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Effect of essential oils extracted from *Satureja calamintha*, *Mentha pulegium* and *Juniperus phoenicea* on *in vitro* methanogenesis and fermentation traits of vetch-oat hay

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The impact of inclusion of essential oils (EO) extracted from *Juniperus phoenicea, Satureja calamintha* and *Mentha pulegium* at three levels: 0, 1.66, 3.33 and 6.66 µl/ml of incubation medium, on methane production and rumen fermentation traits of vetch-oat hay was studied in *in vitro* gas production test, using 200 mg of substrate in a 60 ml graduated syringes. Gas production was recorded at 3, 6, 9, 24, 48, 72 and 96 h. Methane was measured at 3, 24 and 96 h, while pH and ammonia N were analyzed just at 24 h of incubation. At 24 h of fermentation, all doses of EO reduced significantly total gas and methane production (P < 0.05). At the highest concentration and after 24 h, methanogenesis was decreased by 60.98, 57.70 and 64.64% for *J. phoenicea*, *M. pulegium* and *S. calamintha*, respectively. This effect was more pronounced at the end of fermentation (96 h) where reduction in methane production was superior to 72%. However, addition of EO increased pH and decreased significantly ammonia concentration, mainly, for high EO concentrations. The results indicate that these EO had a potential to reduce methanogenesis in the rumen, but further *in vitro* and *in vivo* trials are required to search optimum dose which reduce methane production without adversely changing dietary fermentation and rumen function.

Key words: Methane, ammonia, essential oils, *Satureja calamintha (*Calament), *Mentha pulegium (fliou,* Menthe pouliot*)*, *Juniperus phoenicea (Arar,* Genévrier*)*, *in vitro* gas production test.

INTRODUCTION

Methane (CH_4) is a potent greenhouse gas and it has a global warming potential 25 times than carbon dioxide (CO_2) (Benchaar and Greathead, 2011). In fact, 33% of anthropogenic emissions of this gas are produced by ruminant livestock (Beauchemin et al., 2008). CH_4 is an end-product generating in the rumen under anaerobic conditions by methanogenic *Archaea*, using CO_2 and H_2 which are produced during the fermentation of the feed ingested by the animal. Furthermore, CH_4 represents a significant loss of dietary energy from the production system (Eckard et al., 2010); hence, reducing enteric methanogenesis may also lead to benefits for both the

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environment and the animal producer. Appreciable research efforts have been directed towards reducing CH_4 production by ruminants, many chemical feed additives have been tested to decrease CH_4 production in the rumen, but many of these substances appear to be toxic and some may generate chemical residues in animal derived foods (Patra and Saxena, 2010). Current research efforts are interested in using natural products, such as plant secondary metabolites such as essential oils (EO). EO are complex mixtures of lipophilic, liquid, volatile and often terpenoid compounds that are obtained by many methods of extraction (such as steam distillation, solvent extraction, supercritical CO_2 extraction) and have extensive antimicrobial activity against Gram-positive and Gram-negative bacteria (Calsamiglia et al., 2007; Benchaar and Greathead, 2011).

The objective of this study was to investigate the effectiveness of increasing doses of three EO extracted from *Satureja calamintha*, *Juniperus phoenicea* and *Mentha pulegium* in the inhibition of methanogenesis in the rumen, and their effects on *in vitro* ruminal fermentation traits of vetch-oat hay.

MATERIALS AND METHODS

Extraction procedure of essential oils

EO were extracted from aerial parts (leaves) of *S. calamintha*, *J. phoenicea* and *M. pulegium* by hydrodistillation. 100 g of each plant were mixed with 1400 ml of distilled water and boiled for 3 h. After decantation, EO were recuperated and stored in dark flasks at 4°C until tested.

In vitro fermentation

Ruminal digesta samples were obtained from three sheep (mean weight, 52 kg) and pooled together in order to achieve a homogenous inocula. Rumen digesta was collected 1 h before the morning feeding, placed in a Thermos container that was sealed immediately, and transported to the laboratory. The animals received daily 1200 g of vetch-oat hay (DM, 891.1 g/kg; ash, 50.8 g/kg DM; CP, 67.9 g/kg DM; NDF, 616.2 g/kg DM; ADF, 327.7 g/kg DM; ADL, 43.6 g/kg DM) in two equal meals at 8: 00 h and 16: 00 h. Water was available *ad libitum*. At the laboratory, the ruminal fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks.

The method of Menke et al. (1979) was used for the gas production procedure. Preparation of artificial saliva was done according to the method of Menke and Steingass (1988). The artificial saliva containing buffer solution ($NaHCO_3$, 39 g/l), macrominerals solution (Na_2HPO_4, 5.7 g; KH_2PO_4, 6.2 g and $MgSO_4.7H_2O$, 0.6 g per 1 L of distilled water), microminerals solution ($CaCl_2.2H_2O$, 13.2 g; $MnCl_2.4H_2O$, 10 g; $CoCl_2.6H_2O$, 1 g; $FeCl_3.6H_2O$, 8 g per 100 ml of distilled water) and potential redox indicator (resazurine, 0.1 g/100 ml) was prepared the day before incubation and stored at 39°C. The reducing agent ($Na_2S.9H_2O$, 0.625 g; NaOH 1 N, 4 ml; distilled water, 95 ml) was then prepared dor the incubation of the samples. Artificial saliva and ruminal fluid were mixed in a 2:1 ratio. 200 mg of vetch-oat hay (ground to pass a 1-mm screen) were weighed into 60 ml syringes and incubated at 39°C with 30 ml of inoculum. EO was added before the diluted rumen fluid at doses of 50, 100 and 200 µl/syringe which were equivalent to 1.66, 3.33 and 6.66 µl/ml.

In each trial and for each level, three syringes were used. Three syringes without EO (controls) were incubated in the same conditions at the same time.

Analytical techniques

Manual measurements of gas production were done at 3, 6, 9, 24, 48, 72 and 96 h. CH_4 in gas was determined at 3, 24 and 96 h according to the procedure described by Jouany et al. (1994) by transferring the total gas into syringes containing 4 ml of hydroxide sodium (NaOH, 10N) to absorb carbon dioxide. The gas remaining was assumed to be CH_4. After 24 h of incubation, the medium of each batch was checked for pH and analyzed for ammonia concentration as described by Chaney and Marbach (1962). 2 ml of orthophosphoric acid (50 g/l) were mixed with 10 ml of syringe contents. Samples were then centrifuged at 15,000 g for 15 min at 4°C, and the supernatant was used to determine ammonia concentration. Ammonia of blanks was subtracted from the measured N-NH_3 of samples to obtain the net ammonia concentration.

Calculations and statistical analysis

Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows: $y= a+b\ (1-e^{(-ct)})$ where a=the gas production from the immediately soluble fraction, b= the gas production from the insoluble fraction, c= the gas production rate constant for the insoluble fraction (b), t= incubation time, (a+b) = the potential extent of gas production and y= gas produced at time "t". The estimation of these parameters was made by the Neway excel software developed by Chen (1997). Data on *in vitro* gas production tests (pH, gas and methane productions, exponential parameters) were subjected to ANOVA analysis employing the SAS software (1990). The means were compared by Scott-Knott's test at the level of 5%, using the completely randomized design.

RESULTS

The effect of EO on *in vitro* gas production (ml g^{-1} DM) are presented in Table 1. EO included in the incubation medium did not affect ($P > 0.05$) gas production in the first hours of fermentation, but after 24 h, total gas production was reduced significantly ($P < 0.05$) by the addition of the three EO at all concentrations. The greatest decrease was observed with EO of *S. calamintha.* Similarly, methane production was not affected by concentration of EO during the early hours of fermentation (after 9 h of incubation, $P > 0.05$). However, a significant decrease was noted and compared with the control, at 24, 48, 72 and 96 h (Table 2). This impact was dependent upon the dose of EO administrated in syringes. The greatest decrease was observed for *S. calamintha* (65%) and the lowest for *M. pulegium* (58%). This decrease was greater than 72% for all three of the EO at the end of fermentation (96 h of incubation).

The addition of EO to the incubation medium had a significant effect ($P > 0.05$) at 24 h pH values (Table 2). Compared to the controls, addition of EO increased pH values except at the lowest concentration (1.66 µl/ml) of *J. phoenicea* and *M. pulegium*. Furthermore, inclusion of these EO in culture media reduced significantly ammonia production (Table 2) ($P < 0.05$). This effect was linearly

Table 1. Effect of increasing doses of essential oils on *in vitro* total gas production (ml/g DM).

Incubation time (h)	Control	*Juniperus phoenicea*			*Mentha pulegium*			*Satureja calamintha*			S.E.M	P
	0*	1.66*	3.33*	6.66*	1.66*	3.33*	6.66*	1.66*	3.33*	6.66*		
3	12.00	13.67	12.33	13.00	13.34	13.33	12.67	12.00	12.33	12.67	0.69	0.1531
6	13.67	15.33	15.00	14.67	15.00	14.67	13.67	13.67	15.33	14.00	0.82	0.7931
9	15.35	17.02	16.02	16.68	17.02	16.02	15.02	15.35	17.02	16.02	0.87	0.4950
24	27.35^{a}	24.68^{b}	21.01^{d}	20.01^{de}	24.68^{b}	21.01^{d}	18.68^{f}	23.35^{c}	19.35^{ef}	17.35^{g}	0.58	0.0163
48	36.02^{a}	32.35^{b}	24.68^{e}	20.68^{f}	30.35^{c}	23.02^{e}	20.35^{fg}	28.02^{d}	20.02^{fg}	18.02^{g}	1.05	0.0204
72	37.35^{a}	35.35^{b}	26.68^{d}	20.68^{f}	35.35^{b}	24.68^{e}	20.02^{f}	30.02^{c}	19.68^{f}	17.68^{g}	0.73	0.0000
96	39.35^{a}	35.35^{b}	26.68^{d}	20.68^{f}	35.35^{b}	24.68^{e}	20.02^{f}	30.02^{c}	19.68^{f}	17.68^{g}	0.78	0.0000
a**	7.39^{b}	10.65^{a}	10.84^{a}	9.86^{a}	10.85^{a}	11.38^{a}	10.41^{a}	10.12^{a}	9.74^{a}	8.95^{a}	1.98	0.0304
b**	33.42^{a}	26.58^{b}	16.31^{d}	10.87^{f}	26.66^{b}	13.43^{e}	9.74^{fg}	20.36^{c}	10.69^{f}	9.05^{fg}	1.70	0.0208
c**	0.04^{b}	0.03^{c}	0.04^{b}	0.11^{a}	0.03^{c}	0.05^{b}	0.08^{a}	0.04^{b}	0.10^{a}	0.12^{a}	0.01	0.0345

Means with different letters (a, b, c, d, e, f, and g) within the row are statistically different. S.E. M = standard error of the means. *Concentrations of essential oils (µl/ml). **Data were fitted to the equation $p = a + b\ (1 - e^{-ct})$ as described by Ørskov and McDonald (1979), where *p* is percentage degraded at time t, a is the zero time intercept, a + b is the potential degradability and c is the rate of degradation.

Table 2. Effect of increasing doses of essential oils on methane production (ml/g DM), pH and ammonia N concentrations.

Parameter	Incubation time (h)	Control	*Juniperus phoenicea*			*Mentha pulegium*			*Satureja calamintha*			S.E.M	P
		0*	1.66*	3.33*	6.66*	1.66*	3.33*	6.66*	1.66*	3.33*	6.66*		
	3	3.17	2.70	2.56	2.30	2.17	2.84	2.14	2.17	2.84	2.19	0.50	0.3473
CH_4 (ml/g DM)	24	7.92^{a}	3.75^{b}	3.09^{cde}	2.80^{cde}	2.42^{de}	3.35^{cd}	2.94^{cde}	2.50^{de}	2.80^{cde}	2.21^{e}	0.31	0.0015
	96	15.67^{a}	6.50^{b}	4.84^{c}	2.75^{d}	6.17^{b}	3.25^{c}	2.88^{d}	5.92^{b}	2.24^{d}	2.16^{d}	0.50	0.0114
pH	24	6.53^{c}	6.54^{c}	6.66^{b}	6.66^{b}	6.54^{c}	6.64^{b}	6.64^{b}	6.63^{b}	6.72^{a}	6.72^{a}	0.02	0.0000
NH3-N (10^2 mg/l)	24	7.00^{b}	6.94^{b}	6.86^{b}	7.65^{a}	7.68^{a}	6.64^{c}	6.78^{c}	6.93^{b}	6.45^{c}	5.44^{d}	0.02	0.0000

Means with different letters (a, b, c, d and e) within the row are statistically different. S.E.M= standard error of the means.*Concentrations of essential oils (µl/ml).

related to dose administered (r = 0.99) for *S. calamintha*. Nevertheless, a different trend was observed for *M. pulegium* and *J. phoenicea*. For the first extract, ammonia production was increased with dose of 1.66 µl/ml; however, a significant reduction was recorded with the other doses. While for *J. phoenicea*, this production was intensified in the presence of the high concentration (6.66 µl/ml) and any effect was noted for the other doses.

DISCUSSION

In this experiment, addition of EO in the culture media at all doses reduced methane production and this effect was more pronounced after 24 h of incubation; this impact was mainly attributed to their antimicrobial activity (Hart et al., 2008; Rochfort et al., 2008). In fact, these secondary compounds were constituted from a mixture of chemical molecules that exert antimicrobial activity by multiple mechanisms of action and can inhibit a broad variety of both Gram-positive and Gram-negative bacteria and other ruminal microorganisms (Calsamiglia et al., 2007). According to the literature, the prominent components of *M. pulegium* EO are menthol, menthone, piperitone, piperitenone, 1,8 cineole, α-terpineol and pulegone (Derwich et al., 2010; Mahboubi and Haghi, 2008;

Marzouk et al., 2008), the major component of *J. phoenicea* is α-Pinene and other components may coexist in low percentage such as linalool, α-phyllandrene, mycene (Bouzouita et al., 2008; Derwich et al., 2010; Ennajar et al., 2009a,b) and that of *S. calamintha* were characterized by the presence of thymol, p-cymene and γ-terpinene (Satrani et al., 2001). The impact of these EO on ruminale methanogenesis can be a result from their major compound or from the synergic action of all constituents. The same observations have been observed by several authors who studied the inhibition effect of ruminal methanogenesis by addition of individual or blends of EO. *Eucalyptus* oil inhibited CH_4 production up to 58% at 1.66 ml/L (Kumar et al., 2009) and 90.3% at 2 ml/L (Sallam et al., 2009). *M. piperita* EO at 2 ml/L reduced methanogenesis by 75.6% (Agarwal et al., 2009). A concentration of 20 mg/l of Juniper berry oil decreased CH_4 production up to 50% (Chaves et al., 2008). In other research, Macheboeuf et al. (2008) reported that thymol caused a suppression of CH_4 to the extent of 99% at 6 mM and carvacrol reduced CH_4 production by 98.4% at 5 mM.

Inclusion, EO in culture media at 3.33 and 6.66 µl/ml induced an increase in pH values compared with the control. This increase in pH can be associated with a reduction in total volatile fatty acids (VFA) concentration and/or with a high liberation of carbonate (HCO_3) ions from the buffer solution. The same results have been reported by other authors who have worked in the same conditions. For example using batch cultures, Busquet et al. (2006) noted that addition of eugenol, carvacrol, anethol, anise oil, cinnamon oil and oregano oil (at 3 mg/ml) reduced total VFA and increased pH after 24 h of fermentation. Similarly, Castillejos et al. (2006) reported that thymol, guaiacol, vanillin and eugenol at concentration of 5 mg/ml increased final pH and decreased total VFA concentration in 24 h *in vitro* batch culture incubations.

Ruminants have a low efficiency of N retention (Castillejos et al., 2005); hence, many studies have been directed to improve their ammonia N utilization. Molero et al. (2004) reported that the reduction in losses of N from the rumen can be obtained in different ways, one of them being the feeding of slowly degradable proteins and/or feed compounds that either reduce levels of rumen ammonia N and/or reduce protein degradation in the rumen. In the current study, EO had variable effects on ammonia concentrations that were partly dependent upon the EO dose. In view of the antimicrobial activity of EO, the observed decrease in ammonia N concentrations cannot be attributed to an increase in microbial growth (Macheboeuf et al., 2006). The decrease in ammonia N concentration can have many causes, such as: inhibition of proteolysis, peptidolysis or deamination. Indeed, many studies suggest that most EO reduced ammonia N by inhibition of deamination which is assured principally by ammonia hyper-producing bacteria (Castillejos et al., 2005). The decrease in ammonia concentration could also result from a negative effect on protozoa and thus a decrease in predation of bacteria. Furthermore, the increase in ammonia concentrations observed with EO of *J. phoenicea* (6.66 µl/ml) and *M. pulegium* (1.66 µl/ml) can be attributed to a selective inhibition of microorganisms that are users of ammonia N (Macheboeuf et al., 2006).

Conclusion

The three EO tested decreased CH_4 production at all doses tested and also decreased ammonia N concentrations. These secondary compounds had a potential to reduce methanogenesis in the rumen, but further *in vitro* and *in vivo* trials are required to determine the optimum dose which reduce CH_4 production without adversely affecting fermentation and rumen function. Moreover, it is important to determine their mechanisms of action.

REFERENCES

Agarwal N, Shekhar C, Kumar R, Chaudhary LC and Kamra DN (2009). Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Feed Sci. Technol. 148:321–327.

Beauchemin KA, Kreuzer M, O'Mara F and McAllister TA (2008). Nutritional management for enteric methane abatement: a review. Aust. J. Ep. Agric. 48:21–27.

Benchaar C, Chaves AV, Fraser GR, Yang Y, Beauchemin KA and McAllister TA (2007). Effects of essential oils and their components on *in vitro* rumen microbial fermentation. Can. J. Anim. Sci. 87: 413-419.

Benchaar C and Greathead H (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. Anim. Feed Sci. Technol. 166-167: 338-355.

Bouzouita N, Kachouri F, Ben Halima M and Chaabouni MM (2008). Composition chimique et activités antioxydante, antimicrobienne et insecticide de l'huile essentielle de *Juniperus phoenicea*. J. Soc. Chim. Tunisie. 10:119-125.

Busquet M, Calsamiglia S, Ferret A and Kamel C (2006). Plant extracts affect *in vitro* rumen microbial fermentation. J. Dairy Sci. 89:761-771.

Calsamiglia S, Busquet M, Cardozo PW, Castillejos L and Ferret A (2007). Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation. J. Dairy Sci. 90: 2580-2595.

Castillejos L, Calsamiglia S, Ferrer A and Losa R (2005). Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Anim. Feed Sci. Technol.119: 29-41.

Castillejos L, Calsamiglia S and Ferret A (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* system. J. Dairy Sci. 89:2649-2658.

Castillejos L, Calsamiglia S, Ferret A and Losa R (2005). Effects of a specific blend of essential oil compounds and type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Anim. Feed Sci. Technol. 119:2941.

Chaney AL and Marbach EP (1962). Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Chen XB (1997). Neway Exel: a utility for processing data of feed degradability and *in vitro* gas production (version 5.0). Rowett Research Institute, Aberdeen, UK.

Chaves AV, He ML, Yang WZ, Hristov AN, McAllister TA and Benchaar C (2008).Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. Can. J. Anim. Sci., 89:97-104.

Derwich E, Benziane Z and Boukir A (2010). Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. Int. J. Agric. Biol.12:199-204.

Derwich E, Benziane Z and Boukir A (2010). GC/MS Analysis and Antibacterial Activity of the Essential Oil of *Mentha Pulegium* Grown in Morocco. Res. J. Agr. Biol. Sci. 6(3):191-198.

Eckard RJ, Grainger C and De Klein CAM (2010). Options for the abatement of methane and nitrous oxide from ruminant production: a review. Livest. Sci.130: 47-56.

Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Abderraba M, Raies A and Romdhane M (2009b). Chemical composition and antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L. (Cupressacees). J. Food. Sci. 74 (7):71-364.

Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Arlette S, Manef A, Raies A and Romdhane M (2009a). The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils. J. Sci. Food. Agric. 90:462-470.

Hart KJ, Yáñez-Ruiz DR, Duval SM, McEwan NR and Newbold CJ (2008). Plant extracts to manipulate rumen fermentation. Anim.Feed Sci.Technol. 147:8-35.

Kumar R, Kamra DN, Agrawal N and Chaudhary LC (2009). Effect of eucalyptus (*Eucalyptus globulus*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim. Nutr. Feed Technol. 9: 237-243.

Macheboeuf D, Morgavi DP, Papon Y, Mousset JL and Arturo-Schaan M (2008). Dose-response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Anim. Feed Sci. Technol. 145:335-350.

Macheboeuf D, Papon Y, Artuso-Schaan M, Mousset JL and Cherel R (2006). Utilisation d'extraits végétaux (huiles essentielles et extrait de polyphénols) pour diminuer la dégradation ruminale des protéines-Étude *in vitro*. Renc. Rech. Ruminants. 13:69-72.

Mahboubi M and Haghi G (2008). Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J. Ethnopharmacol. 119:325-327.

Marzouk B, Ben Hadj Fredj M, Chraief I, Mastouri M, Boukef K and Marzouk Z (2008). Chemical composition and antimicrobial activity of essential oils from Tunisian *Mentha pulegium* L. J. Food. Agr. Environ. 6:78-82.

Menke KH and Steingass H (1988). Estimation of the energetic feed value obtained by chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev. 28:7-55.

Molero R, Ibars M, Calsmiglia S, Ferret A and Losa R (2004). Effects of a specific blend of essential oil compounds on dry matter and crude protein degradability in heifers fed diets with different forage to concentrate ratios. Anim. Feed Sci. Technol. 114:91-104.

Orskov ER and McDonal I (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92: 499-503.

Patra AK and Saxena J (2010).A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochem., 71:1198-1222.

Rochfort S, Parker AJ and Dunshea FR (2008). Plant bioactives for ruminant health and productivity. Phytochem., 69:299-322.

Sallam SMA, Bueno ICS, Brigide P, Godoy PB, Vitti DMSS and Abdalla AL (2009). Efficacy of eucalyptus oil on *in vitro* rumen fermentation and methane production. Options Meditérranéennes, 85: 267-272.

Satrani B, Abdellah F, Fechtal M, Talbi M, Blaghen M and Chaouch A (2001). Composition chimique et activité antimicrobienne des huiles essentielles de *Satureja calamintha* et *Satureja alpine* du Maroc. Ann. Fals. Exp. Chim., 94 (956):241-250.