Potential of essential oils from natural populations of Pistacia lentiscus to modify in vitro ruminal fermentation in sheep

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Potential of essential oils from natural populations of *Pistacia lentiscus t*o modify *in vitro* ruminal fermentation in sheep

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Abstract. In order to evaluate the effect of *Pistacia lentiscus* essential oils (EOs) on *in vitro* rumen fermentation in sheep, leaves and twigs were collected from the Eastern Region of Tunisia (Zaghouan) in spring. The EOs were extracted by hydrodistillation and analyzed using GC/MS. Increasing doses of EOs (0 ; 5 ; 10 ; 20 ; 40 ; 80 ; 120 µL/0.5 g DM of substrate) were tested with a ration composed of ray-grass hay and concentrate (50:50 on DM basis) in inoculum according to the technique of Menke and Steingass (1988). Rumen liquor was sampled from three adult Barbarine sheep (Averaged age and body weight: 18 months and 38 kg, respectively) receiving a ration composed of 70% of oat hay and 30% of commercial concentrate. For each dose of EOs, three syringes were reserved to determine true organic matter degradability (TOMD) and two syringes were incubated for subsequent ammonia-N (NH_3-N) analyses. Short chain fatty acid (SC-FA) concentration and partitioning factor (PF) were calculated. Results showed that *Pistacia lentiscus* EOs were composed of monoterpene Hydrocarbons (73%), associated to 16.7 % of α-pinene; 15.9% of sabinene; 12% of cis-ocimène and 11.6% of ϒ-terpinene. After 24 h of fermentation, gas production (GP) decreased (P<0.001) with the increase of EOs doses (i.e. 70.6, 82.9, 51.5 and 46.1 mL/0.5 DM, respectively for doses 20, 40, 80 and 120 µL). The same trend (P<0.0001) was observed for the estimated SC-FA concentrations. Ammonia-N concentration increased (P<0.0001) at 20 µL and reached 151.7 mg/L, and then decreased to 97.2 mg/L at 120 µL of EOs. Concerning TOMD, a trend of decrease (P < 0.0001) was noted when adding EOs and the lowest value was observed at 5 µL of EO. PF values are quite among doses 5,10 and 20 µL (averaged: 3.2), it increased significantly for 40, 80, 120 µL (4.9, 8.5, 10.9 mg/mL respectively). It was concluded that *Pistacia lentiscus* EOs could be envisaged as a potential additive to manipulate rumen fermentation in the optic of improving feed feeding efficiency in ruminant.

Keywords. Essential oil – *Pistacia lentiscus* – *In vitro* Fermentation – Sheep.

Potentiel des huiles essentielles de populations naturelles de* Pistacia lentiscus *pour modifier la fermentation ruminal* in vitro *chez le mouton

Résumé. *Afin d'évaluer l'effet des huiles essentielles de* Pistacia lentiscus *(HE) sur la fermentation ruminale* in vitro *chez les moutons, des feuilles et des brindilles de la plante ont été collectées dans la région de Zaghouan (Nord de la Tunisie, semi-aride) au printemps. Les HE ont été extraites par hydrodistillation et analysées à l'aide de GC / MS. Des doses croissantes d'HE (0; 5; 10; 20; 40; 80; 120 µL / 0,5 g de substrat) ont été testées avec une ration composée de foin de ray-grass et de concentré (50:50% sur la base de MS) dans l'inoculum selon la technique de Menke et Steingass (1988). Le jus rumen a été échantillonné à partir de 3 béliers de race Barbarine (Age moyen et poids vif, respectivement, 18 mois et 38 kg) recevant une ration composée de 70 foin d'avoine et 30% de concentré commercial. Pour chaque dose d'HE, 3 seringues ont été réservées pour déterminer la dégradation vraie de la matière organique (TOMD) et 2 seringues ont été réservées pour analyser l'ammoniac (NH_3-N). La concentration en acides gras à chaîne courte (SC-FA) et le facteur de partage (PF) ont été calculés. Les résultats ont montré que les HE de Pistacia lentiscus étaient principalement composés d'hydrocarbure monoterpène (73%), associé à 16,7% d'α-pinène ; 15,9% de sabinène ; 12% de cis-ocimène et 11,6% d'Y-terpinène. Après 24 h de fermentation, la production de gaz (GP) a diminué (P<0,0001) de 20 à 120 µL de doses (c.-à-d. 70,6, 82,9, 51,5 et 46,1 ml, avec les doses, respectivement, 20, 40, 80 et 120 µL). La même tendance (P <0,001) a été observée pour les concentrations de SC-FA calculées. La concentration d'ammoniac a augmenté de manière significative (P <0,0001) à 20 µL et a atteint 151,7 mg / L, puis a*

atteint 97,2 mg / L avec la dose 120 µL d'HE. Une diminution (P <0,0001) du TOMD a été notée après ajout d'HE sachant que la valeur la plus basse a été observée avec la dose 5 µL d'HE. Les valeurs des facteurs de fractionnement sont presque similaires pour les doses 5,10 et 20 µL (moyenne: 3.2). Le PF a augmenté de manière significative avec les doses 40, 80, 120 µL (4.9, 8.5, 10.9 mg / mL respectivement). On a conclu que l'HE de Pistacia lentiscus *pourrait être envisagé comme un additif potentiel pour manipuler la fermentation au niveau du rumen dans l'optique d'améliorer l'efficacité de l'alimentation animale chez les ruminants.*

Mots-clés. *Huile essentielle* – Pistacia lentiscus – *Fermentation* in vitro – *Mouton.*

I – Introduction

Recently, an increasing concern about synthetic antioxidants safety and efficacy has generated interest in the more efficient use of sources of natural antioxidants and biologically active compounds. The essential oils (EOs) extracted from aromatic and medicinal plants has attracted a great deal of researchers' interest due to their natural antioxidants and biologically active compounds explored in food, pharmaceutical and cosmetic (Hammer *et al*., 1999; Burt, 2004; Bakkali *et al*., 2008; Calo *et al*., 2015). In this context, many EOs have been tested as natural antibiotics growth promoters that can upgrade animal performance without emergence of antimicrobial resistance in humans (Newbold *et al*., 2004; Calsamiglia *et al*., 2007; Patra *et al*., 2014; Roy *et al*., 2014). However, there is only a small number of reports available in the literature, studying the *Pistacia lentiscus* L. effect on rumen fermentation, digestion and products quality.

Pistacia lentiscus L., from the Anacardiaceae family, is a Mediterranean dense bush with a strong characteristic aroma and green leaves (Zrira *et al*., 2003). Since antiquity, its aerial part has been used in the treatment of hypertension and possesses stimulant and diuretic properties (Bentley and Trimen, 1980 in Gardeli *et al*. 2008). This woody species is used browsed by sheep and goats as source of forage. Even though, the effects of the EOs on digestion and production performance of ruminants is not well documented. Therefore, the aim of the current study was to evaluate the effect of increasing doses of *Pistacia lentiscus* EO, on in vitro fermentation parameters in sheep.

II – Material and methods

1. Plant material and essential oils extraction

Pistacia lentiscus leaves and thin twigs (of less than 4 mm of diameter) were collected in spring 2011 from a bushland located in the region of Zaghouan (North of Tunisia, semi-arid). After air-drying of this plant material for 7 days, the EOs were extracted by hydro-distillation. The extracted amount of EOs was stored at until analyzed and used in the vitro trial.

2. Diets and animals

A simulated diet was composed of 50% of ray-grass hay and 50% of commercial ovine concentrate on dry matter (DM) basis, ground and mixed through a 1 mm screen. The chemical composition of diet's ingredients is presented in Table 1.

For the *in vitro* fermentation trial, we used two rumen-cannulated Barbarine rams (averaged age and body weight: 32 months and 45 kg). They were housed in individual pens and received twice per day 70 g $kg^{-1}BW^{0.75}$ of a diet composed of 70% oat-vetch hay and 30% barley grains on dry matter (DM) basis.

Table 1. Chemical composition of feeds (%DM)

	DM (%)	Ash	CP	NDF	ADF	ADL
Pistacia lentiscus leaves and twigs	92.1	2.9	9.02	43.5	32.1	9.6
Concentrate	94.1	6.6	16.1	30.8	6.2	5.1
Ray-grass hay	81.6	12.5	11.3	62.7	34.1	7.2

3. Measurements and calculations

The GC/MS identification of the EOs profile was made by corresponding their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC/MS data system in addition to other published mass spectra (Adams, 2004). The percentage composition was determined depending on peak area normalization without using correction factors.

We investigated the increasing doses of identified Pistacia EOs (0, 5, 10, 20, 40, 80 and 120 µL) on fermentation parameters. The *in vitro* gas production was measured on three 100 ml-glass syringes in two incubation runs as described by Menke and Steingass (1988). For each syringe containing 0.5 g of the simulated diet, we added to the corresponding dose of EOs and the ruminal liquid and buffer solution (1:1) and then we incubated them at 39°C. Gas production was measured after 2, 4, 6, 8, 10, 12 and 24 h of incubation.

Gas production was determinated by using the non-linear model of France et *al*. (2000):

$GP = b*(1- e^{-k(t-L)})$ where: GP = Gas production at time t (ml), b = asymptotic gas production (ml), k = fractional fermentation rate (h^{-1}) and L = lag time (h).

The concentration of short chain fatty acids was estimated as described by Getachew et *al*. (2000) on the basis of the gas production at 24 h incubation:

SCFA (mM/syringe) = 0.0239 GP – 0.0601

At the end of the incubation, the pH fluid samples was measured and the liquid was 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).

4. Chemical analysis

Samples of the extracted EOs were injected in the GC/MS (HP-5MS capillary column) for the determination of their profile. Oven temperature was programmed to rise from 50 to 240 °C at a rate of 5°C/minutes. The used carrier gas was He with a flow rate of 1.2 mL/minutes and a split ratio of 60:1. The experimental feeds were analyzed for their contents in dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall fractions (NDF, ADF and ADL) were analyzed as described by Van Soest et *al.* (1991).

5. Statistical analysis

The data was analysed by The General Linear Model procedure (GLM) of SAS (2009) with the option of LS MEANS multiple ranges. This model has included the effects of dose, incubation and interaction.

III – Results and discussion

1. Essential oils composition

The ten most abundant Pistacia EOs compounds and their classification are listed in Table 2. They represent 84.41% of EOs composition.

Table 2. Main compounds of *Pistacia lentiscus*

Compounds	Chemical classification	%
α-pinene	Monoterpene hydrocarbons	16,7
sabinene	Monoterpene hydrocarbons	15,9
cis-ocimene	Monoterpene hydrocarbons	11,9
ϒ-terpinene	Monoterpene hydrocarbons	11,6
germacrene D	Sesquiterpene hydrocarbons	6,1
α-phellandrene	Monoterpene hydrocarbons	4,6
α-terpinene	Monoterpene hydrocarbons	3,8
δ-cadinene	Sesquiterpene hydrocarbons	3,8
β-caryophyllene	Sesquiterpene hydrocarbons	3,5
α-terpinolene	Monoterpene hydrocarbons	1,9

In total, Pistacia EOs presented 68 identified compounds characterised by a high amount of monoterpene hydrocarbons (73%). The most presented compounds are l'α-pinene (16.7 %); sabinene (15.9 %), cis-ocimene (12 %), ϒ-terpinene (11.6%).

As it was reported by several authors, (Douissa *et al*., 2005, Barra *et al*., 2007, Gardeli *et al*., 2008), we found that the chemotype in *Pistacia lentiscus*, is α-pinene (16.7 %). However, other results reported that the highest compounds are limonene in Italy (Castola *et al*., 2000) and limonene, α-terpineol, β–caryophyllene and β-myrcene in Morocco (Zrira *et al*., 2003; Said *et al*., 2011).

2. Rumen fermentation parameters

Data reflecting the effect of increasing levels of *Pistacia lentiscus* EOs on gas production parameters are reported in Table 3. After 24 h of fermentation, results showed that GP did not change ($P<0.001$) for 5 and 10 μL doses. Doses of 20 μL and beyond had decreasing GP (70.6, 62.9, 51.5 and 46.1, respectively for doses 20, 40, 80 and 120 μL).

To the best of our knowledge, this is the first work that investigated Pistacia EOs effect on fermentation parameters. However, a study conducted on *Myrtus communis* for which chemotype is also α-pinene (29%) showed that GP decreased ($P<0.0001$) from 10 to 120 μL doses (i.e. 93.4, 84.3, 70.3, 35.3 and 25.3 ml, respectively for 10, 20, 40, 80 and 120 μL) (Bettaieb *et al*., 2016). This GP decrease can be explained by the antibacterial effect of EOs against rumen bacteria (Kim et *al.,* 1995).

Our study showed that the final pH value was higher than the blank (i.e. incubated inoculum without substrate) for all doses of Pistacia EOs. It was maximum at 40 μL (6.57) and similar at 5, 10, 20, 40, 80, and 120 μL (averaged: 6.49) ($P<0.0001$). The increase in final pH was associated with a significant reduction in SCFA production, due to a decrease of diet fermentation (Castillejos et *al*., 2006).

The concentration of SC-FA was not affected with EOs doses of 5 and 10 μL (averaged:41.4 mg/L) but decreased with the other levels: 20, 40, 80, and 120 μL (35.3, 21.4, 12.3 and 4.6 mg/L respectively). The same trend was observed by Moujahed et *al.* (2013) for *Rosmarinus officinalis* EOs. Ammonia-N concentration increased ($P<0.0001$) with the administration of 20, 40, 80, and 120 μL (151.7, 135.2, 115.6 and 97.2 mg/L respectively). Ammonia N reduction (from 30 to 50%) was observed by Busquet *et al*. (2006) for carvacrol, carvone, eugenol, oregano, and cinnamon EOs.

Table 3. Effects of increasing doses of *Pistacia lentiscus* EOs on the *in vitro* rumen fermentation parameters

Dose (µL/50 mL)	0	5	10	20	40	80	120	SEM
GP 24 (mL/0.5 mg DM) ***	96.4^{a}	96.1^{a}	91.6^{a}	70.6^{b}	62.9^{c}	51.5^{d}	46.1^{e}	16.55
pH ****	6.20^{b}	6.49^{ab}	6.48^{ab}	6.45^{ab}	6.57^{a}	6.54^{ab}	6.48^{ab}	0.110
SC-FA (mg/L) ****	41.1^{a}	41.6^{a}	41.3^{a}	35.3^{b}	21.4^{c}	12.3^{d}	4.6^{e}	0.55
N-NH_3 (mg/L) ****	179.2^{a}	171.8^{a}	169.3^{a}	151.7^{b}	135.2^{c}	115.6^{d}	97.2^{e}	1.79

a, b, c, d, e Values with different letters in the same line are statistically different, *** P<0.001, **** P<0.0001, SEM: Standard error of the mean.

4. Microbial activity

The effects of Pistacia EOs on TDOM and PF is shown in Table 4. At low doses (0, 10 and 20 µL), the TDOM did not change (averaged 60.4%). Higher doses of EOs (40, 80 and 120 µL) decreased (P<0.0001) TDOM by 49.8, 46.0, and 44.0 % respectively. Other results on the EOs of *Eucalyptus camaldelensis* reported a decrease of TOMD with the increasing doses of EOs (from 25 to 125 µL of EOs). This decrease was not significant (Sallam et *al*. 2009)..

The partitioning factor (PF), which represent the fermentation of nutrients into gas, SCFA and microbial mass, had substantially changed with the increasing levels of EOs. Almost constant for 5,10 and 20 µL (averaged: 3.2), it increased significantly for 40, 80, 120 µL (4.9, 8.5, 10.9 mg/mL respectively).

Table 4. Effects of increasing doses of EOs from *Pistacia lentiscus* on TDOM and PF

Dose (µL/50 mL)	0	5	10	20	40	80	120	SEM
TDOM (%) ****	60.1^{a}	61.4^{a}	59.5^{a}	55.0^{b}	49.8^{c}	46.0^{c}	44.0^{d}	0.008
PF (mg/mL) **	2.7^{d}	3.2^{d}	3.0^{d}	3.4^{d}	4.9^{c}	8.5^{b}	10.9^{a}	0.444

a, b, c, d 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

Our results showed that the administration of increasing doses of Pistacia EOs resulted in a significant decrease in gas production and short chain fatty acid during the in vitro fermentation. In addition, we found that for high doses of Pistacia EOs, the partitioning factor was improved. It was concluded that the Pistacia EOs could be considered as a potential modulator of rumen fermentation and feed efficiency in ruminants. In vivo studies should be emphasized to study the response of sheep to Pistacia EOs.

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