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Chemical composition, *in vitro* digestibility and kinetics of gas production of foliage of some Tunisian shrubs

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SUMMARY – A mixture of leaves and fine green stems of *Erica arborea, Myrtus communis, Phillyrea angustifolia, Pistacia lentiscus* and *Quercus suber*, harvested in spring from the uplands of Taref in the delegation of Nefza (northwest of Tunisia), was analysed for its chemical composition and phenolic compounds. The *in vitro* dry matter digestibility (IVD) and volumes of gas produced, measured at 15 different incubation times (till 144 h) were also assessed. *P. lentiscus* showed the lowest crude protein content (CP), IVD and D144 (potential degradability) and the highest condensed tannins. Potential of gas production (A) was lower in *Q. suber* and higher in *M. communis*. On the basis of their crude protein contents and potential degradability, the ranking order of the shrub species was *M. communis>Ph. angustifolia>E. arborea>Q. suber>P. lentiscus.* It was suggested that some of the studied browse species could be used as supplements to rations rich in highly degradable protein.

Keywords: Shrubs, *in vitro* digestibility, gas production, tannins.

RESUME – *"Composition chimique, digestibilité* in vitro *et cinétique de production de gaz du feuillage de quelques arbustes tunisiens". Un mélange de feuilles et de brindilles de* Erica arborea*,* Mytrus communis*,* Phyllerea angustifolia*,* Pistacia lentiscus *et* Quercus suber*, collectées au printemps dans la montagne de Taref de la délégation de Nefza (nord-ouest de la Tunisie), a été analysé pour sa composition chimique et ses composés phénoliques. La digestibilité* in vitro *(IVD) et les volumes de gaz produits, mesurés à 15 différents temps d´incubation (jusqu'à 144 h), ont aussi été étudiés.* P. lentiscus *a montré le plus faible contenu protéique (CP), IVD et D144 (dégradabilité potentielle) et la concentration la plus élevée en tannins condensés. Le potentiel de production de gaz (A) a été faible chez* Q. suber *et élevé chez* M. communis*. En se basant sur leur contenu protéique et leur dégradabilité potentielle, le classement des arbustes étudiés est :* M. communis>Ph. angustifolia>E. arborea>Q. suber>P. lentiscus*. Ces espèces pourraient être utilisées comme suppléments aux rations riches en protéine fermentescible.*

Mots-clés : *Arbustes, digestibilité* in vitro*, production de gaz, tannins.*

Introduction

In Tunisia, rangelands represent 33% of the total area, widespread mainly in the arid and semi-arid regions. Promotion of suitable and nutritionally better species in these areas could be a practical approach to reduce fodder scarcity during long periods of drought (June-October) and to meet nutritional requirements of many categories of animals. Up to date, the *Acacia* spp. and particularly *A. cyanophilla* has been the most widely studied tree in Tunisia. However, despite their abundance in the rangelands and their evergreen foliage throughout the year, many browse species have been, generally, undervalued mainly because of insufficient knowledge about their potential feeding value. Chemical analysis, particularly in combination with *in vitro* digestibility can help in the preliminary evaluation of the nutritive value of shrub species, which were not investigated previously. Thus, detailed investigation on browse species seems to be very important in order to identify the better ones in terms of nutrient content and digestibility. The objective of this study was to assess the potential nutritive value of some Tunisian browse species based on their chemical composition, polyphenolic concentration and kinetics of *in vitro* gas production. These rapid and low cost methods have been used to screen feed resources before making them available to livestock.

Material and methods

Source of shrubby samples

Leaves and current twigs of the season from the shrub plants: *Erica arborea, Mytrus communis, Phyllerea angustifolia, Pistacia lentiscus* and *Quercus suber* were collected from the uplands of Taref in the delegation of Nefza (Norwest of Tunisia) and taken in spring of 1998. The climate is Mediterranean (mean annual rainfall and temperature is 900 mm and 21ºC, respectively). The browse plants were clipped with scissors harvesting a mixture of leaves and fine green stems (Ø <5 mm). In the laboratory, both leaves and stems were handily separated from the original samples, then they were immediately oven-dried and milled in a hammer mill using a 1-mm sieve for their later analysis.

Chemical analysis

Nitrogen (N) content was measured using the Kjeldahl method (AOAC, 1995). Neutral detergent fibre (NDF) was determined according to the technique proposed by Van Soest *et al*. (1991), whereas the analyses of acid detergent fibre (ADF) and acid detergent lignin (ADL) were carried out using the technique of Goering and Van Soest (1970). In both techniques modifications proposed by ANKOM (1998) were followed. Total phenols (TP) were determined according to the method of Julkunen-Tiitto (1985) using the tannic acid as a standard. Free condensed tannins (FCT) and bound condensed tannin (BCT) were measured using the butanol-HCl assay reported by Porter *et al*. (1986) with the modifications of Makkar (2000) using the purified quebracho tannin as a standard. Concentration of total condensed tannins (TCT) was therefore calculated as follows: TCT = FCT + BCT

In vitro dry matter digestibility

For the *in vitro* dry matter digestibility determination the technique proposed by Goering and Van Soest (1970) was followed. Four Merino sheep housed in individual cages, fitted with rumen fistula and fed 1 kg alfalfa hay daily provided rumen fluid for *in vitro* incubations of the shrub samples. Rumen content was collected before the morning meal in thermos flasks and taken immediately to the laboratory. The rumen liquor sample was strained through four layers of cheesecloth and kept at 39ºC under CO_2 atmosphere. A culture medium containing macro- and micro-minerals solutions, resazurin and a bicarbonate buffer solution was prepared as described by Goering and Van Soest (1970). The medium was kept at 39ºC and saturated with CO_2. Oxygen in the medium was reduced by the addition of a solution containing cysteine-hydrochloride and Na_2S, as described by Goering and Van Soest (1970). Rumen fluid was then diluted into the medium in the proportion 1:4 (v/v). Samples were weighed (250 mg) out into two polyester bags and placed in two incubation jars (1 bag/jar), a 5 litre glass recipient with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases. 2 litres of the buffered rumen fluid were anaerobically transferred into the two incubation jars. The jars were placed in an incubator (DAISY, ANKOM) at 39ºC, with continuous rotation to facilitate the effective immersion of the bags in the rumen fluid. After 48 h of incubation in buffered rumen fluid, samples were followed by a neutral detergent extraction (100ºC, 1 h) as described by Goering and Van Soest (1970) in order to determine the true *in vitro* digestibility (IVD). Four observations per sample were obtained.

In vitro gas production

The method used for the gas production measurements was as described by Theodorou *et al*. (1994). Buffer solutions and rumen liquor/buffer (1:4) were prepared as described above. Ground samples (300 mg) were weighed out into 100 ml serum bottles kept at approximately 39ºC and flushed with CO_2 before use. Three serum bottles were used for each substrate. 50 ml of rumen/buffer mixture were anaerobically dispensed in each bottle at 39ºC. 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 several incubation times (3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h after inoculation time) using a pressure transducer (Theodorou *et al*., 1994). Three serum bottles

containing rumen fluid inoculum were incubated as controls and were used to compensate for gas production in the absence of substrate. At the end of the incubation period, the contents of each serum bottle were filtered using sintered glass crucibles under vacuum and crucibles were oven-dried at 100ºC for 48 h to estimate the potential DM disappearance (D144, g/kg DM). 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): $G = A\ (1\text{-}e^{-c(t-L)})$, 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 triplicate (three observations per sample).

Statistical analysis

Analysis of variance (Steel and Torrie, 1980) was performed on *in vitro* digestibility and gas production kinetics data. The statistical significance of the differences between means was tested using the Duncan test.

Results and discussion

The level of CP in the foliage of Mediterranean shrubs is often lower than 10% DM (Cabiddu *et al*., 2000). This was the case of all the studied species, except for *C. villosa* and *M. communis* (Table 1). The lowest CP content was observed in *P. lentiscus* (below 7%); thus, digestibility of its foliage would be depressed due to lack of nitrogen (Minson and Milford, 1967). Foliage of all the studied species revealed high cell wall contents. This should be attributed mainly to woody twigs included during the analysis. In general, data on chemical composition was consistent with what has been reported earlier in the literature with other Tunisian (Ben Salem *et al.,* 1996) and Spanish species (Hervás *et al*., 2000). Concentration of the phenolic compounds in examined shrubs revealed considerable variation between species with *P. lentiscus* having the highest concentration of TP and FCT followed by *E. arborea*, consistently to the findings reported by Ben Salem *et al*. (1996) and Hervás *et al*. (2000) on *P. lentiscus* and *E. arborea*, respectively. However, *Ph. angustifolia* had low contents of FCT, which would generally be considered unlikely to significantly affect digestion of nutrients in ruminants. With high protein content and low CT (Table 1) this shrub would be regarded as having potentially high nutritive value, which would justify its consumption, by sheep and goat in the studied area. It is noteworthy that a high proportion of TCT content was recovered as FCT, except for *Ph. angustifolia*. It is believed, therefore, that any detrimental effect of CT on microbial fermentation of nutrients in the rumen should be explained by the concept of 'free tannins' (Barry and McNabb, 1999).

Table 1. Chemical composition (g/kg DM) and phenolic compound contents (g/kg DM, equivalent standard) of foliage (leaves and twigs) of the shrub species

Species	CP	NDF	ADF	ADL	TP	FCT	BCT	TCT
E. arborea	80	524	398	297	249	221	58	279
M. communis	130	383	200	110	227	49	55	104
Ph. angustifolia	110	445	354	260	230	2	10	12
P. lentiscus	60	433	252	240	261	363	42	405
Q. suber	70	551	395	360	161	103	48	151

Furthermore, the considerable variation displayed between the different phenolics should be related not only to browse species but also to the method of analysis (properties of the standard used). Data on *in vitro* dry matter digestibility and parameters of gas production are depicted in Table 2. As it was expected, foliage of *P. lentiscus* showed the lowest IVD. This has been attributed partly to the poorly digestible compounds such as NDF and lignin, and partly to their secondary chemical

compounds such as tannin since their inhibitory action reduces rumen microbial and enzymatic activity. On the other hand, it is well accepted that the chemical linkages between lignin and hemicellulose are the principal plant factors restricting digestion of the plant cell wall by ruminants (Manley and Duncan, 1986). It is pertinent to mention that IVD of *P. lentiscus* was higher than what has been expected from its chemical composition. This would be attributed mainly to a possible solubilisation of indigestible components in the neutral detergent solution.

Table 2. Kinetics of gas production from the foliage of shrub species

Species	IVD (g/kg DM)	D144 (g/kg DM)	A (ml/g DM)	G24 (ml/g DM)	c (h^{-1})
E. arborea	530 b	520 b	217 b	126 c	0.036 c
M. communis	670 a	680 a	292 a	151 b	0.030 d
Ph. angustifolia	680 a	650 a	291 a	197 a	0.047 a
P. lentiscus	520 b	370 c	190 c	127 c	0.046 ab
Q. suber	540 b	480 b	187 c	122 c	0.044 b
s.e.d.	16.3	7.2	4.3	2.7	.0010

[a, b, c, d] Within the same column, means without a common superscript letter are significantly different ($P<0.05$).

The *in vitro* gas production method was shown to be a reliable tool in feed evaluation because gas production was well correlated with microbial protein synthesis (Krishnamoorthy *et al*., 1990), *in vivo* (Khazaal *et al*., 1993) and *in vitro* (Ammar, 2002) digestibility. As it is depicted in Table 2, with the exception of the rate of gas production, all the lowest parameters of gas production were found in *P. lentiscus* and *Q. suber*. These lower values are indicators of poor digestibility that conform their low organic matter digestibility (unpublished data). However, *Ph. angustifolia* and *M. communis* revealed relatively higher dry matter disappearance and potential of gas production. This should be due to their lower cell wall and tannin contents (Table 1) and higher degradability of insoluble fraction (unpublished data). So the cell wall fraction may have a negative influence on forage digestibility as generally described in conventional feedstuffs (Van Soest, 1994) and particularly in browse plants (Wilson, 1977). Although D144 and A were slightly higher for foliage of *M. communis,* the corresponding gas production rate was the slowest (0.03%/h). This result suggests that the rate of gas production is affected by tannin and indigestible components of the cell wall and its lignification to a greater extent than the others parameters. On the other hand the present result may indicate an adaptation of microorganisms to structural carbohydrates and tannins with a delay in degrading them.

Conclusions

The studied species constitute a source of medium-to-low quality forage. They could help ruminants to meet their nitrogen demands, when other palatable plants are not available, or added to ruminant fed on rations rich in rumen degradable protein. On the basis of their crude protein contents and potential degradability, the ranking order of the shrub species was *M. communis>Ph. angustifolia>E. arborea>Q. suber>P. lentiscus.*

More research has to be conducted to better understand the nutritional value of these browse species and the potential effects of any non-nutritive factors on the *in vitro* digestibility and kinetics of gas production.

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