Potential of Eucalyptus (Eucalyptus camaldulensis) essential oil to modify in vitro rumen fermentation in sheep

Attia K., Chouchen R., Darej C., Moujahed N.

in

Napoléone M. (ed.), Ben Salem H. (ed.), Boutonnet J.P. (ed.), López-Francos A. (ed.), Gabiña D. (ed.).
The value chains of Mediterranean sheep and goat products. Organisation of the industry, marketing strategies, feeding and production systems

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 115

2016
pages 525-529

Potential of Eucalyptus (*Eucalyptus camaldulensis*) essential oil to modify *in vitro* rumen fermentation in sheep

K. Attia , R. Chouchen , C. Darej and N. Moujahed*

Laboratoire des Ressources Animales et Alimentaires
Institut National Agronomique de Tunisie, 43 Av. Ch. Nicolle, 1082, Tunis Belvedere (Tunisie)
*e-mail: Nizar.moujahed@yahoo.fr

Abstract. The current study aimed at evaluating the effects of increasing doses (0, 10, 20, 40, 80 and 120 µl/50 ml of buffered rumen fluid) of essential oil (EO) extracted from the leaves of Eucalyptus (*Eucalyptus camaldulensis*) on some *in vitro* rumen fermentation characteristics. Doses of EO were added to 0.5 g of a diet composed of 50% of rye-grass hay and 50% of concentrate. The medium of incubation consisted on ruminal liquid extracted from sheep, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C). At 24 h of incubation gas production (GP) was measured, a part of the liquid was collected for analysis of ammonia nitrogen (NH_3-N) and 3 syringes were reserved to determine true organic matter degradability (TOMD).Then the partitioning factor (PF) was estimated after 24 h of incubation. Results showed that, GP was not affected at 10 µl of EO, but decreased significantly (P<0.0001) for 20, 40, 80 and 120µl doses by 16, 29.7, 46.3 and 54.5 % respectively, comparatively with the dose 0 µl. NH_3-N concentration was reduced (P<0.0001) by 8.2, 18.6, 21.6 and 25 % when 20,40, 80 and 120 µl of EO were added, comparatively with dose 0 µl. TOMD values were equivalent among the doses 0, 10 and 20 µl of EO, but decreased significantly (P<0.0001) at doses 40, 80 and 120 µl, which were equivalent. The PF values were 2.93, 3.68, 3.81, 4.48, 5.26 and 7.43 for the increasing doses of EO. The only significant difference (P<0.05) was observed between doses 0µl and 120 µl. It was concluded that EO modified fermentation trends in the rumen, mainly by reducing GP and protein deamination. At low doses, TOMD seemed not to be drastically affected. *In vivo* studies are needed to demonstrate eventual benefits from utilization of EO by ruminant sand to situate the compromise between reducing gas emission and positive effects on digestion.

Keywords. Essential oil – Eucalyptus – Fermentation – Sheep – *In vitro*.

Effet de doses croissantes des huiles essentielles d'Eucalyptus (*Eucalyptus camaldulensis*) sur les fermentations mesurées* in vitro *(cas du mouton)

Résumé. *L'effet de doses croissantes (0, 10, 20, 40, 80 et 120 µl /50 ml) des huiles essentielles (HE), extraites des feuilles d'eucalyptus (Eucalyptus camaldulensis), sur les caractéristiques de fermentation du rumen a été évalué in vitro. Les doses d'HE ont été ajoutées à 0,5 g d'un régime composé de 50% du foin de ray-grass et 50% de concentré. Le milieu d'incubation se compose du liquide ruminal extrait des moutons, mélangé en proportions égales avec une solution tampon et introduit dans des seringues de 100 ml en verre (39°C). Après 24 h d'incubation, la production de gaz (PG) a été mesurée, une partie du liquide a été prélevée pour l'analyse de l'azote ammoniacal (NH_3-N), et 3 seringues ont été réservées pour déterminer la valeur de la dégradabilité réelle de la matière organique (DMO). Le facteur de partition (FP) a été ensuite estimé. Les résultats ont montré que la PG n'a pas été affectée par la dose 10 µl d'HE, mais a diminué de manière significative (P <0,0001) avec les doses 20, 40, 80 et 120 µl, cette diminution a été respectivement de 16; 29,7 ; 46,3 et 54,5%, comparativement à la dose 0 µl. La concentration en NH_3-N a diminué (P <0,0001) lorsque 20,40, 80 et 120 µl d'HE ont été ajoutées, respectivement de 8,2; 18,6; 21,7 et 25%, comparativement à la dose 0 µl. Les valeurs de la DMO étaient équivalentes pour les doses de 0, 10 et 20 µl de l'HE, mais a diminué de manière significative (P <0,0001) aux doses de 40, 80 et 120 µl, qui étaient équivalentes. Les valeurs du FP étaient de 2,93 ; 3,68 ; 3,81 ; 4,48 ; 5,26 et 7,43 pour les doses croissantes de l'HE. Une différence significative (P <0,05) a été observée entre les doses 0 et 120 µl. On pourrait conclure qu'à faibles doses, la DMO semble ne pas avoir été considérablement affectée. Des études* in vivo *sont nécessaires pour démontrer les éventuels avantages découlant de l'utilisation de l'HE par les ruminants et de distinguer le compromis entre la réduction des émissions de gaz et des effets positifs sur la digestion.*

Mots-clés. *Huile essentielle – Eucalyptus – Mouton – Fermentation* – In vitro.

I – Introduction

For many years, antibiotics have been used as growth promoters for livestock (Corpet, 1996). In 2001 and according to the World Health Organization, this use was estimated to 50% of the worldwide produced antibiotics. However, these substances seemed to have favored the emergence of a large number of resistant bacterial strains and allergic reactions for consumers (Corpet, 1996). In 2006, the use of antibiotics to improve growth and animal performances was prohibited in the European Union. This led to the reappearance of pathogens responsible of causing diseases and economic losses (Alloui, 2011). Consequently, considerable efforts were deployed to develop alternatives to substitute antibiotics. Among these alternatives, essential oils (EO) are receiving a growing attention as natural antibiotic substitutes and also as beneficial additives for the manipulation of rumen fermentations (Wallace,2004). In this connection, the Eucalyptus is a native tree widely available in Tunisia, with several species that could provide relatively important amounts of EO. The effects of these EO are related to their active components and chemo-types. For example, in the case of *Eucalyptus camaldulensis*, previous studies carried out in Tunisian laboratory showed that the main compound was eucalyptol (20.6%, Mediouni Ben Jemâa *et al*.,2013 and in our laboratory 18.65 % Bettayeb *et al*., unpublished).Also, the literature reported some data which had shown the potential of EO from Eucalyptus to favorably affect rumen metabolism such as results of Sallem *et al*. (2009) and Patra and Yu (2012) for *Eucalyptus globulus* In the current study, we evaluated the impact of EO extracted from *Eucalyptus camaldulensis*, on *in vitro* fermentation parameters in sheep.

II – Material and methods

EO was extracted by hydro-distillation (Peyron, 1992) from *Euclayptus camaldulensis* brought from the regions of Zaghouan (North of Tunisia, semi-arid). The studied diet was composed of 50% of rye-grass hay and 50% of commercial concentrate (bovine fattening concentrate: corn grain, soybean meal and MVS) on dry matter (DM) basis, ground and mixed through a 1mm screen. Chemical composition of Eucalyptus and diet components is presented in Table 1.

Table 1. Chemical composition of feeds (%DM)

Feeds	DM (%)	Ash	OM	CP	NDF	ADF	ADL
Eucalyptus leaves and twigs	47.3	8.2	91.8	6.7	46.7	29.6	13.6
Concentrate	89	7.6	92.4	15.5	23.8	3.9	3.6
Ray-grass hay	95.2	14.4	85.6	16.8	52.9	30.9	–

Rumen content for inoculum preparation was collected from four adult male Barbarine sheep (age and live weight averaged 12 months and 30 kg) slaughtered at the slaughterhouse of Ariana municipality. The data relative to sheep were checked from the owners and for all the incubations we used animals receiving diets composed of oat hay supplemented with barely grain in order to standardize rumen fluid. The rumen contents of the 4 slaughtered sheep is collected immediately after evisceration in preheated thermos (39°C) and transferred rapidly to the laboratory where the contents were mixed and filtered through 4 layers of surgical gas.

We measured the effect of growing doses (0, 10, 20, 40, 80 and 120µl) of EO on *in vitro* rumen fermentation parameters. The medium of incubation consisted on ruminal liquid, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C) as described by Menke and Steingass (1988). Each dose of EO (5 replications, through 2 successive incubations) was dissolved in methanol and added immediately before incubation to 0.5 g of experimental diet D. During the incubation, gas production was measured (after 2, 4, 6, 8, 10, 12 and 24 h) and pH were determined after 24 h. At the end of the incubation, the fluid samples from two syringes were collected

and conserved for ammonia-N (NH_3-N) determination and the three other syringes were used to determine the truly organic matter degradation (TOMD) and to calculate the partitioning factor (PF) as described by Blümmel et *al*. (1997):

PF (mg/ml) = TOMD (mg) /gas volume produced at 24 h (ml).

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984).Cell wall fractions (NDF, ADF and ADL) in feeds were determined as described by Van Soest et *al*. (1991). Ammonia-N was analyzed according to the method of Conway (1962).

The General Linear Model procedure (GLM) of SAS (2009) with the option of LSMEANS multiple ranges was used to analyze data. The model included effects of dose, incubation and interaction. The control syringes (T: containing buffered solution with inoculum) were used as co-variable in order to control rumen liquid variation.

III – Results and discussion

1. Rumen fermentation parameters

The effect of different levels of EO on gas production (GP) parameters is presented in Table 2. The results showed that after 24 h of incubation, the increase of EO doses resulted in a significant ($P<0.0001$) decrease of GP by7.4 (at 20 µl of EO) to 54.5 % (at 120µl of EO) comparatively with doses 0 and 10 µl which were equivalent. This result confirmed the trends observed by Patra and Yu (2012) who studied the effect of growing doses of essential oil from *Eucalyptus globulus* on *in vitro* GP. Busquet *et al*. (2005) indicated that the *in vitro* GP decreases with the increase of the doses of EO. This decrease can be explained by the antibacterial effect exerted by EO, inhibiting the growth of some bacterial (*E. Coli* and *Staphylococcus aureus,* Bachir Raho and Benali, 2012) and protozoal (Sallem *et al*., 2009) populations.

After 24 h of incubation, the effect of EO on pH (Table 2) was significant ($P <0.0001$) only for high doses (80 and 120µl). Indeed, pH values were equivalent from the dose 0 to 40 µl (averaged 6.35) and increased in 80 and 120µl (averaged 6.44). It is worthy to note that for all the doses, the pH values remains favorable for normal rumen fermentations. Our results confirmed those of Kumar *et al*. (2012), who noted an increase of pH by about 2.2 and 1.7% when they added 4 mg Eucalyptus powder to a moderate and high fiber content diets. Similarly, Patra and Yu (2012) found that the addition of 0.5 and 1 g/l of Eucalyptus EO resulted in increased pH values, comparatively with the control. This increase is related to the intense antimicrobial property of EO. According to Castillejos *et al*. (2006), the increase of pH is associated to a significant reduction in total volatile fatty acid production, reflecting a decline in substrate fermentation because of the antimicrobial effect of EO compounds (Fraser *et al*., 2007).

Ammonia-N concentration (Table 2) increased significantly ($P<0.0001$) at 10µl comparatively with the dose 0µl (48.2 and 45.9 respectively). Thereafter, NH_3-N decreased to 42.1 mg/100ml for 20 µl and then to a mean value of 35.9 mg/100ml for 40, 80 and 120 µl of EO. A similar trend was observed by Sallam *et al*. (2009) for EO effect on NH_3-N. Also, the use of Eucalyptus powder instead of EO resulted in equivalent observations (Kumar et *al*., 2012). In contrast, when higher doses were used, Gunal *et al*. (2014) found that the addition of 125, 250 and 500 mg/ml of EO increased the concentration of NH_3-N by about 29; 24 and 17% respectively. The NH_3-N concentration in the rumen can increase or decrease according to the amount of deaminated protein and depending on the amount and type of dietary carbohydrate available for microbial fermentation (Russell *et al*., 1983).These results leads to suggest a selective positive effect of EO on microorganisms noted at low doses (10 µl). However, as claimed by Castillejos *et al*.(2005) at higher doses, most EO cause an inhibition of deamination and a decrease in NH_3-N production provided primarily by the hyper-producing ammonia bacteria.

Table 2. Effects of increasing doses of EEO on *in vitro* rumen fermentation parameters

Dose (µl/50 ml)	0	10	20	40	80	120	SEM
GP 24(ml)****	110.3[a]	102.1[a]	92.6[b]	77.5[c]	59.2[d]	51.3[d]	4.898
pH****	6.32[b]	6.33[b]	6.36[b]	6.38[b]	6.45[a]	6.43[a]	0.011
NH_3-N (mg/100ml)****	45.9[a]	48.2[a]	42.1[b]	37.4[c]	36[c]	34.4[c]	1.327

[a, b, c, d] Values with different letters in the same line are statistically different.

**** P<0.0001, SEM: Standard error of the mean.

2. Microbial activity

The effect of EO on TOMD and PF is consigned in Table 3. At low doses (10 and 20 µl) TOMD values were equivalent to the dose 0µl (averaged 79.9%). A significant (P<0.0001) decrease by about 14% was observed at the higher other doses (40, 80 and 120µl) which were equivalent (averaged 69.7%).Results of Sallam *et al*. (2009) showed decreasing but not significant trend of TOMD at high levels of EO comparatively with the control. Russell and Strobel (1989) reported that the supplementation of Eucalyptus oil led to the inhibition of the cellulolytic rumen bacteria, such as *Ruminococcus albus* and *Butyrivibrio fibrosolvens*. According to Dorman and Deans (2000), mechanisms of antibacterial activity of EO are related to their active compounds such as terpenoids and phenylpropanoids. Bacteria are affected through interaction with the membrane cell, causing conformational changes in the membrane structure.

Partitioning factor values, which reflects substrate-dependent variation in the *in vitro* partitioning of degraded substrate between short chain fatty acids, gases and microbial biomass, seemed to increase according to EO growing. However this increase was not statistically distinct. Indeed, for the three first doses (0, 10 and 20µl) PF values were equivalent (averaged to 3.47 mg/ml, P<0.01), for the doses 40 and 80 µl were also equivalent (4.87 mg/ml) and a significant difference were observed between the dose 120 and 0µl. This wide variation observed in our results (PF ranged between 2.93 and 7.43 mg/ml) was also noted by Sallam *et al*. (2009) for EO supplementation. In connection with this, Kumar *et al*. (2012) found that supplementation of diets by Eucalyptus powder increased PF parameter comparatively to the control. Such results are difficult to interpret since TOMD values interfere with other phenomenons such as solubilized particle losses (Sallem *et al*., 2009). This means that the decrease in organic matter degradation was not biologically proportional to the decrease of gas production.

Table 3. Effects of increasing doses of EO from *Eucalypus camaldulensis* on TOMD and PF

Dose (µl/50 ml)	0	10	20	40	80	120	SEM
TOMD (%) ****	80.1[a]	79.8[a]	79.9[a]	72.6[b]	68.7[b]	67.8[b]	0.0125
PF (mg/ml) **	2.93[b]	3.68[b]	3.81[b]	4.49[ab]	5.26[ab]	7.43[a]	0.424

[a, b] Values with different letters in the same line are statistically different.

**** P<0.0001, ** P <0.01, SEM: Standard error of the mean.

IV – Conclusion

It can be concluded that EO modify fermentation trends in the rumen, mainly by reducing gas production and protein deamination. At low doses, truly degraded organic matter seemed not to be drastically affected. Volatile fatty acid determination is needed to better understand the fermentative trend of carbohydrates and the effect on energetic statue of the animal when supplemented with EO. *In vivo* trials are also requested to investigate the effect of EO on intake, digestion and performances and to control the doses of EO affecting sheep favorably.

References

Alloui M.N., 2011. Les phytobiotiques comme alternative aux antibiotiques promoteurs de croissance dans l'aliment des volailles. *Livestock Research for Rural Development*, 23 (6), p. 133.

AOAC,1984. *Official's methods of analysis*. Association Official Analytical Chemists, Wasghington, DC,14th edn.

Bachir Raho G. and Benali M., 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, 2012, p. 739-742.

Blümmel M., Makkar H.P.S. and Becker K.,1997. In vitro gas production: a technique revisited. *J. Anim. Physiol. Anim. Nutr.*, 77, p. 24-34.

Busquet M., Calsamiglia S., Ferret A., and Kamel C., 2005. Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. *Anim. Feed Sci. Technol.*, 123, p. 597-613.

Castillejos L., Calsamiglia S., Ferret 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. *Animal Feed Science and Technology,* 119, p. 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* systems. *Journal of Dairy Science*, 89, p. 2649-2658.

Conway E.J. 1962. *Microdiffusion Analysis and Volumetric Error*, 5th edition. Crosby Lockwood and Son, London.

Corpet M., 1996. Microbiological hazards for humans of antimicrobial growth promoter use in animal production. *Rev. Med. Vet.*, 147, p. 851-862.

Dorman J.J.D. and Deans S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl. Microbiol.,* 88, p. 308-31.

Fraser G.R., Chaves A.V., Wang Y., McAllister T.A., Beauchemin K.A. and Benchaar C., 2007. Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. *J. Dairy Sci.,* 90, p. 2315-2328.

Gunal M., Ishlak A., AbuGhazaleh A.A. and Khattab W., 2014. Essential oils effect on rumen fermentation and biohydrogenation under *in vitro* conditions. *Czech J. Anim. Sci.,* 59, 2014, 10, p. 450-459.

Kumar S.S., Navneet G., Mehta M., Mohini M., Pandey P., Shete S. and Brisketu K., 2012. Efficacy of garlic, eucalyptus and neem powders on rumen modulation, methanogenesis and gas production kinetics in wheat straw based diet evaluated *in vitro*. *Wayamba Journal of Animal Science,* p. 347-355**.**

Mediouni Ben Jemâa J., Haouel S. and Khouja M.L., 2013. Efficacy of Eucalyptus essential oils fumigant control against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) under various space occupation conditions. *Journal of Stored Products Research*, 53, 2013, p. 67-71.

Menke KH. and Steingass H., 1988. Estimation of the energic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Dev.*, 28, p. 7-55.

Patra A.K. and Yu Z., 2012. Effects of essential oils on methane production and fermentation by, and abundance and diversity of, rumen microbial populations. *Applied and Environmental Microbiology*, vol. 78 (12), p. 4271-4280.

Peyron L., 1992. Techniques classiques actuelles de fabrication des matières premières naturelles aromatiques. Chapitre 10, p. 217-238. Cited In : *Les arômes alimentaires*. Richard H. and Multon J.-L. (coords). Ed. Tec & Doc-Lavoisier and Apria. 438 p.

Russell J.B., Sniffen C.J. and Van Soest P.J., 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. *J. Dairy Sci.*, 66, p. 763-775.

Russell J.B. and Strobel H.J., 1989. Effect of Ionophores on ruminal fermentation. *Applied and Environmental Microbiology Journal,* 55, p. 1-6.

Sallam S.M.A., Bueno I.C.S., Brigide P., Godoy P.B.,Vitti D.M.S.S. and Abdalla A.L., 2009. Efficacy of eucalyptus oil on *in vitro* ruminal fermentation and methane production. In: Nutritional and foraging ecology of sheep and goats. *Options Méditerranéennes*, Series A, no. 85, p. 267-272.

SAS, 2009. *Users Guide version 9.0.* Statistical Analysis Systems Institute, Inc.

Van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74 (10), p. 3583-3597.

Wallace R.J., 2004. Antimicrobial properties of plant secondary metabolites. *Proc. Nutr. Soc.*, 63, p. 621-629.