Effect of a thymol-based additive on rumen fermentation, on methane emissions in eructed gas and on milk production in Holstein cows

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Abstract

The objective of this study was to assess the effect of a thymol-based feed additive as a means of modulating the rumen fermentation, the methane emissions and milk production in dairy cows. The additive was made by mixing 7% thymol with 48 % of bentonite clay, 30% stevensite clay, 10% *Rosmarinus* essential oil and 5% of olive oil. The first experiment was to evaluate in a rumen *in vitro* system the effect of different concentrations of the additive on gas production. In the second experiment, the additive was fed to Holstein dairy cows to test the effect on the emission of methane and milk production. Total gas production and percent methane in the gas in the *in vitro* rumen incubation was reduced curvi-linearly by increasing concentrations of the thymol additive. Supplementing the diet of lactating Holstein cows with 100 g/d of the additive reduced methane in eructed gas by 33% and increased milk production by 10%.

Keywords: *antimicrobial, fermentation, gas production, milk production*

Introduction

In Morocco, most of the milk production comes from small regional farms (FAO 2019), but this production remains low knowing the expenses incurred by the breeders in the feeding of dairy cows. The low zootechnical productivity of these small cattle farms is the result of several factors related to the feeding of cows and livestock management (FAO 2019). The problem of methane emissions is neglected by farmers although if this could be reduced the benefits would be reduced greenhouse gas emissions with potential for associated improvement in ruminant production.

Current strategies to reduce methane focus primarily on understanding the microbial rumen ecosystem. Several studies have shown that essential oils can reduce enteric methane (Evans and Martin 2000; Benchaar et al 2006; Calsamiglia et al 2007) and increase milk production (Kung et al 2008).

Thymol is a common component of essential oils derived from *Thymus* and *Origanum* plants conferring on them antimicrobial properties (Burt 2004; Calsamiglia et al 2007; Marchese 2016). Thymol has been tested directly for its antibacterial activity on a broad spectrum of bacteria including rumen microorganisms (Evans and Martin 2000; Marchese 2016). Because of this antimicrobial action, thymol has bee shown to be capable of modifying the ruminal flora (Busquet et al 2006; Calsamiglia et al 2007).

Microbial digestion constitutes a major particularity in the ruminant species, characterized by the phenomenon of fermentation. This fermentation can be modified through feed and feed additives to create a better equilibrium of the microbial population in the rumen, thus optimizing growth and milk production (Benchaar et al 2008; Patra and Yu 2013).

The objective of this research was to evaluate the efficacy of a thymol-based additive to modulate the fermentation of the rumen fluid and to evaluate *in vivo* its effect on methane emissions and milk production in dairy cows.

Materials and methods

In vitro effect on gas production in the rumen fluid

The thymol-based additive was elaborated in the laboratory by mixing 7% of thymol with 48% bentonite clay, 30% stevensite clay, 10% of *Rosmarinus* essential oil and 5% of olive oil.

Ruminal content was collected by esophageal sampling from Holstein dairy cows and filtered through two layers of sterile gauze strips to remove plant debris and isolate the rumen fluid. Mueller Hinton agar (MHA) (biokar, France) and Mueller Hinton broth (MHB) (biokar, France) alone or supplemented with ruminal liquid were used for the gas production test.

In screw tubes containing 9 ml of MHB and 1 ml of ruminal liquid, the thymol additive was added at concentrations of 2.5, 5, 10 and 20 mg/ml of substrate. Three tubes were prepared for each concentration. Product activity was studied by the inverted tube technique in anaerobic jars (Durham 1898). The system consists of inverting the tube containing the fermentation mixture in the form of a bell in sterile tubes and incubating under anaerobiosis for 24h at 37°C, at the end of which gas production is measured.

Effect of thymol additive on enteric methane emissions and milk production in Holstein cows

The 10 dairy cows of Holstein breed had a live weight between 360 and 420 kg. They were fed a daily ration of 3kg of alfalfa hay, 3kg wheat straw and 5kg of commercial concentrate. The ration was distributed in two meals per day.

The experiment was divided into three periods of three weeks each (2 weeks for adaptation to the feeding ration and one week for the measurement of the enteric methane emission). To test the dose response effect of the product on enteric methane production two different dose rates, 50g/cow/day in (Period 2) and 100g cow/day (Period 3) were used.

- Period 1: Control period: The cows received the standard ration only
- Period 2: The cows received 50 g of additive mixed with the feeding ration
- Period 3: The cows received 100 g of additive mixed with the feeding ration.

The production of methane was measured by indirect calorimetry (open circuit system) with a gas mask put on the face of the animal for several hours after the morning feeding. The gas handling system was essentially the same as that described by Kempton et al (1976). In this system air is drawn across the nose and mouth of the animal. A suction pump circulates the extracted gases in the tubes at an adjusted flow rate of 152 liters/min). A second sampling pump, connected to the system, takes exhaled air samples, which after dehumidification are returned to the methane analyzer which is previously calibrated. Calibration of the system consists of injecting nitrogen into the methane analyzer.

Effect of thymol additive on milk production

The trial was with 30 cows in lactation stage between 190 and 210 days after calving. The cows were divided into 2 groups: The experiment was carried out in two phases: a first pre-experimental phase of 30 days. A second experimental phase of 30 days. The control group received the standard ration alone; the thymol group received in addition to the standard ration, 100 g/cow/day of the additive. Individual daily milk production was measured at 8:00 am and 4:00 pm during the 60 days of the experiment.

Statistical analyses

The data were analyzed by the ANOVA program in the Sigma Stat 4.0 software.

Results

There was a linear decline in gas production as the concentration of the thymol additive was increased to 20 mg/ml in the fermentation medium (Table1; Figure 1).

Table 1. Effect of thymol additive on gas production in the *in vitro* rumen incubation

Thymol additive, mg/ml	Gas production, ml
0	5 ± 0.5
2.5	4.2 ± 0.1
5	3 ± 0.62
10	2.3 ± 0.27
20	1.0 ± 0.5

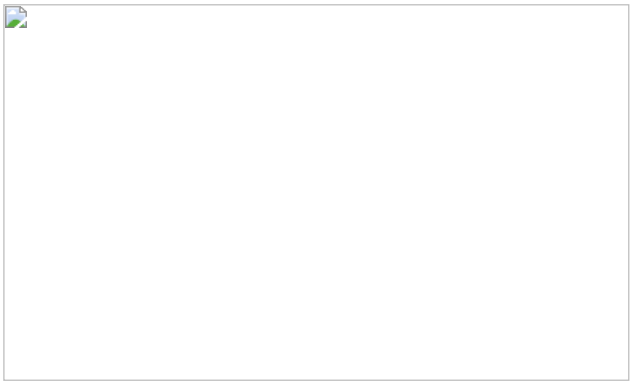

Figure 1. Gas production was reduced with a curvilinear trend as the concentration of thymol additive was increased

Addition of 50 g/d thymol additive to the feed reduced the enteric methane emissions by 22% (Table 2); increasing the dose to 100 g/d reduced methane by 33%.

Table 2. Mean values with SE for methane emissions in cows fed 50 or 100g/day of thymol additive or no additive (control)

	Thymol additive, g/d			
	0	**50**	**100**	***p***
Methane, L/d	200±9.8	154±7.61	134±6.68	<0.05

Supplementing the dairy cow diet with 100 g/d of thymol additive increased milk production by 10% (Table 3).

Table 3. Mean values for milk production prior to and during inclusion of 100 g/d of thymol additive in the diet

	Pre-experiment	Experimental
Control	19.3±2.10	17.8±1.46
Thymol additive.	20.7±1.8	20.2±2.64

Discussion

Thymol is a molecule that possesses high antimicrobial properties (Hart et al 2008, Calsamiglia et al 2007; Marchese et al 2016; Kokoska et al 2018). Our findings support those of many researchers (Evans and Martin 2000; Chaves et al 2008; Macheboeuf et al 2008; Rochfort et al 2008; Jahani Azizabadi et al 2011) that thymol reduces methane production in rumen *in vitro* incubations.

However, to our knowledge the rumen *in vitro* effects of thymol have not previously been projected to an *in vivo* production system, such as milk production in dairy cows. The increase in milk production recorded in our experiment, as a result of feeding a compound that depresses rumen gas production, in general, and methane production in particular, is similar to the improvements in N retention observed in goats supplemented with foliage from cassava varieties rich in precursors of hydrogen thiocyanate – substances known to have a depressing effect on rumen gas production and on methane in particular (Binh et al 2018; Thuy Hang et al 2019; Phuong ett al 2019). This unexpected relationship in which a depression of rumen fermentation is reflected in improved animal productivity was ascribed by Phonethep et al (2016) to be due to the more efficient utilization of protein by intestinal digestion versus rumen fermentation, and the fact that carbohydrate-rich substrate escaping the rumen would be fermented more efficiently in the cecum-colon in which the end product of fermentation is acetic acid – not methane (see Demeyer 1991; Immig 1996; Popova et al 2013).

Conclusions

- In an *in vitro* rumen incubation, total gas production and percent methane in the gas was reduced by a clay-based additive containing 7% thymol.
- Supplementing the diet of lactating Holstein cows with 100 g/d of the additive reduced methane in eructed gases by 33% and increased milk production by 10%.

Acknowledgments

This research is a partial achievement of Houda Hamdani's PhD thesis. The authors thank Miss Imane Remmal and Mrs Hélène Mock for their assistance in checking the English of the manuscript.

References

Banchereau T H and Mousset J-L 2005 Ruminant feed additive for increasing milk producton. EP 1609372A2 https://patentimages.storage.googleapis.com/44/d8/3a/c3e0e0b2eccb64/EP1609372A2.pdf

Benchaar C, Petit H V, Berthiaume R, Whyte T D and Chouinard P Y 2006 Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. Journal of dairy science ; 89(11):4352-64.

Binh P L T, Preston T R, Van H N and Dinh V D 2018 Methane production in an in vitro rumen incubation of cassava pulp-urea with additives of brewers' grain, rice wine yeast culture, yeast-fermented cassava pulp and leaves of sweet or bitter cassava variety. Livestock Research for Rural Development. Volume 30, Article #77. http://www.lrrd.org/lrrd30/4/binh30077.html

Burt S 2004 Essential oils: Their antibacterial properties and potential applications in foods—A review. Int. J. Food Microbiol.94:223–253. http://www.jonnsaromatherapy.com/pdf/Burt_Essential_Oils_A_Review_2004.pdf

Calsamiglia S, Busquet M, Cardozo P W, Castillejos L and Ferret A 2007 Invited review: Essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science. 90: 2580–2595.

Chaves A V, He M L, Yang W Z, Hristov A N, McAllister T A and Benchaar C 2008 Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. Canadian Journal of Animal Science, 88(1), 117-122. https://www.nrcresearchpress.com/doi/pdfplus/10.4141/CJAS07061

Demeyer D 1991 Differences in stoichiometry between rumen and hindgut fermentation. Adv. 1023 Animal Physiology and Animal Nutrition 22:50-66

Evans J D and Martin S A 2000 Effects of thymol on ruminal microorganisms Current Microbiology 41 : 336–340. https://www.ncbi.nlm.nih.gov/pubmed/11014870

FAO 2019 Gateway to dairy production and products. http://www.fao.org/dairy-productionproducts/en/?page=2&tx_dynalist_pi1%5Bpar%5D=ytoxontzoje6ikwio3m6mtoimci7fq%3D%3D

Hart K J, Yáñez-Ruiz D R, Duval S M, McEwan N R and Newbold C J 2008 Plant extracts to manipulate rumen fermentation Anim.FeedSci.Technol 147:8-35. https://www.researchgate.net/profile/Neil_Mcewan2/publication/37147100_Plant_extract_to_manipulate_rumen_fermentation/links/59e5bc9f458515250250965 2/Plant-extract-to-manipulate-rumen-fermentation.pdf

Immig I 1996 The rumen and hindgut as source of ruminant methanogenesis. Environmental Monitoring and Assessment Volume 42, Issue 1-2, pp 57-72

Jahani Azizabadi H, Danesh Mesgaran M, Vakili S A, Rezayazdi K and Hashemi M 2011 Effect of various medicinal plant essential oils obtained from semi-arid climate on rumen fermentation characteristics of a high forage diet using *in vitro* batch culture. African Journal of Microbiology Research 5(27) 4812-4819.https://academicjournals.org/journal/AJMR/article-full-text-pdf/6B67D7816122

Kempton T J, Murray R M and Leng R A 1976 Methane production and digestibility measurements in the grey kangaroo and sheep. Australian Journal of Biological Sciences 29(3), 209-214. http://www.publish.csiro.au/bi/pdf/BI9760209

Kokoska L, Kloucek P, Leuner O and Novy P 2018 Plant-Derived Products as Antibacterial and Antifungal Agents in Human Health Care Current medicinal chemistry. https://www.ingentaconnect.com/content/ben/cmc/pre-prints/content-30182844

Kung Jr L, Williams P, Schmidt R J and Hu W 2008 A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. Journal of Dairy science 91(12) 4793-4800.

Macheboeuf D, Morgavi D P, Papon Y, Mousset J L and Arturo-Schaan M 2008 Dose response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Anim FeedSci Technol 145, 335-350

Marchese A, Orhan I E, Daglia M, Barbieri R Di, Lorenzo A, Nabavi S F and Nabavi S M 2016 Antibacterial and antifungal activities of thymol: A brief review of the literature. Food chemistry, 210, 402-414.

Patra A K and Yu Z 2013 Essentials oils modify rumen bacterial compositions *in vitro* as revealed by microarrayanalysis J. Anim. Sci. 91 (E-Suppl. 2/J), J. Dairy Sci., 96 (E-Suppl. 1), pp. 713. https://www6.inra.fr/productionsanimales/content/download/6913/93095/version/1/file/Prod_Anim_27_4_06.pdf

Phonethep P, Preston T R and Leng R A 2016 Effect on feed intake, digestibility, N retention and methane emissions in goats of supplementing foliages of cassava (Manihot esculenta Crantz) and Tithonia diversifolia with water spinach (Ipomoea aquatica). Livestock Research for Rural Development. Volume 28,

Article #72. http://www.lrrd.org/lrrd28/5/phon28072.html

Phuong L T B, Preston T R, Van N H and Dung D V 2019 Effect of additives (brewer's grains and biochar) and cassava variety (sweet versus bitter) on nitrogen retention, thiocyanate excretion and methane production by Bach Thao goats. Livestock Research for Rural Development. Volume 31, Article #1. http://www.lrrd.org/lrrd31/1/phuong31001.html

Popova M, Morgavi D P and Martin C 2013 Methanogens and methanogenesis in the rumen and cecum of lambs fed two different high concentrate diets Applied Environmental .Microbiology doi:10.1128/AE

Rochfort S, Parker A J and Dunshea F R 2008 Plant bioactives for ruminant health and productivity.https://researchonline.jcu.edu.au/10183/1/Plant_bioactives_review.pdf

Thuy Hang L T, Preston T R, Ba N X and Dung D V 2019 Effect of biochar on growth and methane emissions of goats fed fresh cassava foliage. Livestock Research for Rural Development. Volume 31, Article #67. http://www.lrrd.org/lrrd31/5/thuyhang31067.html

Received 2 May 2019; Accepted 12 June 2019; Published 2 July 2019

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