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Antimethanogenic activity of essential oils extracted from *Rosmarinus officinalis* and *Lavandula officinalis*

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ABSTRACT

Methane constitutes the main way of hydrogen elimination in the rumen during the microbial digestion of food. Its eructation by ruminants led to both energy loss for animals and a worsening of the greenhouse by its radioactive power. The aim of this study focuses on exploring the ability of essential oils (EO), extracted from rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula officinalis*), in reducing ruminal methanogenesis. Activity of essential oils extracts was compared to that of purified essential oils (carvacrol, cinnamaldehyde and thymol).

The aerial parts of plants (rosemary and lavender) are used fresh. Extraction of EO was made by the hydrodistillation process. Antimethanogenic activity of EO extracts was measured *in vitro* in batch systems (polypropylene syringes, 60 ml capacity). EO extract of each plant and purified EO (carvacrol, cinnamaldehyde and thymol) were tested at different doses: 10, 20 and 40 µl. Gas production was monitored at different time intervals: 2, 4, 6, 8, 24, 48 and 72 h. The qualitative and quantitative analysis of fermentative gas (CH_4 and CO_2) was chemically determinate after 24 hours incubation.

The results indicate that addition of crude oils of *Rosmarinus officinalis* and *Lavandula officinalis* and the purified oils did not affect significantly the pH values ($P > 0.05$). The addition of EO of both plants did not affect gas production after 2 hours of fermentation ($P > 0.05$) for the three doses. Between 4 and 6 h of incubation, increasing EO dose of *Lavandula officinalis* induces a continuous decline (but not significant) of the gas production compared with the control (no additives). Similarly, addition of *Rosmarinus officinalis* essential oils had not significant effect on gas production for 10 and 40 µl doses compared with control. However, higher gas production was recorded for 20µl dose. Addition of *Lavandula officinalis* EO at 40µl level caused significant decrease in gas production after 24h incubation ($P < 0.05$). Methane production was significantly reduced in the presence of EO extracted from *Rosmarinus officinalis* (28.1% ; $P < 0.05$). Besides, EO extracted from *Lavendula officinalis* decrease significantly methane production during 24 hours of incubation for 10 and 40µl doses. In general, EO used in our study showed a significant effect on reducing methane. Thus, we can focus future research on composition characterization of each essential (CG—MS) and to determine minimal inhibitory concentration (MIC) allows maximum methane reduction without adverse effects on digestibility.

KEYWORDS

Carvacrol;
Cinnamaldehyde;
Methane;
Rumen;
Thymol.

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INTRODUCTION

In recent years, the gradual warming of our planet caused by the greenhouse effect becomes a reality as much political as scientific. This effect is due to the accumulation in the atmosphere of gases that trap infrared radiation. These are mainly carbon dioxide, methane, chlorofluorocarbons and nitrogen oxides whose contributions to the greenhouse effect have been estimated respectively at 49, 18, 14 and 6%[5].

Methane is the major way of H_2 elimination produced in the rumen during the microbial digestion of food. Its eructation by ruminants led to both energy loss for animals and a worsening of the greenhouse by its radioactive power. Indeed, its atmospheric concentration has increased from 0.70 to 1.68 ppm during the last two centuries[2] and continues to increase annually by 0.6%[13].

Different strategies were used to minimize the methane emissions. They were mainly interested in the modification of fermentation conditions and/or change the balance of ruminal microbiota population, especially protozoa. Besides, synthetic molecules responsible for microbial activity regulation (antibiotics, halogenated compounds, etc. ...) have been widely used. Biological interventions (defaunated, ruminal acetogenesis) were also applied since the 70s[10]. However, the new European legislation and the recommendations from organizations of consumer protection have greatly limited the use of these processes. Therefore, the search for new alternatives is primordial. In this context, additives natural extracts from plants continue to attract the attention of researchers. The aim of this study focuses on exploring the ability of essential oils (EO), extracted from rosemary (*Rosmarinus officinalis*) and lavender (*Lavandula officinalis*), in reducing ruminal methanogenesis. Activity of EO extracts was compared to that of purified essential oils (carvacrol, cinnamaldehyde and thymol)

MATERIALS AND METHODS

Plant material

Plants used for the extraction of essential oils are lavender (*Lavandula officinalis*) and rosemary (*Rosmarinus officinalis*). They were chosen for their pharmacological use (especially antiseptic property). Both plants are collected in the region of Tebessa and El Hammamet, where the climate is semi-arid. They were collected during the month of April 2009 by hand cutting of the aerial part of plants.

Extraction of crude oils

The aerial parts of plants (rosemary and lavender) are used fresh. Extraction of EO was made by the hydrodistillation process. 100 g of the plant (rosemary or lavender) were mixed with 500 ml of distilled water. The EO were collected by condensing and then separated by settling. They were stored in dark bottles at 4°C.

In vitro Experiment

Antimethanogenic activity of EO extracts was measured *in vitro* by the method described by Menke et al[11]. Fermentation was carried out in polypropylene syringes (60 ml capacity. 200mg of alfalfa hay were introduced into each syringe and left to ferment with 30 ml of the mixture (10 ml of rumen fluid + 20 ml buffer solution). The rumen fluid used as inoculum was collected from sheep fed alfalfa hay *ad libitum* and filtered through 4 layers of surgical gauze and placed in pre-warmed Thermos at 39°C and satured with CO_2. Introduction of EO extract in syringes was performed at the time of incubation. EO extract of each plant and purified essential oils (carvacrol, cinnamaldehyde and thymol) were tested at different doses: 10, 20 and 40 µl. For each sample and for each dose, three syringes were incubated. Under the same conditions, three syringes without substrate and without additives (rumen fluid + buffer solution) and three syringes without additives but containing the substrate were incubated. Syringes were incubated at 39°C for 72h.

Gas production was monitored at different time intervals: 2, 4, 6, 8, 24, 48 and 72 h. The qualitative and quantitative analysis of fermentative gas (CH_4 and CO_2) is performed after 24 hours incubation. This analysis is performed using the methodology described by Jouany[9].

Statistical analysis

Gas production results were analyzed by ANOVA with two factors. The means were compared using the

Dunnette test (comparing the results to that of the control) and the SDPP test for comparing additives effect. Analysis was made by the computer program STATISTICA (Version 6).

RESULTS AND DISCUSSION

pH values of inoculum and that recorded after 72h of incubation were illustrated in TABLE 1. Overall, the average pH of inoculum (freshly ruminal fluid) was 6.48. This value indicates physicochemical conditions favorable to a good fermentation activity of the ruminal microbiota. The addition of crude oils of *Rosmarinus officinalis* and *Lavandula officinalis* and the purified oils (carvacrol, cinnamaldehyde and thymol) did not affect significantly the pH values ($P > 0.05$) (TABLE 1). Similar results were also obtained by Cardozo et al[4] after addition of essential oils of *anise*, *capsicum* and a mixture of cinnamaldehyde and eugenol. The same results were also noted by Noirot and Bayourthe[12] which have used carvacrol in their experiment.

Effect of essential oils extracted from *Rosmarinus officinalis* (EO-RO) and *Lavandula officinalis* (EO-LO) on gas production was shown in figure 1. It appears that addition of essential oils of both plant and for three doses did not affect gas production after 2 hours of fermentation ($P > 0.05$). Between 4 and 6 h of incubation, increasing essential oil dose of EO-LO induces a continuous decline (but not significant) of the gas production compared with the control (no additives). Similarly, addition of EO-RO had not significant effect on gas production for 10 and 40 µl doses compared with control. However, higher gas production was recorded for 20µl dose. Addition of EO-LO at 40µl level caused significant decrease in gas production after 24h incubation ($P < 0.05$). At noted earlier, addition of EO-RO extract at 20 and 40 µl doses had also no significant effect on gas production, but 10 µl dose reduces significantly gas production ($P < 0.05$). Similar results were also recorded by Benchaar et al (2007). These authors have note that the addition of essential oils extracted from thyme and oregano (20 µl) had no effect on gas production.

Effect of purified essential oils on gas production at the same doses was presented in figure 2. After 2 hours of incubation, addition of thymol and cinnamaldehyde leads to low gas production at 10 and 40 µl doses. However, gas production was increased with 20µl dose. Nevertheless, gas production was significantly decreased in presence of carvacrol ($P < 0.05$). The same pattern was recorded for cinnamaldehyde at 24 h incubation. Between 4 and 6 hours of incubation, for cinnamaldehyde at 20 µl dose reduced significantly gas production ($P < 0.05$). However, this effect was not noted for 10 and 40 µl doses ($P > 0.05$). The same

TABLE 1 : pH values recorded after 72h of fermentation in syringes incubated with crude and purified EO

Essential oils	Doses			
	0µl	10µl	20µl	40µl
Rosmarinus officinalis	7.12	6.98	6.97	7.15
Lavandula officinalis	7.12	7.15	7.02	6.90
Carvacrol	7.12	7.13	6.95	7.04
Cinnamaldéhyde	7.12	7.44	7.54	7.05
Thymol	7.12	6.96	7.02	7.59

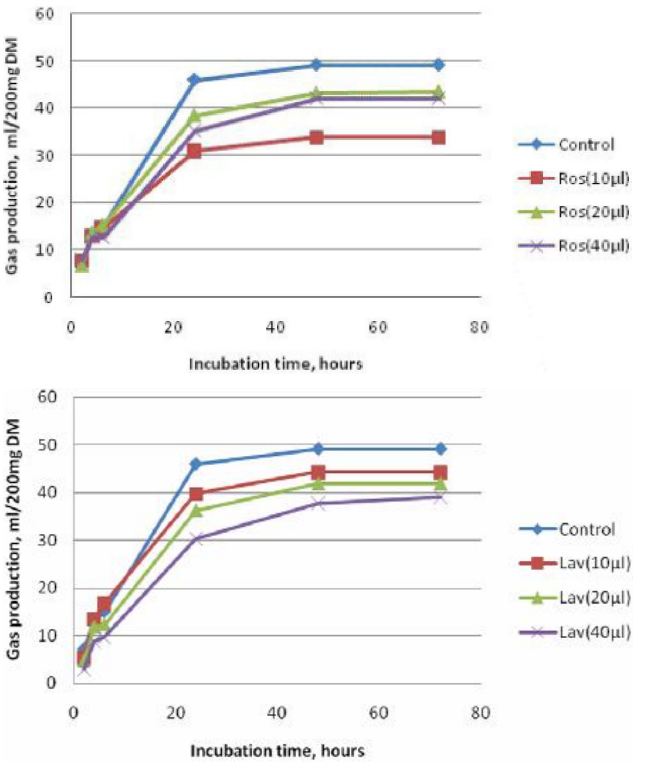

Figure 1 : Influence of EO-RO (Ros.) and EO-LO (Lav.) used at three doses (10µl, 20µl and 40µl) on *in vitro* gas production

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TABLE 2 : Effect of EO-RO, EO-RO and purified essential oils on CH_4 and CO_2 production (ml ± SEM) after 24h and 72h of fermentation of alfalfa hay.

	Dose (µl)	24h		72h	
		CH_4	CO_2	CH_4	CO_2
Control	00	$15,3^{a}$ ±2.1	$30,8^{a}$ ±4.3	$16,3^{a}$±2.0	$32,8^{a}$ ±4.5
	10	$09,9^{b}$ ±10.3	$22,1^{b}$ ±09.2	$10,8^{b}$ ±10.1	$23,0^{b}$ ±11.3
Rosmarinus officinalis	20	$17,1^{a}$ ±9.2	$24,1^{b}$ ±8.1	$18,2^{a}$ ±8.9	$25,3^{b}$ ±9.3
	40	$09,4^{b}$ ±2.6	$29,6^{a}$ ±9.5	$10,3^{b}$ ±3.5	$31,8^{a}$ ±9.4
	10	$2,1^{c}$ ±7.6	$38,6^{a}$ ±7.7	$13,4^{a}$ ±8.1	$31,0^{a}$ ±8.2
Lavandula officinalis	20	$4,1^{c}$ ±3.5	$34,9^{a}$ ±9.6	$6,7^{c}$ ±3.7	$35,3^{a}$ ±9.5
	40	$5,7^{c}$ ±4.2	$30,8^{a}$ ±11.3	$5,5^{c}$ ±4.0	$33,6^{a}$ ±10.9
	10	$2,1^{c}$ ±0.8	$5,7^{c}$ ±1.2	$2,4^{c}$ ±1.0	$6,1^{c}$ ±1.3
Carvacrol	20	$2,7^{c}$ ±1.3	$5,8^{c}$ ±1.5	$2,9^{c}$ ±1.5	$6,1^{c}$ ±2.0
	40	$1,3^{c}$ ±0.4	$3,0^{c}$ ±0.6	$1,4^{c}$ ±0.6	$3,3^{c}$ ±0.7
	10	$09,5^{b}$ ±1.5	$28,2^{a}$ ±1.9	$10,3^{b}$ ±0.5	$30,2^{a}$ ±2.0
Cinnamaldehyde	20	$5,7^{b}$ ±3.5	$22,6^{b}$ ±7.4	$6,1^{c}$ ±4.4	$24,4^{b}$ ±6.0
	40	$19,3^{a}$ ±10.7	$24,4^{b}$ ±3.3	$21,3^{a}$ ±11.4	$26,3^{a}$ ±4.3
	10	$1,2^{c}$ ±0.4	$2,7^{c}$ ±0.8	$1,2^{c}$ ±0.5	$2,7^{c}$ ±1.0
Thymol	20	$0,5^{c}$ ±0.7	$1,7^{c}$ ±1.2	$0,5^{c}$ ±0.5	$1,7^{c}$ ±1.2
	40	$0,7^{c}$ ±0.9	$3,5^{c}$ ±1.5	$0,7^{c}$ ±0.6	$3,5^{c}$ ±1.4

Means assigned with different letters in the same column are significantly different (P < 0.05)

trend was observed for carvacrol and thymol. These results were consistent with those reported by Busquet and Calsamiglia[3] who also used the same essential oil but at higher levels. This reduction can be due to the deficiency of hydrogen, carbon and energy which was essential for gas production and can creates competition between the constituents of the rumen microbiota. Similarly, addition of thymol affected significantly gas production (P <0.05). The same observation was made by Evans and Martin[7]. These authors have found significant effect of thymol on gas production. Concerning carvacrol, similar observations were done by Benchaar et al[1] where carvacrol and eugenol have been used at 400ml/l concentration (v/v). These effects may be due to a deficit of carbon and hydrogen which creates competition between the constituents of the rumen microbiota. Moreover, these essential oils (thymol and carvacrol) were rich in OH group[6]. These hydroxyl compounds exert an antimicrobial activity by disrupting the proton concentration at the cell membrane which affects flow electron and active content cell coagulation.

Effect of EO-RO on methane production was shown in TABLE 2. It appears that methane production was significantly reduced in the presence of this essential oil (P < 0.05). However, it was noted that 20µl level of this plant extract induced an increase in volume of methane compared with the control. This result could be explained by the favorable ruminal environment (low

redox potential) which permits terpenes degradation. Under these conditions, the redox equivalents from the oxidation of terpenes can lead to methane production[8]. Influence of essential EO-LO on methane production is also shown in TABLE 2. It indicates significant methane reduction during 24 hours of incubation for 20 and 40µl doses. This reduction may be due to the toxicity of essential oil on the ruminal microbiota, in particular, protozoa and/or unavailable hydrogen (H_2) in ruminal media.

Effects of purified extracts are also reported in TABLE 2. It shows that addition of cinnamaldehyde had significant effect on methane reducing methane, except 40µl dose. Different trend was noted for carvacrol and thymol addition. These oils caused significant reduction in methanogenesis compared to the control (no additives) ($P < 0.05$). This decrease can be explained by the fact that essential oils inhibit the synthesis of acetate acid and thus limit *in vitro* methane production.

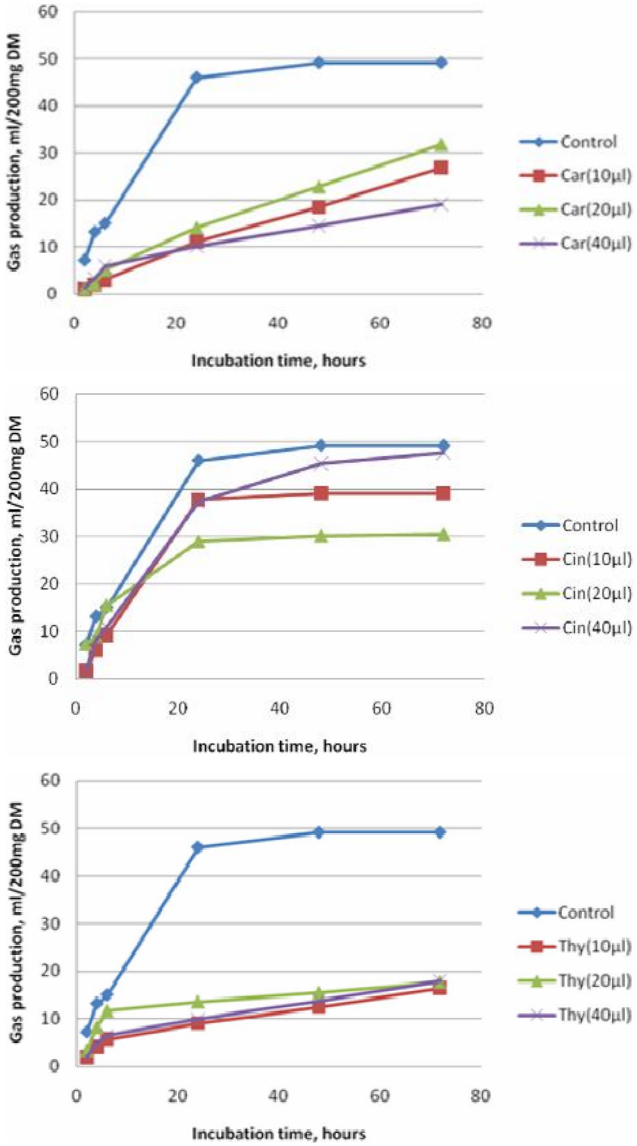

Figure 2 : Effect of purified essential oils [carvacrol (Car.), cinnamaldehyde (Cin) and thymol (Thy.)] used at different doses (10, 20 and 40µl) on *in vitro* gas production

CONCLUSION

The results obtained in this study indicate clearly that both purified or extract essential oils, used at different levels, do not modified pH ruminal media. It means that these compounds could control the release of proton from buffer solution and/or minimize effect of volatile fatty acids production.

Addition of carvacrol and thymol leads to lower gas production during all incubation times. Levels used in this study for both purified oil appear to be lethal for ruminal microbiota because they stopped completely the fermentation process. Methane production was also significantly reduced in presence of these two essential oils. The same pattern was observed for plant essential oils extract and cinnamaldehyde, except 10µl and 20µl levels for *Lavandula officinalis* and to cinnamaldehyde, respectively.

In general, essential oils used in our study showed a significant effect on reducing methane. Thus, we can focus future research on composition characterization of each essential (CG—MS) and to determine minimal inhibitory concentration (MIC) allows maximum methane reduction without adverse effects on digestibility.

REFERENCES

[1] C.Benchaar, H.V.Petit, R.Berthiaume, D.R.Ouellet, J.Chiquette, P.Chouinard; Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, and milk composition in dairy cows fed alfalfa silage or corn silage. Journal of Dairy Science, http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS002203020 7715722.pdf, **90**, 886-897 **(2007)**.

[2] D.R.Blake, F.S.Rowland; Global Atmopsheric Concentrations and Source Strength of Ethane. Nature, **321**, 231-233 **(1986)**.

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[3] M.Busquet, S.A.Calsamiglia, K.Ferret; Plant extracts affect *in vitro* rumen microbial fermentation. Journal of Dairy Science, http://download.journals.elsevierhealth.com/pdfs/journals/0022-0302/PIIS0022030206721373.pdf, **89**, 761-771 **(2006)**.

[4] P.W.Cardozo, S.A.Calsamiglia, K.Ferret, C.Kamel; Effects of alfalfa extract, anise, capsicum and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. Journal of Animal Science, http://jas.fass.org/cgi/reprint/84/10/2801.pdf, **84**, 2801-2808 **(2006)**.

[5] Y.Chouinard; Production et émission du méthane et du gaz carbonique par les ruminants. 65éme congrès de l'ordre des agronomes du Québec OAQ, 10 **(2004)**.

[6] H.J.D.Dorman, S.G.Dean; Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2672.2000.00969.x/pdf, **88**, 308-316 **(2000)**.

[7] J.D.Evans, S.A.Martin; Effects of thymol on ruminal microorganisms. Current of Microbiology, **41**, 336-340 **(2000)**.

[8] J.Harder, C.Probian; Microbial degradation of monoterpenes in the absence of molecular oxygen. Applied and Environemental Microbiology, http://aem.asm.org/cgi/reprint/61/11/3804.pdf, **61**, 3804–3808 **(1995)**.

[9] J.P.Jouany, L.Broudiscou, R.A.Prins, S.Komisarczuk-Bony; Metabolism and nutrition of the rumen microbial population. In: Nutrition of farm animals. J.P.Jouany (Eds.), INRA Paris, 350-381 **(1994)**.

[10] H.P.S.Makkar; *In vitro* gas methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology, 123-124, 291-302 **(2005)**.

[11] K.H.Menke, L.Raab, A.Salewski, H.Steingass, D.Fritz, W.Shneider; The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor. Journal of Agricultural Science, **93**, 217-222 **(1979)**.

[12] V.Noirot, C.Bayourthe; Effect of carvacrol on ruminal fermentation *in vitro*. Journal of Dairy Animal 89 (supplement 1):238 http://adsa.asas.org/meetings/2006/abstracts/233.pdf, **(2006)**.

[13] L.P.Steele, E.J.Dlugokencky, P.M.Lang, P.P.Tans, R.C.Martin, K.A.Masarie; Slowing down of the global accumulation of atmospheric methane during the 1980s. Nature, **358**, 313–316 **(1992)**.