{b}	49.8^{a}	-6.5^{def}	53.5^{bcdef}	0.05^{a}	47.1^{bcde}
CUM-100	-5.2^{ab}	80.4^{cd}	0.12^{ab}	75.2^{a}	-11.8^{g}	62.1^{a}	0.15^{a}	50.2^{a}	-5.2^{bcde}	54.1^{bcde}	0.05^{a}	48.8^{bcd}
CUM-150	-4.5^{a}	79.1^{cde}	0.12^{a}	74.6^{a}	-8.4^{efg}	61.2^{a}	0.14^{a}	52.8^{a}	-5.1^{bcd}	55.0^{bcd}	0.05^{a}	50.0^{b}
ANI-50	-14.6^{e}	75.7^{cde}	0.10^{bcde}	61.2^{efg}	-7.1^{cdefg}	43.8^{e}	0.09^{cdef}	36.7^{e}	-6.1^{cdef}	40.4^{i}	0.03^{cde}	34.3^{g}
ANI-100	-20.8^{f}	80.8^{cd}	0.10^{bcde}	60.0^{fg}	-10.5^{fg}	46.9^{cde}	0.09^{cdef}	36.4^{e}	-5.9^{cde}	47.9^{fgh}	0.02^{fg}	42.0^{ef}
ANI-150	-21.3^{f}	82.6^{bc}	0.09^{ef}	61.3^{defg}	-7.7^{defg}	46.8^{cde}	0.06^{g}	39.0^{de}	-5.9^{cde}	55.7^{b}	0.02^{gh}	49.8^{bc}
SEM	0.607	0.724	0.002	0.571	0.540	6.637	0.003	0.421	0.142	0.531	0.001	0.512
EFFECTS EO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EFFECTS Dose	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.98	0.00	0.00	0.00	0.00
EFFECTS EO x dose	0.00	0.00	0.01	0.00	0.02	0.02	0.00	0.01	0.00	0.00	0.00	0.00

EO - essential oils; ORE - oregano EO; BSD - blackseed EO; LAU - laurel EO; CUM - cummin EO; GAR - garlic EO; ANI - anise EO
CIN - cinnamon EO; 50-50 ppm dose, 100-100 ppm dose, 150-150 ppm dose
a - the gas production from the immediately soluble fraction (ml)
b - the gas production from the insoluble fraction (ml), a+b - potential gas production (ml), c - the gas production rate constant for the insoluble fraction (ml/h); SEM - stantard error of means; ^{abc} $P<0.05$

(a+b) of barley with CUM-100 and CUM-150 was higher than that of the control group, but lower with the addition of ORE-150 ($P<0.05$). The rate of gas production (c) in barley was higher with CUM-150, but was lower with ORE-50, ORE-100, ORE-150 and GAR-150 than the control group ($P<0.05$). Doses of BSD, LAU, CIN and ANI had no effect on the values of a+b or c ($P>0.05$).

No differences were found among the doses of any of the essential oils in terms of the a + b value in soyabean meal ($P>0.05$). Potential gas production, the (a+b) value, was higher with CUM-50, CUM-100 and CUM-150 and lower with ANI-50 and ANI-100 as compared with the control group. In soyabean meal, dose treatments with BSD, LAU and CIN did not differ in terms of c value. Higher 'c' values were obtained, however, when CUM-100 and CUM-150 were added; on the other hand, lower values were found with the addition of GAR-150, ANI-150, ORE-150 and BSD-150 ($P<0.05$).

Treatment with BSD, LAU, CIN, CUM and ANI had significant effects in terms of a+b value for wheat straw ($P<0.05$). LAU-150, GAR-150, CUM-100, CUM-150 and ANI-150 produced higher a + b values, whereas ANI-50 and ORE-150, lower values ($P<0.05$) in comparison with the control group. The 'c' value was not affected by treatments with BSD, CIN and CUM. As compared with the control group, addition of CUM-50, CUM-100 and CUM-150 resulted in higher values of c, while that of ORE-100, ORE-150, LAU-100, LAU-150, GAR-100, GAR-150 and ANI-100, ANI-150 led to lower values ($P<0.05$).

DISCUSSION

Cardozo et al. (2004) reported that CIN, GAR, ORE and ANI changed rumen VFA proportions and that the results obtained by adding ANI and GAR suggested that deamination was inhibited. In this study, ORE-150 decreased *in vitro* gas production of all feeds. GAR-150 decreased *in vitro* gas production except for soyabean meal, in which it had no effect. These results are in agreement with the findings of Cardozo et al. (2004).

Decreased *in vitro* gas production by ORE may indicate more efficient utilization of energy due to the inhibited loss of energy as methane. An increasing trend for liveweight gain in lamb fattening by using oregano, oregano oil, and a commercial essential oil mix can be attributed to this effect (Akkan et al., 2006). *In vitro* gas production decreased for all feeds by increasing doses of essential oils in this study. Morover, ORE-150 showed the lowest *in vitro* gas production, which was similar to literature findings.

Benchaar et al. (2007) reported that *in vitro* gas production of carvacrol, thymol and eugenol decreased compared with controls. Those results are in agreement with the findings for ORE and CIN in this study. ORE and CIN possess more carvacrol and eugenol compounds respectively than other EOs.

UM increased, whereas ANI, GAR and ORE decreased *in vitro* gas production in barley. It is well known that there is a high correlation between *in vitro* gas production and both *in vivo* digestibility and/or microbial growth (Menke et al., 1979). These results showed that CUM could be used to improve digestion of slowly degradable starch, and

ANI, GAR and ORE, to control degradation of highly degradable starch sources in the rumen to maintain ruminal pH within the physiological range. Cardozo et al. (2004) reported that GAR and CIN could inhibit deamination in the rumen. Similarly, lower gas production for soyabean meal with ANI and ORE and higher gas production with CUM may also suggest that protein degradation could be controlled by ANI and ORE. Furthermore, CUM might improve nitrogen utilization in the rumen if the diet was based on slowly degradable protein sources. *In vitro* gas production was decreased significantly by ANI, GAR and ORE, and only CUM increased gas production significantly. CUM may be used to improve cellulose digestion and could be considered a feed additive.

The decrease in total gas and methane production observed with the use of garlic oil confirms its ability to inhibit methanogenesis. Busquet et al. (2005) reported that *in vitro* gas production decreased as doses were increased. Patra et al. (2006) investigated the effects of water, methanol and ethanol extracts of garlic on rumen fermentation and methanogenesis. An aqueous garlic extract caused higher gas production and the ethanol and methanol extracts of garlic secondary metabolites appeared to have a potential to reduce rumen methanogenesis without adversely affecting rumen fermentation. The effects of *Cordia verbenacea D.C*. essential oil (EO) on ruminal fermentation were determined by using the *in vitro* gas production technique (Araujo et al., 2010). Inclusion of EO inhibited methanogenesis when hay was used as the substrate, but this effect was not seen with concentrate. These results showed that EO from *Cordia verbenacea D.C.* was able to modify *in vitro* ruminal fermentation when hay was the substrate. In this study, GAR-150 decreased *in vitro* gas production compared with controls, and 'c' values decreased as doses increased.

The effects of essential oils on rumen fermentation differ significantly. Carvacrol and thymol have strong antimicrobial activity against a wide range of gram-positive and -negative bacteria. Both are found in ORE (Sivropoulou et al.,1996). Castillejos et al. (2006) reported that low doses of thymol (50 mg/l) had no effects on *in vitro* rumen microbial fermentation. But at higher doses of thymol or ORE total VFA decreased (Castillejos et al., 2006) and decreased total gas production (Akkan et al., 2006; Benchaar et al., 2007; Kamalak et al., 2011). Furthermore, several *in vitro* studies have suggested that the effects of thymol are diet and pH dependent (Cardozo et al., 2005; Castillejos et al., 2006).

In the current experiment, the addition of ORE (thymol) decreased *in vitro* gas production. These findings are in agreement with the cited-above literature. On the other hand, the addition of ORE also decreased the gas production rate (h-1). This result is in agreement with Kamalak et al. (2011), but not with Benchaar et al. (2007).

Anethol is the main active component of ANI (79.56%) and is responsible for its antimicrobial activity. *In vitro* studies with rumen fluid showed that anethol and ANI decreased total VFA production Calsamiglia et al. (2007) reported that ANI reduced the acetate-to-propionate ratio in the rumen, and may be beneficial in a beef production system. In this study, ANI-100 for barley, ANI-50 for soyabean and wheat straw decreased *in vitro* gas production compared with the control. These results are in agreement with the findings of Calsamiglia et al. (2007).

Eugenol is one of the main active components of CIN (accounts for up to 69.57%). The eugenol content in CIN in this study is in agreement with the findings of Davidson and Naidu (2000), who suggested that, when used at optimal doses, the efficiency of energy and protein utilization in the rumen was improved. Eugenol may improve VFA production and profile, and N utilization in the rumen of lactating animals (Castillejos et al., 2006). Different doses of CIN essential oils led to similar *in vitro* gas production levels in barley and soyabean meal. *In vitro* gas production levels of wheat straw were found to be lower for CIN-150 dose at 12 h incubation. CIN containing EUG might be used for improving rumen fermentation in forage-based diets. Therefore, this result is in agreement with the findings of Castillejos et al. (2006).

A commercial blend of essential oil compounds, the major components of which are carvacrol, thymol, eugenol, vanillin and limonene, can be used to manipulate rumen fermentation (McIntosh et al., 2003; Benchaar et al., 2007). In this study, CUM showed the highest *in vitro* gas production compared with controls for all of the feeds. ORE-150 showed the lowest *in vitro* gas production for all of the feeds. Moreover, there were important and varied interactions of feeds, doses and incubation times, which is in agreement with the findings of McIntosh et al. (2003). Benchaar et al. (2007) reported that the changes in rumen fermentation caused by essential oil compounds (thymol, carvacrol and eugenol) may not beneficial for dairy cattle. They suggest that the types and concentrations of EO and EO compounds must be carefully defined.

CONCLUSIONS

The results of this study suggest that the tested essential oils (EO) and their combinations influence rumen fermentation in a manner depending upon the EO and feeds used. *In vitro* results should be evaluated in *in vivo* studies.

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