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Effects of essential oils from *Rosmarinus officinalis* and *Thymus capitatus* on *in vitro* rumen fermentation in sheep

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Abstract. This study aimed to evaluate the effects of increasing doses (0, 5, 10, 20 and 40µl/50 ml of buffered rumen fluid) of essential oils (EO) extracted by steam distillation from the leaves and twigs of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus capitatus*)on *in vitro*gas production and the fermentation characteristics. Doses of EO were added to 500 mg of a diet composed of 50% of rye-grass hay and 50% of concentrate on dry matter (DM) basis. The medium of incubation consisted on ruminal liquid extracted from 2 cannulated sheep, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C). At 24 h of incubation gas production was measured and part of the liquid was collected for analysis of ammonia nitrogen (NH_3-N). Volatile fatty acid (VFA) concentration was calculated on the basis of total gas production. Rosemary EO had no effects, neither on total gas production nor VFA accumulation. However, at 24 h of incubation, rosemary EO increasedNH_3-N concentration ($P<0.001$) when administered at the doses of 20 and 40 µl. Thyme EO decreased ($P<0.0001$) gas production starting from 10 µl dose. An increase ($P<0.001$) of NH_3-N concentration was recorded from the low dose of thyme EO, while the medium and high doses declined ($P<0.001$) NH_3-N concentration. VFA concentration decreased linearly from 5 µl to 40 µl doses of thyme EO. Medium doses from thyme EO might reduce carbohydrate fermentation and protein deamination. It was concluded that the EO from thyme have a more drastic anti-bacterial effect on rumen fermentation than those from rosemary.

Keywords. Essential oil – Rosemary – Thyme – Fermentation – Sheep.

Effets des huiles essentielles de* Rosmarinus officinalis *et* Thymus capitatus *sur les fermentations ruminales* in vitro *chez le mouton

Résumé. *Cette étude avait pour but d'évaluer les effets de doses croissantes (0, 5, 10, 20 et 40 µl/50 ml de fluide du rumen) d'huiles essentielles (HE) extraites par distillation à la vapeur à partir des feuilles et brindilles de romarin (*Rosmarinus officinalis*) et de thym (*Thymus capitatus*) sur la production de gaz et les caractéristiques de fermentation* in vitro*. Les doses d'HE ont été ajoutées à 500 mg d'un régime composé de 50% de foin d'avoine et 50% de concentré sur la base de la matière sèche (MS). Le milieu d'incubation est composé du liquide ruminal extrait à partir de 2 moutons canulés, mélangé dans des proportions égales à une solution tampon dans des seringues en verre de 100 ml (39°C). À 24 h d'incubation, la production de gaz est mesurée et une partie du liquide a été conservée pour l'analyse de l'azote ammoniacal ($N\text{-}NH_3$). La concentration en acide gras volatil (VFA) a été calculée sur la base de la production totale de gaz. Les HE de romarin n'ont eu aucun effet, ni sur la production totale de gaz ni sur la concentration des AGV. Cependant, à 24 h d'incubation une augmentation de la concentration de $N\text{-}NH_3$ ($P<0,001$) a été notée aux doses de 20 et 40 µl d'HE de romarin. Les HE du thym ont réduit ($P<0,0001$) la production de gaz à partir de la dose de 10 µl. Une augmentation de la concentration en N-NH3 a été notée à partir de la faible dose d'HE de thym, alors que les doses intermédiaires et élevées ont réduit ($P<0,001$) la concentration en $N\text{-}NH_3$. La concentration en AGV a diminué linéairement entre les doses de 5 et 40 µl d'HE de thym. Les doses intermédiaires d'HE du thym auraient réduit la fermentation des carbohydrates et la désamination des protéines. Il est à conclure que les HE du thym auraient un effet antimicrobien plus intense sur les fermentations que celles du romarin.*

Mots-clés. *Huiles essentielles – Romarin – Thym – Fermentation – Moutons.*

I – Introduction

Antibiotics are frequently administrated to animals in order to prevent metabolic diseases and troubles and to improve feeds valorisation as well as animal performances. However, during the last years, the use of these substances as an improving growth factor is more and more contested because of emergence of antimicrobial resistance in humans, leading to their ban in European Union since 2006 (Regulation 1831/2003/EC). Consequently considerable efforts were deployed to develop alternatives to substitute antibiotics. Among these alternatives, essential oils (EO) are used as feed additives to improve growth performances of ruminants raised under intensive conditions. These compounds consist of complex mixtures of various aromatic and volatile substances extracted from various plant materials mainly by steam distillation. The antimicrobial effect of EO is mainly related to their capacity to modify the permeability of microbial cells (Conner, 1993; Helander *et al.*, 1998). Consequently, these additives were used at low doses, to modify the fermentation trends in the rumen because of their toxicity for some unfavourable bacterial species in the rumen, such as methanogens (Wallace, 2004). In the current study we aimed to determine the effect of increasing doses of EO from frequent pasture species in the central region of Tunisia (*Thymus capitatus* and *Rosmarinus officinalis)* on *in vitro* rumen fermentation parameters of moderate beef cattle diet.

II – Materials and methods

1. Plant material

Thymus capitatus and *Rosmarinus officinalis* leaves and twigs were collected from the region of Kairouan (central region of Tunisia, arid). Samples from each species were taken from different places and mixed to make an overall sample. Dry matter (DM) was determined at 105°C in a forced-air oven. A subsample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* assays. Fresh samples were stored at –20°C for essential oil extraction using steam distillation methods.

2. Diets, animals and measurements

A composed diet (D: 50% of ray-grass hay and 50% of commercial concentrate) was used to determine the effect of growing doses (0, 10, 20, 40, 80 and 120µl) of essential oil from *Rosmarinus officinalis* and *Thymus capitatus* on *in vitro* rumen fermentation parameters. Diet mixture was made of ground feeds using a mixer.

Two adult local sheep ("Noire de Thibar" breed) with rumen cannula (average age and live weight: 24 months and 48.5 kg, respectively) were used for *in vitro* determinations. They received twice per day a ration (70 g kg-1LW0.75) composed of 70% of oat-vetch hay and 30% of barely concentrate on dry matter (DM) basis. The medium of incubation consisted on ruminal liquid, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C). Each dose of EO was dissolved in 200 µl of methanol and added immediately before incubation to 500 mg of experimental diet. Incubation lasted 24h, then gas production and pH were measured and fluid samples were taken for ammonia-N (NH_3-N). Each dose of EO was incubated in triplicate through two successive incubations.

3. Chemical analysis

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).Short Chain Fatty Acids (SCFA) at 24 h of incubation calculated as:

SCFA (mM/syringe) = 0.0239 GP – 0.0601 (Getachew *et al.*, 2000)

4. Statistical analysis

The General Linear Model procedure (GLM) of SAS (1996) with the option of LSMEANS multiple ranges was used to analyze data. The model included effects of dose and incubation.

III – Results and discussion

Chemical composition of feeds is presented in Table 1. Chemical composition of *Thymus capitatus* is relatively equivalent to hay except for total wall content (NDF): Lignocellulose fractions (ADF) were equivalent between the two species but the lignification level of *Thymus capitatus* was higher compared to *Rosmarinus officinalis*. Extracted EO content was higher in *Rosmarinus officinalis* than in *Thymus capitatus*.

Table1. Chemical composition of feeds (g/kg DM)

Feeds	Ash	CP	NDF	ADF	ADL	EO
Ray-grass hay	112	99	689	363	53	-
Concentrate	57	169	342	53	-	-
Thymus capitatus	102	88	409	326	226	2.5
Rosmarinus officinalis	62	58	389	301	167	4.3

Gas production, NH_3-N and SCFA concentrations are reported in Table 2. Rosemary EO had no effects, neither on total gas production nor short chains of VFA accumulation. However, at 24 h of incubation, rosemary EO were associated to an increase of NH_3-N concentration ($P<0.001$) observed at the doses of 20 and 40 µl. This result confirmed the findings of Noirot *et al.* (2007), who classified *Rosmarinus officinalis*, according to its main component (α-pinen), as the lowest bactericide compounds within EO. The authors suggested that component may be responsible of the deamination and /or of the limitation of N bacterial uptake in culture medium.

Table 2. Effects of increasing doses of EO from *Rosmarinus officinalis* on gas production and NH_3-N and SCFA concentrations

Dose (µl/50 ml)	0	5	10	20	40	SEM
Gas 24 (ml)	114.1	100	108.5	112.6	110.2	2.02
NH_3-N (mg/l)***	116^{b}	117^{b}	118.6^{b}	127^{ab}	138^{a}	1.82
SCFA (mM/50ml)	53.3	46.6	50.7	52.6	51.5	1.34

a,b. Values with different letters in the same line are statistically different, *** $P<0.001$, SEM: Standard error of the mean.

Table 3 reports the gas production and NH_3-N and SCFA concentrations generated from the administration of increasing doses of EO from *Thymus capitatus*. Thyme EO decreased ($P<0.0001$) gas production starting from 10 µl dose. An increase ($P<0.001$) of NH_3-N concentration was recorded from the low dose of thyme EO, while the medium and high doses declined ($P<0.001$) NH_3-N concentration. VFA concentration decreased linearly from 5 to 40 µl doses of thyme EO. This is consistent with the results found by Macheboeuf *et al.* (2007) who studied the effect of carvacrol, which we identified as carvacrol chemotype (Moujahed *et al.*, 2011) on *in vitro* fermentation parameters and noted that changes of pH values followed a growing tendency with increased doses. The effect of growing doses of thyme EO on NH_3-N concentration followed a decreasing trend. This result confirmed those observed by

Macheboeuf *et al*. (2007) and Castillejos *et al*. (2007) who studied the effect of several doses of essential oil of *Thymus capitatus* and *Thymus vulgaris* respectively, on in vitro NH_3-N concentration.

Table 3. Effects of increasing doses of EO from *Thymus capitatus* on gas production and, NH_3-N and SCFA concentrations

Dose (µl/50ml)	0	5	10	20	40	ESM
Gas 24 (ml)***	100 [a]	88.9 [b]	67.6 [c]	54 [d]	41[e]	2.03
NH_3-N (g/ml)***	91.5 [b]	110 [a]	110.3 [a]	67.6 [c]	73 [c]	1.31
SCFA (mM/50 ml)***	46.6[a]	41.3[a]	31.2[b]	24.6[c]	18.4[d]	1.01

a,b,c. Values with different letters in the same line are statistically different, *** P<0.001, SEM: Standard error of the mean.

IV – Conclusions

The EO from thyme have a more drastic anti-bacterial effect on rumen fermentation than those from rosemary. *In vivo* trials are currently carried out to investigate the effect of EO on intake, digestion and performances and to study the form of adding EO in diets of sheep.

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