INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 4, p. 150-156, 2015

RESEARCH PAPER OPEN ACCESS

# Effects of pastoral plants essential oil redirecting rumen fermentation to reduce methanogenesis using *in vitro* gas production method

**H. Bouchiha[1,2,3], R. Rouabhi[1,3*], K. Bouchama[1], H. Berrebbah[3], M.R. Djebar[3]**

[1]*Applied Biology Department, SNV Faculty, Larbi Tebessi University, Algeria*

[2]*Laboratoryof Microbiological Applications, Biology Department, Constantine University, Algeria*

[3]*Laboratory of Cellular Toxicology, Biology Department, Annaba University, Algeria*

**Key words:** Methane, Ruminants, Essential oïl, Hydro-distillation, *Artemisia Herba alba, rosmarinus officinalis.*

http://dx.doi.org/10.12692/ijb/7.4.150-156 Article published on October 25, 2015

## Abstract

The aim of the present study was to evaluate interactions occurring between microbial degradation and essential oil extracted by hydro-distillation of two plants with three doses. Plants selected for this study are: *Artemisia herbaalbaand Rosmarinusofficinalis*.The effects of EO as additives were evaluated in vitro by using *batch systems* (*in vitro* gas production technique). Cumulative gas production (CGP) was recorded at 2, 4, 6,8,48 and 72 hours of incubation. The qualitative analysis of gas produced (carbon dioxide ($CO_2$) and methane ($CH_4$) was recorded after 24 h of incubation and measured according to procedure described by Jouany (1994). Cumulative gas production profiles were fitted to the exponential model y = a + b (1 - e-c*t). At the end of incubation, pH values were noted.Chemical analysis shows that the reduction of total gas production is detected with all plants (P < 5%). the largest reduction is recorded with *Rosmarinusofficinalis* (34.73%)after the addition of 14 µL of EO, the other doses decrease methane production by5.1% and 26.08% with 10 and 19 µL respectively.This *in vitro* continuous culture suggests that gas production byrumen microbial populations may be affected by essentials oils. Therefore, essentials oils hold promise as feed additives in ruminant nutrition to improve feed efficiency and control of methane production in livestock. The consequences of the observed effects need to be evaluated with different doses or their active components and if possible conducted *in vivo* before final conclusions are drawn.

* **Corresponding Author:** R. Rouabhi ✉ r_rouabhi@yahoo.fr,

## Introduction

Methanogenic Achaea account for an annual global production of some 400 million metric tons of methane (Ferry, 1997). This has a profound impact on the global change, because the atmospheric methane, which originates from these biological sources, can contribute for about 20% to global warming (Lindau and al., 1993). Since atmospheric methane concentrations have increased steadily by about 1-2% per year through the last decades, (Ferry 1997), Methane formation by methanogenic Achaea occurs in nearly all anaerobic environments such as rumen. Many rumen methane inhibitors, such as ionophores and nitrite, are being explored as feed additives to inhibit rumen methane production, but most of them tend to be eliminated due to safety concerns over animal-derived food (Szumacher and al., 2010). Other alternatives are, therefore, required for example natural essential oils (EO) extracted from plants which have been used to reduce methane production, inhibit feed protein degradation (Calsamiglia and al., 2007). It is reported that the anti-microbial activity of EO is related to the chemical structure of their main active components (Castillejos and al., 2006). Methane emissions can be decreased by supplementing the diet with certain additives and ingredients. Adding fats to the diet can reduce methane emissions by lowering ruminal ferment ability, and to a lesser degree, through hydrogenation of the unsaturated fats (Johnson and Johnson, 1995). However, added fat can also lower feed intake and fiber digestibility (McGinn and al., 2004), potentially negatively affecting animal performance. Alternative natural feed additives that shift ruminal fermentation may show promise (Wallace, 2004). When an EO is used to inhibit methane production, the hydrogen produced byfibre digestion will accumulate in rumen fluids. An alternative pathway allowingfor the capture of reducing equivalents spared from methanogenesis is, therefore,needed.Thus, selective trials would be needed to determine the optimal EO and addition level that caninhibit methanogens but have little negative effects on ruminal fibre digestion.In our studies, we investigate the impact of different doses of EO on reducing ruminal methaneproduction. It is still unclear, however, what the effect on rumen fermentation and methane production will be when the natural essential oils are added (Newbold and al., 2004; Spanghero and al., 2008).

## Materials and methods

*The samples*

Rumen fluid is taken at random from 3 adult sheep sacrificed; they received a free diet and undefined. The samples are taken in the morning and the contents of three sheep mixed was filtered through 4 layers of surgical gas and introduced to laboratory into a pre-heated thermos (saturated with $CO_2$).

*Inoculum preparation*

The inoculum was prepared following the procedures of (Menkeand*al.,* 1979). It consisted of the rumen liquor mixed with anaerobic artificial saliva (1: 2 v/v). The latter was prepared as described by (Menke and Steingass 1988).

The syringes, prewar med at 39°C and contained two hundred grams of single substrates *vetch-oat hay*, were inoculated with 30 ml inoculum under continuous $CO_2$ reflux. They were incubated in an incubator at 39°C for 72 hours.

The EO is injected to the reaction mixture just before the incubation fomenters, while all treatments were subjected to three doses (10, 14 and 19 ul). In each test, three fomenters with control substrate also incubated but without additive (three syringes containing incubation medium without any substrate and additives were incubated as blanks to correct the total gas production resulting from the activity of the rumen fluid).

*Gas production calculation and statistical analysis*

*Gas production measurements*

At the end of incubation, the pH and gas production were measured and analyzed the composition of gases ($CO_2$ and $CH_4$) are the main fermentation gases produced by microbial degradation. The reading is carried out of the gases produced at various kinetic points (2, 4, 6, 8, 24, 48 and 72). The carbon dioxide

and methane productions were evaluated after 24h of incubation by injection in each syringe 4 ml of sodium hydroxide (NaOH, 10N) (Jouany, 1994).

*Gas data calculation and statistical analysis*

Mean gas production data of blanks were subtracted from the recorded gas production of fomenters with control substrate and additive to get net gas production values. These calculated CGP were fitted with the monomolecular model (Ørskov and McDonald, 1979):

(y = a+b (1- -c*t)) where: "a" is the gas production from the readily fermented fraction, "b" the gas production from the slowly fermented fraction and "c" the rate of fermentation.

**Results and discussion**

*Effect of essentials oils on pH*

The results of pH affected by *Artemisia herbaalba*, *Rosmarinusofficinalis*and pH ofcontrol are presented in Table 1.

**Table 1.** pH affected by EO of Artemisia herbaalba and Rosmarinusofficinalis.

| Treatments | Artemisia herba alba | Rosmarinusofficinalis | Control |
|---|---|---|---|
| Value of pH | 6.96 | 6.54 | 6.74 |

It appears that the average pH value of rumen juice in the various tests varies between 6.96 and 6.54. So the treatments did not affect pH change or variance.

These values are similar to those reported by Chenost and*al.* (2001) and Adoui (2001) noted average value of 6.42. Benchaarand*al.* (2006, 2007) showed a slight increase in pH, whereas Newboldand*al.* (2004), Beauchemin and McGinn (2006), and Castillejosand*al.* (2007) were unable to detect any differences in rumen pH when essential oil mixtures were administered.

The range of measured pH is considered favorable for the cellulolytic activity of rumen microorganisms (Hungate, 1966). For Hoover (1986) the pH was within the range considered optimal for microbial digestion [6.0 to 7.0].

Successors are several factors influencing the pH such as the nature of the substrate, additives and inoculum. Thus, neutral pH values measured can be explained by the nature of our extracts EO low water content and also prevent the attachment of bacterial cells to the substrates. Indeed, Tassouand*al.*, (1995) reported that the different properties of EO due to contact with the macromolecules such as lipids or proteins that protect bacteria.

*Effect on gas production*

The results of gas production GP at various kinetic points (2,4,6,8,24,48 and 72 hours) affected by *Artemisia herba alba* and *Rosmarinusofficinalis* with different doses as compared to control are shown in Figure 1.

The fermentation is relatively intensive during the first 24 hours of incubation; after which it reaches a stationary phase. The kinetics of gas production appears to be determined by two distinct phases; the first one corresponds to the degradation of the soluble fraction of substrates *vetch-oat hay*and the second to the insoluble but potentially fermentable fraction. The changeprofile in the gas production kinetics after the use of EOS is probably due to their chemical composition.

This last indicates that the different mechanisms of action of these EOS against microbes may have contributed to the differing fermentation profiles obtained with different plants and doses. Macheboeufand*al.* 2008 reportedthat this difference may be attributed to the varying anti-microbial activities among aldehyde and phenolic-based oils. The mechanism of EO action was closely related to their lipophilic characteristics. Some of these oils can destroy the cell membrane of microbes, while some function through binding proteins and disturbing the

metabolism of cells (Gill and Holley, 2004).

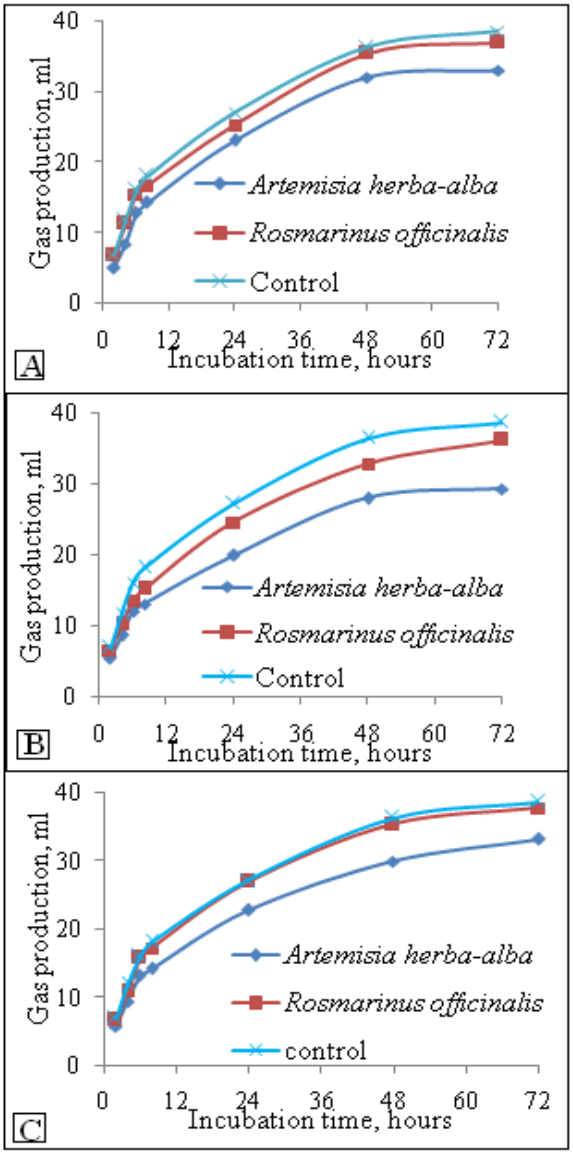


**Fig. 1.** A, B and C represent experimental gas production kinetics and exponential model profile of essential oils with (10, 14 and 19 µl) respectively.

*Effect on methane production*

The decrease in methane emission compared with the control tended ($P < 0.005$) to be maintained with all the EO. However, doses of EO had no effect significatively ($P > 0.005$) on methane productions (Fig. 2).

After Adding essential oil of *Artemisia herbaalba*to the diet decreased methane ($CH_4$) production then a control by 23.03%,27.91%and27.91%with 10, 14 and19µL respectively.However,after Addition essential oil of *Rosmarinusofficinalis* to the diet Methane ($CH_4$) productiondecreased then a control by 5.1%,34.73%and 26.08% with 10, 14 and19 µL respectively.

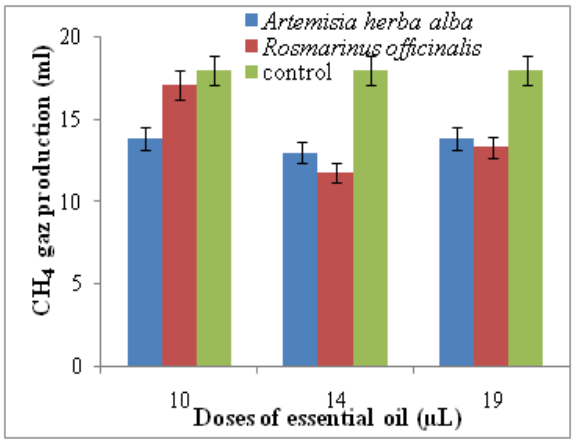


**Fig. 2.** Represent $CH_4$ production affected by essential oils with different doses.

This difference in reduction of $CH_4$ between different essential oils added is probably due to the difference in their effects on ruminal fermentation, it depends on several factors influence the effect of our EO such as the type and molecular structure of the components assets based plant, the added dose and type of target microorganisms and their possible adaptation to EO.

Calsamigliaand*al.* (2007) reported that terpenoids are the most important active components of EO;terpenoids is a set of materials with the skeleton of terpenes with one or more chemical functional groups (alcohol, aldehyde, ketone, acid, lactone, etc.).

*In vitro*, a higher antimicrobial activity compared oxygenated terpene hydrocarbon terpenes was observed. Lis-Balchinand*al.* (1998) reported that pure oxygenated components also showed higher activity compared to EO where they are. On this basis, the order of antimicrobial activity of these compounds is the following: Phenols> aldehydes> ketones> alcohols> ethers> hydrocarbons (Amaral and*al.* 1998).

The molecular structure therefore seems to be of importance as the presence of oxygen in the molecule of terpene role of the hydrocarbon backbone lipophilic characteristic and the hydrophilic property

of the functional groups are critical against the antimicrobial activity (Kalembaand Kunicka, 2003).

Another important parameter determining the antimicrobial activity of EO is the type of target microorganisms. Generally, various microorganisms do not have a similar sensitivity with respect to EO (Rouabhi *et al.*, 2006).

Fungi generally show a greater sensitivity to bacteria vis-à-vis the EO (Amaraland*al.* 1998.).Finally, a higher sensitivity of anaerobic bacteria was observed regardless of the EO compared to those living under aerobic conditions (Amaraland*al.*1998).

**Reference**

**Amaral JA, Ekins AS, Richards SR, Knowles R.** 1998. Effect of selected monoterpenes on methane oxidation, denitrification and aerobic metabolism by bacteria in pure culture.Applied Environ. Microbiol. **64,** 520-525. PMCID: PMC106766.

**Beauchemin KA, McGinn SM.** 2006. Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. Journal of Animal Science **84,** 1489-1496. http://dx.doi.org/2006.8461489x

**Benchaar C, Petit V, Berthiaume R, Ouellet R, Chiquette J, Chouinard Y.** 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. Dairy Science **90,** 886-897. http://dx.doi.org/10.3168/jds.S00220302(07)71572-2

**Benchaar C, Duynisveld L, Charmley E.** 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle.Canadian Journal of Animal Science **86,** 91-96. http://pubs.aic.ca/doi/pdf/10.4141/A05-027

**Calsamiglia S, Busquet M, Cardozo PW, Castillejos L, Ferret A.** 2007. Invited Review: Essential oils as modifiers of rumen microbial fermentation.Dairy Science **90,** 2580-2595. http://dx.doi.org/10.3168/jds.2006-644

**Castillejos L, Calsamiglia S, Ferret A, Losa R.** 2007. Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. Animal Feed Science **132,** 186-201. http://dx.doi.org/10.1016/j.anifeedsci.2006.03.023

**Castillejos L, Calsamiglia S, Ferret A.** 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in vitro systemsDairy Science **89,** 2649-2658. http://dx.doi.org/10.3168/jds.S00220302(06)72341-4

**Ferry JG.** 1997. Methane: small molecule, big impact. Science **278,** 1413-1414. http://dx.doi.org/10.1126/science.278.5342.1413

**Gill AO, Holley RA.** 2004. Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against L. monocytogenes and Lactobacillus sakei.Applied Environment Microbiology **70,** 5 750-5755. http://dx.doi.org/10.1128/AEM.70.10.57505755.2004

**Hoover H.** 1986. Chemical factors involved in ruminal fiber digestion. Dairy Science **69,** 2755-2766. http://dx.doi.org/10.3168/jds.S00220302(86)80724-X

**Hungate RE.** 1966. The rumen and its microbes. Academic press, New York, USA. **3,** 27-38.

**IPCC, Intergovernmental Panel on Climate Change.** 2007. Climate Change.The Scientific Basis.Cambridge UniversityPress, Cambridge (UK).

**Johnson KA, Johnson DE.** 1995. Methaneemissions from cattle. Journal Animal Science **73,** 2483-2492.

http://dx.doi.org/1995.7382483x

**Jouany JP.** 1994. Les fermentations dans le rumen et leur optimisation. INRA Productions Animales **7,** 207-225.

**Juven B, Kanner J, Schved J, Weisslowicz H.** 1994. Factors thatinteract with the antibacterial action of thyme essential oil and its active constituents. AppliedMicriobiogy **76,** 626-631. http://dx.doi.org/10.1111/j.1365-2672.1994.tb01661.x

**Kalemba D, Kunicka A.** 2003. Antibacterial andantifungal properties ofessential oils.Current Medicinal Chemistry **10,** 813-829. http://dx.doi.org/10.2174/0929867033457719

**Lin B, Wang JH, Lu Y, Liang Q, Liu JX.** 2011. In vitro rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. Animal Physiology and Animal Nutrition **97,** 1-9. http://dx.doi.org/10.1111/j.1439-0396.2011.01236.x

**Lindau CW, Patrick Jr. WH, DeLaune RD.** 1993. Factors affecting methane production in flooded rice soils. In: Agricultural Ecosystem Effects on Trace Gases and Global Climate Change (Eds. D. E. Rolston, L. A. Harper), American Society of Agronomy special publication **55,** 157-165. http://dx.doi.org/10.2134/asaspecpub55.c11

**Lis-Balchin M, Deans SG, Eaglesham E.** 1998. Relationship between the bioactivity and chemical composition of commercial plant essential oils.Flavour and Fragrance **13,** 98-104. http://dx.doi.org/10.1002/(SICI)10991026(199803/04)

**Macheboeuf D, Morgavi DP, Papon Y, Mousset JL, Arturo-Schaan M.** 2008. Dose-response effects of essential oils on in vitro fermentation activity of the rumen microbial population. Animal Feed Science **145,** 335-350. http://dx.doi.org/10.1016/j.anifeedsci.2007.05.044

**McGinn SM, Beauchemin Coates KAT, Colombatto D.** 2004. Methane emissions from beef cattle: Effects of monensin, sunfloweroil, enzymes, yeast, and fumaric acid. J. Animal Science **82,** 3346–3356. http://dx.doi.org/2004.82113346x

**Menke KH, Steingass H.** 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development **28,** 7-55.

**Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W.** 1979.The estimation of the digestibility and metabolizable energy content of ruminant feedings tuffs from the gas production when they are incubated with rumen liquor.Agricultural Science **97,** 217-222.

**Newbold CJ, McIntosh FM, Williams P, Losa R, Wallace RJ.** 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Animal Feed Science and Technology **114,** 105-112. http://dx.doi.org/10.1016/j.anifeedsci.2003.12.006

**Orskov ER, Mc Donald I.** 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Agricultural Science **92,** 499-503. http://dx.doi.org/10.1017/S0021859600063048

**Rouabhi R, Djebar-Berrebbah H, Djebar MR.** 2006. Toxic Effect of a Pesticide, Diflubenzuron on Freshwater Microinvertebrate (Tetrahymena pyriformis). Chinese Journal Of Applied & Environmental Biology **12(4),** 514-517. http://dx.doi.org/10.3321/j.issn:1006687X.2006.04.016

**Spanghero M, Zanfi C, Fabbro E, Scicutella N, Camellini C.** 2008. Effects of a blend of essential oils on some end products of *in vitro* rumen fermentation. Animal Feed Science and Technology **145,** 364-374. http://dx.doi.org/10.1016/j.anifeedsci.2007.05.048

**Szumacher-Strabel M, Cieślak A.** 2010. Potential of phytofactors to mitigate rumen ammonia and methane production.Journal of Animal Feed Science **19,** 319-337.

**Tassou C, Drosinos E, Nychas G.** 1995. Effects of essential oil from mint (Menthapiperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 jC and 10 jC. Applied Bacteriology **78,** 593- 600. http://dx.doi.org/10.1111/j.13652672.1995.tb03104.x/abstract.

**Wallace RJ.** 2004. Antimicrobial properties of plant secondary metabolites. Proceedings of the Nutrition Society **63,** 621-629. http://dx.doi.org/10.1079/PNS2004393