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Impact of PEG 6000 on *in vitro* gas production kinetics of some Mediterranean shrubs in sheep

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Abstract. Samples of foliage from some Tunisian shrub species (*Erica arborea, Myrtus communis, Pistacia lentiscus* and *Phillyrea angustifolia*) harvested in summer from a semi arid grazing mountain area of Tunisia were examined for their chemical composition and kinetics of gas production in presence and absence of polyethylene glycol (PEG 6000). Total condensed tannin contents (TCT) were also analysed. Crude protein (CP) content varied widely (40-93 g/kg DM) NDF content ranged from 440 g/kg DM in *M. communis* to 587 g/kg DM in *P. lentiscus*. The highest ADF content was for *E. arborea* (410 g/kg DM) and the lowest for *M. communis* (270 g/kg DM). High levels of CT were observed in *E. arborea* followed by *P. lentiscus*, and *M. communis*, whereas CT was almost absent in *Ph. angustifolia*. Asymptotic gas production (*A*), rate of gas production (c) and gas produced at 24 h of incubation (G24) were generally low in *E. arborea* and high in *Ph. angustifolia*. Addition of PEG to the incubation medium increased the parameters of gas production (A, G24 and c). This increase was more noticeable in *E. arborea* and less detected in *Ph. angustifolia.* Based on their crude protein and tannin contents, *M. communis* and *P. angustifolia*, may play an important role in providing fodder for ruminants even during the dry season when the other forages are scarce. However the foliage of *E. arborea* and *P. lentiscus* could have a potential nutritive value as when its tannin content is biologically inactivated by using a tannin-binding agent.

Keywords. Shrubs – Chemical composition – *In vitro* – Gas production – PEG.

Utilisation du PEG 6000 dans la détermination de l'activité biologique des tannins de certains arbustes méditerranéens

Résumé. *La composition chimique et la cinétique de production de gaz en présence et en absence du PEG ont été examinées sur des échantillons de certaines espèces arbustives (*Erica arborea*,* Myrtus communis*,* Pistacea lentiscus *and* Phillyrea angustifolia*) collectées en été sur la montagne du nord ouest (semi-aride) de la Tunisie. Les teneurs de ces espèces en tannins condensés (TC) ont été aussi déterminées. Le contenu en protéine brut (PB) oscille entre 40 et 93 g/kg MS Le contenu en fibre neutre digestible (FND) varie de 440 g/kg MS (*M. communis *) à 587 g/kg MS (*P. lenticus*). Le contenu le plus élevé en fibre acide digestible (FAD) a été enregistré au niveau de* E. arborea*. Cependant les TC ont été pratiquement absents dans le feuillage de* Ph. angustifolia*. La production de gaz assymptotique (A), le rythme de production de gaz (c) et la production de gaz à 24h d'incubation (G24) ont été généralement faibles pour* E. arborea *et élevés pour* Ph. angustifolia*. L'addition du PEG au milieu d'incubation s'est accompagnée par une augmentation des paramètres de production de gaz (A, G24 and c). Cette élévation a été plus notable pour* E. arborea *et moins détectée pour* Ph. angustifolia*. En se basant sur les teneurs en PB et en TC, les feuillages de* M. communis *et* Ph. Angustifolia *pourraient jouer un rôle important dans l'approvisionnement des aliments aux ruminants même durant la période de sécheresse lorsque les autres fourrages sont rares. Cependant, les feuillages de* E. arborea *et* P. lentiscus *pourraient avoir une valeur nutritionnelle importante une fois leurs tannins sont biologiquement désactivés en utilisant un agent détecteur des tannins.*

Mots-clés. *Arbustes – Composition chimique – Digestibilité –* In vitro *– Gaz – PEG.*

I – Introduction

Most of the rangelands in Tunisia are conformed by shrubby vegetation which provides a source of forage for ruminants. The rangelands area represents 33% of the total area widespread mainly in the arid and semi arid regions. In these zones, a major constraint to livestock production is the scarcity and fluctuating quantity and quality of the year-round feed supply. Many of the shrub species are problematic as feed supplements because they often contain antinutritional compounds such as tannins, saponins and non-protein amino acids, which are either toxics to rumen microbes or to the animal, or their metabolic products are toxic (Lowry *et al.*, 1996). Recently, it has been suggested that occurrence of tannins and their effect on the kinetics of *in vitro* fermentation can be assessed using the gas production technique coupled with the use of a tannin-complexing agent such as polyethylene glycol 6000 (PEG 6000). The objectives of the present study were to detect the nutritive value of some Tunisian shrubs using *in vitro* techniques and determine biological activity of tannins. Effect of PEG on the different parameters of gas production was also assessed.

II – Material and methods

1. Source of shrubby samples

Branches and twigs of several specimens of seven browse species namely *Erica arborea* L., *Pistacia lentiscus* L., *Myrtus communis* L. and *Phillyrea angustifolia* L. were collected in summer from the uplands of northwest Tunisia under the Mediterranean climate (mean annual rainfall and temperature are 900 mm and 21ºC, respectively). In the laboratory, both leaves and fine green stems (Ø <2 mm) were handily separated from the original samples and immediately oven-dried at 40ºC and finely ground (1 mm screen). The vegetation cover is mainly composed by *E. arborea* (22%) (Ammar *et al.*, 2005).

2. Chemical analysis

Dry matter (DM, method ID 934.01), ash (method ID 942.05) and crude protein (CP, method ID 984.13) contents were determined following the methods of AOAC (1999). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991). Extractable condensed tannins (CT) were measured using the butanol–HCl assay (Porter *et al.*, 1986) with the modifications of Makkar (2003) and using purified quebracho tannin as standard. Concentrations of CT were expressed in g/kg DM, standard equivalent.

3. *In vitro* gas production assay

Rumen fluid was obtained from four rumen cannulated Merino sheep fed by one kg alfalfa hay daily and with free access to water and mineral/vitamin licks. A sample of rumen content was collected before the morning meal in thermos flasks and taken immediately to the laboratory where it was strained through four layers of cheesecloth and kept at 39ºC under a CO_2 atmosphere. For the assessment of the kinetics of gas production, technique proposed by Theodorou *et al.* (1994) was followed. DM (300 mg) was weighed in triplicate into serum bottles kept at approximately 39ºC and flushed with CO_2 before use. Buffer solutions and rumen liquor/buffer (1:4) were prepared as described above. 50 ml of rumen/buffer mixture was anaerobically dispensed in each bottle with or without addition of 2 ml of aqueous solution of PEG (25%, g/ml). Six bottles were used for each substrate, three for each treatment (with or without PEG). All the bottles were crimped and placed in the incubator at 39ºC, being shaken at regular times. The volume of gas produced in each bottle was recorded at different inoculation times (3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h) using a pressure transducer (Theodorou *et al.*, 1994). In order to compensate for gas production in the absence of substrate

three serum bottles containing rumen fluid inoculum with or without PEG were incubated as controls. In order to estimate the kinetics of gas production, data of the cumulative gas volume produced were fitted to the exponential model proposed by France *et al.* (2000): G = A [1-e-c(t-L)], where G (ml) denotes the cumulative gas production at time t; A (ml) is the asymptotic gas production; c (h^{-1}) is the fractional rate of gas production and L (h) is the lag time. A, c and L are constant parameters. Incubations were performed in one triplicate run (3 bottles/sample/treatment).

4. Statistical analysis

Data on *in vitro* ruminal gas production kinetics was analyzed as a 4×2 factorial experiment (4 tree foliages species × 2 treatments (i.e., with or without PEG)) using the "GLM" option of SAS (1999) with methods of Steel and Torrie (1980), to determine differences due to tree species and PEG. In the case of significant (i.e., P <0.05) interactions, Duncan's multiple-range test (Duncan, 1955) was used to separate means within tree species.

III – Results and discussion

Although many Mediterranean shrubs gain increasing significance as the nutritional value of grass drops, usually browse does not play a prominent role in the diet for a number of reasons such as the low CP content of browse of some shrubby species (Ammar *et al*., 2005). In the present study, CP content was particularly low (<100 g/kg DM) in all of the browse samples (Table 1) in agreement with data reported for other Mediterranean shrubs (Ammar *et al.*, 2005). However, it is well reported that feeds containing less than 8% CP can not provide the minimum ammonia levels required by rumen microorganisms to support optimum activity according to Norton (2003). Therefore, by the exception of *Ph. angustifolia* leaves of the other studied species are likely to require protein supplementation when they are the only feed consumed by ruminant animals. In our previous study (Ammar *et al.*, 2005), these same species collected in spring were studied and their CP contents were higher than those recorded herein. Therefore it appears that the CP content declined through the growing season as a response to tissue ageing, particularly during the autumn, when nutrients are transferred to perennial tissues before abscission (Ammar *et al*., 2005). All of the shrubs contained high fibre (NDF and ADF) contents. Similar results were reported for foliage of these and other Tunisian and Mediterranean fodder shrubs (Ammar *et al.*, 2004a,b, 2005).

Table 1. Chemical composition of the shrubs species collected in summer

Samples	OM	CP	NDF	ADF	CT
E. arborea	960	65	540	410	570
Ph. angustifolia	948	93	460	375	20
M. communis	942	50	440	282	380
P. lentiscus	970	45	587	390	389

Concentration of phenolics varied widely among browsed species. The lowest CT contents were found in *Ph. angustifolia* (20 g/kg DM, quebracho tannin equivalent), whereas the highest levels were observed in *E. arborea* (570 g/kg DM, quebracho tannin equivalent), consistently with the results pointed out in the literature (Ammar *et al.*, 2005). A part the use of analytical methods to the quantification of tannins, recently there has been interest in the use of tannin-binding agents such as PEG for the quantification and neutralization of tannins and their negative effects on animals. Based on this principle, the relative increase in gas production as a consequence of the addition of PEG represents the quantitative effect of tannins: the higher the biological activity of tannins on rumen microbes, the higher the increase in gas production as a result of

the neutralization of tannins by PEG. In our present study addition of PEG to the incubation medium was traduced by a significant increase (as percentage of the control) of the volume of the gas produced, the fermentation rate and the gas produced at 24 h of incubation (Table 2). The largest improvement was generally recorded with *P. lentiscus* (38, 28 and 56% for A, c and G24, respectively). Similar results were reported earlier (Ammar *et al*., 2004b) on other Mediterranean browse species.

Table 2. *In vitro* ruminal gas production parameters† of tree foliage species in the absence (-) or presence (+) of PEG after 24 h of incubation with sheep rumen fluid

	G24		A		c	
	-	+	-	+	-	+
E. arbórea (EA)	125 ^{b}	186 ^{a}	227 ^{b}	260 ^{a}	0.033 ^{b}	0.052 ^{a}
Ph. angustifolia (PA)	198 ^{b}	213 ^{a}	274 ^{b}	293 ^{a}	0.053	0.054
M. Communis (MC)	155 ^{b}	214 ^{a}	281 ^{b}	303 ^{a}	0.033 ^{b}	0.051 ^{a}
P. lentiscus (PL)	140 ^{b}	217 ^{a}	206 ^{b}	284 ^{a}	0.047 ^{b}	0.061 ^{a}
SEM	2.70		4.16		0.001	
Probability						
F	<.0001		<.0001		<.0001	
P	<.0001		<.0001		<.0001	
F x P	<.0001		<.0001		<.0001	
Ranking of foliages (P <0.05) ††	PA>MC>PL>EA		MC>PA>PL=EA		PL=PA>EA=MC	

†'A' is the asymptotic gas production (ml/g DM); 'c' is the rate of gas production (/h); 'L' is the initial delay before gas production begins (h).
††Foliages are only ranked overall in the absence of an interaction P<0.05.
Different superscripts following means within a row and foliage indicate differences at P<0.05.

IV – Conclusion

The *in vitro* gas production technique coupled with the use of PEG appears to have promising potential for the assessment of phenolic-related antinutritive effects in feeds. It is suggested that understanding structure-activity relationships of tannins is particularly important, with the development of new methods of quantification that relate chemical structure to biological activity.

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