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Nutritive value and essential oils characterization of *Rosmarinus officinalis* and *Thymus capitatus* from the central region of Tunisia

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Abstract. *Rosmarinus officinalis* (rosemary) and *Thymus capitatus* (thyme) leaves and twigs were collected from the central region of Tunisia in March and April 2008. Samples were immediately stored at –10°C. Chemical composition and *in vitro* gas production parameters were determined. Essential oils (EO) from the two species were extracted by steam distillation and analyzed using gas chromatography (FID), followed by a Carbon-13 NMR spectroscopy procedure to confirm the major compound structures. Rosemary and thyme were both low in crude protein (58 and 88 g/kg DM, respectively). The ADF content was similar and close 313 g/kg DM in both plant species, whereas the lignin content in thyme was higher than in rosemary (226 and 167 g/kg DM, respectively). Rosemary showed lower *in vitro* asymptotic gas production than thyme (27.7 *vs* 49.6 ml). *In vitro* organic matter digestibility after 24 h of incubation ($IVOMD_{24h}$) and metabolisable energy (ME) estimated from the chemical composition and gas production followed a similar pattern than that of *in vitro* fermentation parameters. Extracted EO content in rosemary was approximatively the double of that determined in thyme (0.43 and 0.25% of DM, respectively). Thyme EO was found to be high in the active monoterpen phenol carvacrol (70%), which is the chemotype of thyme. Other secondary compounds detected in thyme were β-caryophyllen (8.5%), γ-terpinen (4.3%) and p-cymen (3.8%). On the other hand, major compounds identified in rosemary EO were 1-8 cineol (44.2%), camphor (12%) and α-pinen (11.6%). It was concluded that both native medicinal plant species would be of low nutritive value if pastured by sheep and/or goat. The EO chemotypes detected, mainly carvacrol and 1-8 cineol could be envisaged as potential additives to manipulate rumen fermentation to improve feed efficiency in ruminants and/or as a chemical tracers for trazability of meat produced from grazing sheep and goat.

Keywords. *Rosmarinus officinalis* – *Thymus capitatus* – Essential oil – Chemotype.

Caractérisation de la valeur nutritive et des huiles essentielles de* Rosmarinus officinalis *et* Thymus capitatus *provenant de la région centrale de Tunisie

Résumé. *Les feuilles et brindilles de* Rosmarinus officinalis *(romarin) et* Thymus capitatus *(thym) ont été collectés dans une région du centre de la Tunisie en mars et avril 2008. Les échantillons ont été immédiatement stockés à –10°C. La composition chimique et les paramètres in vitro de la production de gaz ont été déterminés. Les huiles essentielles (HE) des deux espèces ont été extraites par distillation et analysées en utilisant la chromatographie en phase gazeuse (FID), suivie d'un procédé de spectroscopie au Carbon-13 NMR pour confirmer les structures des composés principaux. Le romarin et le thym étaient pauvres en protéine brute (MAT : 58 et 88 g/kg MS, respectivement). Les teneurs en ADF étaient très proches dans les deux espèces (en moyenne 313 g/kg MS), Ce pendant, le degré de lignification était plus élevé dans le cas du thym que dans celui du romarin (ADF : 226 et 167 g/kg DM, respectivement). Le romarin a montré une plus basse production asymptotique de gaz que le thym (b : 27,7 et 49,6 ml, respectivement). La digestibilité in vitro de la matière organique après 24 h ($DIVMO_{24h}$) d'l'incubation et l'énergie métabolisable (EM) estimées à partir de la production de gaz et de la composition chimique ont suivi une tendance semblable à celle des paramètres in vitro de fermentation. La teneur en HE dans le romarin était approximativement le double de celle déterminée dans le thym (0,43 et 0,25% de MS, respectivement). L'es HE du thym se sont avérés riche en carvacrol (70%), qui est le chimiotype de l'espèce. Les autres composés secondaires détectés dans le thym sont le β-caryophyllene (8,5%), le γ-terpinene (4,3%) et le p-cymen (3,8%). D'autre part, les composés principaux identifiés dans les HE du romarin étaient le cineol 1-8 (44,2%), le camphre (12%) et le α-pinene (11,6%). En conclusion, les deux espèces médicinales natives étaient d'une faible valeur nutritive pour les*

petits ruminants en pâturage. Les chimotypes des HE détectés, principalement le carvacrol et le cineol 1-8 peuvent être envisagés en tant qu'additifs potentiels pour manipuler les fermentations ruminales et améliorer l'efficacité d'alimentation chez les ruminants et/ou comme traceurs chimiques pour le traçabilité de la viande produit à partir de ovins et des caprins sur parcours naturels.

Mots-clés. Rosmarinus officinalis –Thymus capitatus – *Huiles essentielles – Chimiotype.*

I – Introduction

In Tunisia, *Rosmarinus officinalis* and *Thymus capitatus* are growing wild in bioclimatic zones extending from the sub-humid to the arid (Nabli, 1995) and are largely grazed by sheep and goats. In addition, these species are recognized as aromatic and medicinal plants. Recent studies have shown that EO extracted from plant species could be envisaged as potential additives to manipulate rumen fermentation, to improve feed efficiency in ruminants (Benchaar *et al*., 2007) and/or as chemical tracers for trazability of meat produced from grazing sheep and goat. They are extensively exploited for the production of essential oils known to have antibacterial, antiseptic and antioxidant properties (Lee *et al*., 2004). These compounds are very complex mixtures which can contain about 20-60 components at quite different concentrations. They are characterized by two or three major components, commonly called chemotypes at relatively high concentrations (20-70%) compared to the other components present in trace amounts (Bakkali *et al*., 2007). The aim of the current study was to investigate the chemical composition and *in vitro* nutritive value of *Rosmarinus officinalis* and *Thymus capitatus* collected from the central regions of Tunisia and to characterize their extracted EO.

II – Material and methods

1. Plant material

Thymus capitatus and *Rosmarinus officinalis* leaves and twigs were collected from the region of Kairouan in March 2008 (semi-arid central region of Tunisia). Samples from each species were taken from different sites and pooled to make a composite. The dry matter (DM) was determined at 105°C in a forced-air oven and a part of each sample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* determinations. Fresh samples were stored at –20°C for essential oil extraction using steam distillation methods.

2. Animals and measurements

Three adult local sheep (24 months and 48.5 kg BW) with rumen cannula were used for *in vitro* determinations. They were housed in individual pens and received twice per day 70 g $kg^{-1}LW^{0.75}$ of a diet composed of 70% oat-vetch hay and 30% barely grains on dry matter (DM) basis.

Samples (300 mg DM) of thyme, rosemary, ray grass hay and an ovine-type concentrate were incubated in 100 ml glass syringes according to the technique of Menke and Steingass (1988). The incubation medium (30 ml) was a mixture of rumen fluid and Menke buffer solution (1:1). Gas production was measured at 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 h of incubation. Diets were incubated in triplicate and two successive incubations were carried out.

3. Chemical analysis

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall composition (NDF, ADF and ADL) in feeds were analyzed as described by Van Soest *et al*. (1991).

Extracted EO were analyzed using gas chromatography (FID), followed by a Carbon-13 NMR spectroscopy procedure to confirm the major compound structures.

4. Calculation and statistical analysis

Gas production was fitted by using the non-linear model of Ørskov and McDonald (1979): $p=a+b(1-e^{-ct})$, where: "p" is the gas production at time t; "a" is the immediate gas production; "b" is the asymptotic gas production; (iv) "c" is the fractional rate of gas production; and "a+b" is the total gas production. Parameters were calculated using PROC NLIN SAS (SAS, 1996). I*n vitro* organic matter digestibility at 24h ($IVOMD_{24h}$) and metabolisable energy (ME) were calculated according to the specific equation of Menke and Steingass (1988).

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 cell wall content (NDF: 40.9% and 68.89%, respectively). The ADF content was similar for both plant species (averaged 31.35% DM), but the lignification level of *Thymus capitatus* was higher compared to *Rosmarinus officinalis* (ADL: 22.6 and 16.7% DM, respectively). Gasmi-Boubaker *et al*. (2009) studied the chemical composition of *Rosmarinus officinalis* growing in the pastures of central Tunisia and found close values (ash: 7.75%, CP: 8.36%, NDF: 45.9% and ADF: 34.5% DM).

Table1. Chemical composition of feeds (% of DM)

Feed	%Ash	%CP	%NDF	%ADF	%ADL	%EO (DM)
Ray grass hay	11.2	9.9	68.9	36.3	5.3	–
Concentrate	5.7	16.9	34.2	5.3	–	–
Thymus capitatus	10.2	8.8	40.9	32.6	22.6	0.25
Rosmarinus officinalis	6.2	5.8	38.9	30.1	16.7	0.43

In vitro fermentation parameters of feeds are presented in Table 2. Parameters found in thyme are relatively comparable to those of hay, except for the rate of degradation "c", which was higher in thyme. Rosemary exhibited the lowest values; this result could be explained by its highest EO content which could depress microbial fermentation (Macheboeuf *et al*., 2007). The values of OMD and ME followed the same trend as *in vitro* parameters.

Table 2. Parameters of *in vitro* fermentation

Feeds	a (ml)	b (ml)	c (h^{-1})	a+b (ml)	OMD	$IVOMD_{24h}$ (%)	ME (kcal/kg DM)
Thymus capitatus	-7.9	62.2	0.077	54.3	53.4	53.39	1704
Rosmarinus officinalis	0.2	27.5	0.091	27.7	34.4	34.39	1123
Ray grass hay	-3.9	66.4	0.053	62.0	51.8	51.85	1648

P: the gas production at time t; a: the immediate gas production; b: the slowly fraction of gas production; c: is the rate of gas production; a+b: the total gas production.

Extracted EO content in rosemary was approximatively the double of that found in thyme (0.43 and 0.25% of DM, respectively). The main components of extracted EO are presented in Table 3. Thyme EO was found to be high in the active monoterpen phenol (carvacrol 70%) which could represent thyme EO chemotype. The other main compounds were β-caryophyllen (8.5%), γ-terpinen (4.3%) and p-cymen (3.8%). These results confirmed those found by Akrout (2004) who

studied some EO from pastoral plants in the region of Matmata (south east of Tunisia) and found that the main component of *Thymus capitatus* is carvacrol (66%); the other components were γ-terpinen (3.86%), sabinen (3.84%), p-cymen (5.38%). Amarti *et al*. (2008) found also the same chemotype (70.92%) in *Thymus capitatus* growing in Morroco.

On the other hand, major compounds identified in rosemary EO were 1-8 cineol (44.2%), camphor (12%) and α-pinen (11.6%). These results are in accordance with those found by Zaouali and Boussaid (2008) who studied essential oil variations from Tunisian natural populations of *Rosmarinus officinalis.* They found that EO extracted from populations in the sub humid and the upper semi-arid areas located in the regions of Cap Bon and Tunisian Dorsal were rich in 1,8-cineol, while those from the upper arid bio-climate (Matmata regions) were characterized by high content of camphor. Approximatively, the same results have been found by Touafek *et al*. (2004) who identified 1,8-cineole (29.5%), 2-ethyl-4,5-dimethylphenol (12.0%) and camphor (11.5%) as the major components of *Rosmarinus officinalis* EO.

Table 3. *Rosmarinus officinalis* and *Thymus capitatus* essential oils main components

Rosemary EO	%	Thyme EO	%
1,8 cineol	44.2	Carvacrol	69.7
Camphor	12	β-caryophyllen	8.5
α-pinen	11.6	γ-terpinen	4.3
Verbenon	5.2	p-cymen	3.8
Camphen	4	α-pinen	0.75
β-caryophyllen	3.5	α-terpinen	0.71
α-thyjen	2.2	Borneol	0.4
p-cymen	1.7	Camphor	0.4
α-terpineol	1.7	thymol	0.09

IV – Conclusions

It was concluded that both native medicinal plant species would be of low nutritive value if pastured by sheep and/or goat. The EO chemotypes detected, mainly carvacrol and 1-8 cineol could be envisaged as potential additives to manipulate rumen fermentation to improve feed efficiency in ruminants and/or as chemical tracers for trazability of meat produced from grazing sheep and goat.

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