Effect of a natural food additive rich in thyme essential oil on methane emissions in dairy cows

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Abstract

The objective of this study was to evaluate the effect of a natural feed additive rich in thyme essential oil on the methane emission by the dairy cows. The methane is a powerful greenhouse gas representing a true energy loss for the ruminants and its reduction is beneficial for the animals and the environment. In order to test the effect of this natural feed additive, rich in thyme essential oil on the emission of methane by ruminants, five dairy cattle of Holstein breed were used and received a ration composed of 4 kg of industrial concentrated feed, 2 kg of alfalfa hay and 2 kg of wheat straw with free access to drinking water. After two weeks of adaptation to the feed, measurements of the production of methane were carried out without feed additive. Then, 50 g of the product rich thyme essential oil were daily added to the basic ration (7.15 g/kg DM) during two weeks of adaptation and measurement of methane was taken in the third week. In the third period, 100 g of the same product was added to the same ration (14.3 g/Kg DM), and measurements of methane were carried out after the two weeks of adaptation. The quantity of methane produced by the five cows was estimated to average 195.9 liter/day. The addition of the product rich in thyme essential oil to the basic ration reduced methane emission on average by 21.6% when the feed additive was added with an amount of 7.15 g/kg dry matter, and a reduction on average of 31.8% with the amount was of 14.3 g/kg of dry matter.

Keywords: Feed additive, Methane production, Thyme essential oil, Dairy cow, Greenhouse effect.

Résumé

L'objectif de ce travail consiste à évaluer l'effet de l'addition à la ration alimentaire, d'un additif alimentaire naturel riche en huile essentielle de thym sur la quantité de méthane émise par les vaches laitières. Le méthane est à la fois un puissant gaz à effet de serre et une vraie perte énergétique pour les ruminants. Sa réduction est bénéfique pour les animaux et pour l'environnement. Afin de tester l'effet de cet additif alimentaire naturel riche en huile essentielle de thym sur l'émission du méthane par les vaches laitières, cinq vaches de race croisées Holstein ont été utilisées, la ration alimentaire quotidienne est composée de 4 kg d'aliment concentré industriel, 2 kg de foin de luzerne et 2 kg de paille de blé. Après une adaptation de deux semaines au régime alimentaire, des mesures de la production du méthane ont été réalisées. Ensuite, 50 g du produit riche en huile essentielle de thym ont été ajoutés quotidiennement à la ration de base (soit 7,15 g/kg MS), et après deux semaines d'adaptation, une deuxième mesure du méthane a été effectuée. Un troisième essai a été réalisé en ajoutant 100 g du même produit à la même ration de base (soit 14,3 g/Kg MS), et une troisième mesure de méthane a été réalisée après les deux semaines d'adaptation. La quantité journalière de méthane produite par les cinq vaches a été estimée à environ 195,9 l/j. L'addition du produit riche en huile essentielle de thym à la ration de base a été à l'origine d'une réduction de méthane produit en moyenne de 21,6% lorsque le produit a été ajouté à la dose de 7,15 g/kg de matière sèche et de 31,8% à la dose de 14,3 g/kg de MS.

Mots clés: Additif alimentaire, Production du méthane, Huile essentielles de thym, Vache laitière, Effet de serre.

INTRODUCTION

Over the past few years, the reality of global warming has become unequivocal. The ruminants contribute to climate change by the emission of the methane which results from the anaerobic degradation of plant biomass in the digestive tract. This gas represents at the same time a high warming power (25 times superior to that of CO_2) (Benchaar and Greathead, 2011) and a true feed energy loss for the animals. On a global scale, livestock participate in the total methane emission of up to 18% (FAO, 2006). In France, the enteric methane from livestock represents about 50 % of total emissions of that gas, while its contribution is around 90% in New Zeland where ruminants livestock is important (CITEPA, 2006). The focus of recent research in animal science has been to provide mitigation strategies to reduce CH_4 production from ruminants. This study consists of measuring the quantities of methane emitted by the animal and to test the effect of a feed additive rich in thyme essential oil on the emission of CH_4 in dairy cattle.

MATERIAL AND METHODS

Five Holstein cows were used during this experimentation and their characteristics are reported in table 1.

The cows were fed a ration composed of 4 kg commercial concentrate, 2 kg of alfalfa hay, 2 kg of wheat straw with free access to drinking water. The ration was distributed in two meals per day, the concentrate and the alfalfa around 9 a.m. and the straw around 3 p.m. The level of feeding was controlled daily by measuring the refusal. The composition of the ration as dry matter and fresh mater is reported in table 2.

Thyme essential oil which is a Phenolic Essential Oil (PEO) were used as feed additive in the present study. The

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PEO are volatile aromatic compounds, which have an oily appearance and produced from aromatic plants by several extraction processes. They are soluble in lipids and organic solvents and have lower density than water (Bekkali et *al.*, 2008). Due to their antimicrobial, antifungal and anti-parasitic activity, essential oils are used in pharmaceutical, food and cosmetics. A product rich in phenolic essential oil of thyme was added to the basic ration to test its effect on the production of methane. Fifty grams of the product was added to the ration daily corresponding to 7.15 dry matter g/kg of the diet in the first test, and then 100 grams of the product was added to the ration daily corresponding to 14.3 g/kg of the diet in the second test. The production of methane was measured by indirect calorimetry (an open circuit) with the use of a collecting gas mask put on the face of the animal for several hours after the morning feeding. Details on the methane measurement system are shown in diagram 1. The calibration of the system consists of the injection of nitrogen in the methane analyzer to adjust for the zero methane gas then the injection of gas containing methane with a known concentration to adjust for span gas.

The experimentation was divided into 3 periods: In the first period the cows were adapted to the feeding ration, and the port of the mask during 2 weeks, then the measurement of the emission of methane was performed without feed additives for the five cows during the third week. During the second period, the cows received 50 grams of the product, rich in thyme essential oil, mixed to the feeding ration for 3 weeks with two weeks of adaptation and a week of measurement of the methane emitted. In the third period, the cows received 100 grams of the same product mixed to the feeding ration during 3 weeks with two weeks of adaptation and a week of measurement of the methane emitted.

Table 1: Characteristics of the animals of the experimentation

Cow	Age (years)	Weight (kg)	Physiological stage	BCS	Parity
A	10	408	Non pregnant and non lactating	2.5	Multipare
B	9	358	Non pregnant and non lactating	2.5	Multipare
C	6	432	Non pregnant and non lactating	3.0	Multipare
D	7	504	Non pregnant and non lactating	2.0	Multipare
E	7	487	Non pregnant and non lactating	2.0	Multipare

Table 2: Ration composition offered to the cows in dry mater and fresh mater

Feed ration composition	Fresh Matter (kg)	Dry matter (%)	Dry matter (kg)
Concentrate	4	87	3.84
Hay of alfalfa	2	88	1.75
Straw	2	88	1.76
TOTAL	8	-	6.99

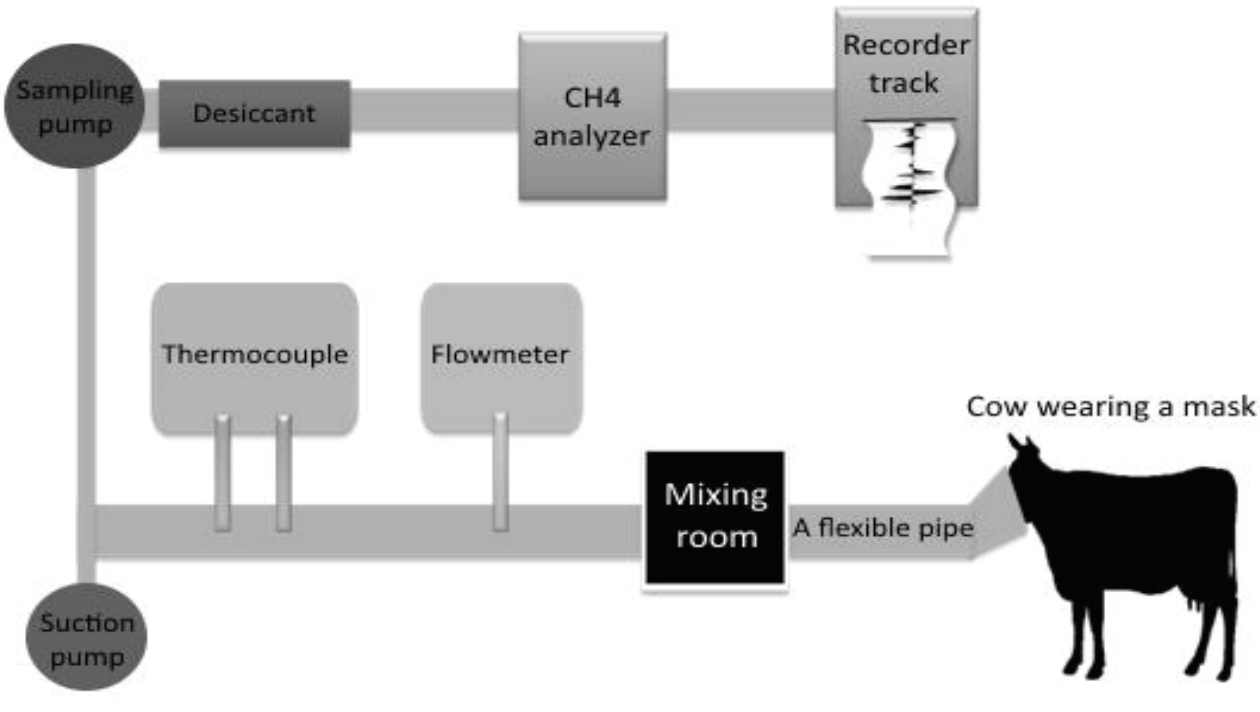

Diagram 1: Methane measurement system

RESULTS

In the first period, methane was in majority emitted by eructation (87%) and the rest (13%) was eliminated through respiration. There was a minimum of 31.5 eructation per hour in the cow A and a maximum of 42.5 eructation per hour in the cow B (Table 3).

Table 3: Eructation per cow per hour (period 1)

Cows	Number of eructation per hour
A	31.5
B	42.5
C	36.7
D	37.7
E	39.1
Average	37.5

The quantities of methane produced during the eructation and respiration were originally recorded in ppm and converted to liters/hour, then extrapolated to 24 hours and expressed in liter/day then as liter/kg dry matter intake for each animal (Table 4).

Methane emission was on average 196 liter per day with a minimum of 177 liter per day in cow D and a maximum of 208 liter per day in cow A. The cows were producing on average 28 liter of methane per kg of dry matter intake.

During the second period, 50 g of a product rich in thyme essential oil was incorporated in the basic ration and methane production was measured. In general, the methane production of the cows showed no change in the number of eructation (compared to period 1) but a reduction of the amplitude of eructation (Table 5).

Table 5: Number of eructation per cow per hour (period 2)

Cow	Number of eructation per hour
A	33.0
B	45.5
C	33.0
D	37.0
E	40.0
Average	37.7

Methane emission was estimated to average 154 liter per day with a minimum of 128 liter per day in cow E and a maximum of 172 liter per day in cow B. The cows were producing on average 22 liter of methane per kg of dry matter intake. The cows showed a reduction in methane production. The rate of reduction varies from one cow to another. The highest response was recorded in the cow E, with a reduction in methane of approximately 30.6%, the lowest responses to the product were recorded in the cows B and D with reductions of 14.9% and 15.3% respectively (Table 7). All the cows showed a significant reduction in methane emission with an average of 21.6 %.

Table 7: Percentage of reduction in methane production after the addition of 50 g of the product rich in thyme essential oil

Cow	Reduction (%)
A	21.5
B	14.9
C	25.9
D	15.3
E	30.6
Average	21.6

Table 4: Methane production in the cows during the first period

Cow	Weight (Kg)	liter /hour	liter/day	liter/kg DM
A	408.0	8.68	208.3	29.8
B	358.0	8.41	202.0	28.9
C	432.0	8.62	206.8	29.6
D	504.0	7.39	177.4	25.4
E	487.0	7.70	184.8	26.4
Average	437.8	8.16	195.8	28.0
Standard Deviation (SD)	59.4	0.60	13.9	2.0

Table 6: Methane production in the cows during the second period

Cow	Weight (kg)	liter /hour	liter/day	liter/kg DM
A	408	6.90	165.6	23.7
B	358	7.16	171.9	24.6
C	432	6.38	153.2	21.9
D	504	6.26	150.3	21.5
E	487	5.35	128.3	18.4
Average	438	6.41	153.9	22.0
Standard Deviation (SD)	59	0.69	16.8	2.4

In the third period, the incorporation of 100 g of this product did not affect the number of eructation in the 5 cows (compared to period 1 and 2). However, high reduction of the eructation amplitude was observed (Table 8).

Table 8: Number of eructation per cow per hour (period 3)

Cow	Number of eructation per hour
A	32.5
B	44.5
C	32.0
D	35.0
E	41.5
Average	37.1

Methane emission was estimated to average 134 liter per day with a minimum of 119 liter per day in cow D and a maximum of 152 liter per day in cow B. The cows were producing on average 19 liter of methane per kg of dry matter intake (Table 9). All the cows showed a reduction in methane production after adding 100 g of the product in the basic ration. A maximum reduction of 40,1% was recorded in cow A, and the minimum reduction was recorded in the cow B with a 24,5% (Table 10). All the cows showed more reduction in methane emission when increasing the dose of the product rich in thyme essential oil. The percentage of reduction of methane emission in all cows averaged 31,8.

Table 10: Percentage of reduction in methane production after the addition of 100 g of the product rich in thyme essential oil

Cow	Reduction (%)
A	40.1
B	24.5
C	28.6
D	32.7
E	33.1
Average	31.8

DISCUSSION

In the present study, methane was emitted mostly during periods of eructation (87%), while a small portion (13%) was eliminated with respiratory gases. These results were similar to those obtained by Murray et *al*., (1976) indicating that methane in cows was released by eructation (83%), respiration (16%) and anus (1%). Production of methane in the five cows of our experimentation was well below the values reported in the literature. In fact, Johnson et *al.,* (2002) have reported a methane production of 543 l/day in dairy cows with an average weight of 600 kg and a milk production of 32,3 liter/day. Similarly, Sechen et *al*., (1989) reported a methane production of 557 l/day in dairy cows with average weight of 603 kg and a milk production of 37,1 l/d. This difference with our results can be explained by the fact that the five cows of our experiment were dry and having lower body weight (440 kg vs 600 kg). Vermorel et *al*., (2008) reported that the methane emission increased linearly with milk production, the level of dry matter intake and the percentage of the concentrate in the diet. In fact, Baxter and Clapperton (1965) have shown a positive relationship between methane emission in cattle and the level of feed intake and Sauvant et *al*., (2012) reported that the intake of concentrate feed affect methane production to certain limits. Our cows were fed the same diet and showed a small differences in methane production which can be attributed to the characteristics of the digestive tract and feeding behavior of the cows (Boadi and Wittenberg, 2002).

The majority of studies reported in the literature indicated that phenolic essential oils have the ability to reduce enteric methane. Thymol and carvacrol, cinnamaldehyde, eugenol and anethol are the most studied molecules in this field. They all have a significant depressive effect on methane production, which can reach total inhibition in the case of high concentrations (Calsamiglia et *al*., 2007; Chaves and *al*., 2008; Macheboeuf et *al*., 2008). The inhibitory effect of the phenolic essential oils on methane production can be attributed to their antibacterial activity against Gram+ bacteria, Gram – bacteria and methane bacteria. In an *in vitro* study done on pure cultures, the growth of the species such as *Methanobrevibacter Smithii* was not inhibited with a dose of 160 ppm of phenolic essential oils mixture but it was inhibited with a higher concentration (Amlan et *al*., 2010). In the study of Agarwal et *al*.,(2009), the inclusion of peppermint oil to 0,33 ml/l of rumen juice has doubled the number of methanogenic bacteria, while CH_4 production was reduced by 20% without affecting the total concentration of volatile fatty acids. Higher doses (1 and 2 ml/l) had a toxic effect on methanogenic bacteria

Table 9: Methane production in the cows during the third period

Cow	Weight (Kg)	liter /hour	liter/day	liter/kg DM
A	408.0	5.19	124.8	17.8
B	358.0	6.35	152.5	21.8
C	432.0	6.15	147.7	21.1
D	504.0	4.97	119.3	17.1
E	487.0	5.15	123.6	17.7
Average	437.8	5.56	133.6	19.1
Standard Deviation (SD)	59.3	0.63	15.3	2.2

accompanied by a decrease in methane production. The negative effects on CH_4 production at low doses can be explained by a resistance of bacteria to the product with less efficient production of CH_4 by the methanogenic species. Several theories were proposed to explain the mechanism by which phenolic essential oils exert their antimicrobial activity. The complex composition of phenolic essential oils tends to prove that this activity is due to several different mechanisms of action, related to the chemical nature of these compounds (Skandamis et *al*., 2001; Carson et *al*., 2002; Burt, 2004). Most of the mechanisms of action are attributed to the interaction of the components of the phenolic essential oils with the cell membrane (Benchaar et *al.,* 2008). Phenolic essential oils consist of lipophilic molecules able to penetrate the phospholipid bilayer, their accumulation between phospholipid membranes leads to a change of the conformation and permeability of the cell membrane which disrupt membrane transport of nutrients (Sikkema et *al*., 1994; Ultee et *al*., 1999). Phenolic essential oils can also disrupt the ionic gradient on both sides of the cytoplasmic membrane, which decreases the membrane stability and also disrupts the membrane transport. The antimicrobial effect of thymol (a phenolic monoterpene and main active compound of thyme) is attributed to the disruption of the plasma membrane of bacteria and a reduction in the uptake of glucose (Calsamiglia et *al*.,2007; Benchaar et *al*.,2008). The phenolic essential oils from cinnamon and garlic may inhibit the enzymatic activity of rumen bacteria such *Enterobacter aerogenes*. Other phenolic essential oils inhibit microbial growth by inactivation of nucleic acids (Calsamiglia et *al*., 2007). The action of phenolic essential oils also depends on the nature of the targeted microorganisms. Gram positive bacteria are more susceptible to the action of phenolic essential oils as compared to gram negative bacteria. This can be explained by the presence of the outer membrane in Gram negative bacteria which represented a barrier that can reduce the permeability of the hydrophobic compounds (Calsamiglia et *al*., 2007). However, low molecular weight of PEO of thymol and carvacrol can cross this barrier (Hart et *al*., 2008). Protozoa have an important role in the production of methane. It was established that all the attached bacteria to protozoa are methanogens (Bergey, 1994) and these bacteria are responsible for 25-37% of total methane production (Finlay et *al*., 1994; Newbold et *al*., 1995). The effect of PEO on the population of protozoa is still debated. Some studies have reported no effect on the number of protozoa (Newbold et *al*., 2004; Benchaar et *al*., 2007) while others have shown a stimulating effect of PEO on protozoa population (Patra and Saxena, 2009). A depressive effect of PEO on protozoa was also reported (Ando et *al*., 2003; Cardozo et *al*., 2006; Fandino et *al*., 2008) and defaunated animals produced less methane.

In the present study the incorporation of a natural feed additive rich in thyme essential oil to the feed of the cows was behind a significant reduction of the amount of methane emitted depending on the PEO amounts used. With an amount of 7.15 g/kg of dry matter a 20% reduction in methane emission was obtained, while the amount of 14.3 g/kg of dry matter allowed a reduction of 30% of methane production in the same cows. It should be noted that the feed additive used in the present work, once generalized on the Moroccan livestock, will allow a significant reduction of methane emission and high contribution to the national strategy of greenhouse gases attenuation.

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