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Chemical and biological characterisation of some woody species browsed by goats in the North-West of Tunisia

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SUMMARY – A study was conducted during spring in the North-West of Tunisia to determine: (i) the chemical composition; (ii) the *in situ* disappearance; and (iii) the *in vitro* fermentation of eight dominant woody species in the region of experimentation (*Arbutus unedo*, *Calicotome villosa*, *Cistus salvifolius*, *Erica arborea*, *Phyllirea angustifolia*, *Pistacia lentiscus*, *Myrtus communis* and *Quercus suber*). Dry matter (DM), organic matter (OM) and crude protein (CP) degradation of each species were measured by the nylon bag technique on three rumen-fistulated local goats. Animals were browsing freely daily between 07:00 and 17:00 h. The same animals provided the rumen fluid used for the fermentation study in syringe. Samples of oat hay served as a control treatment for evaluating the methodological accuracy of the experiment system. Shrubs had moderate levels of CP (from 5.9% DM for *A. unedo* to 11.1% DM for *Erica arborea*) except *C. villosa*, which had the highest (22.1% DM). The range in lignocellulose (acid detergent fibre, ADF) was from 20.1% DM for *Calicotome* to 39.8% DM for *Erica*. The degree of lignification (acid detergent lignin, ADL) was important in all shrubs and represented 20 to 50% of neutral detergent fibre (NDF). *In situ* DM degradation after 120 h varied from 33.9% to 69.6% and volume of gas produced after 120 h varied from 41.1 to 68 ml/300 mg. The lowest DM disappearance and gas production was recorded with *Q. suber*, which had the highest ADL content (36.3% DM). There were relationships between lignin content and *in situ* degradation and *in vitro* fermentation. Ranking of shrubs according to their DM and OM degradation was similar using *in vitro* gas production. Further work is needed to account for the anti-nutritional factors content.

Key words: Woody species, *in situ* disappearance, *in vitro* gas production, goats.

RESUME – "Caractérisation chimique et biologique de quelques espèces arbustives broutées par les caprins dans le Nord-Ouest de la Tunisie". La composition chimique, la dégradabilité in sacco de la matière sèche (MS) et la production totale de gaz dans des seringues sont étudiées sur huit espèces arbustives du maquis du Nord-Ouest de la Tunisie. Les espèces sont : *Arbutus unedo*, *Calicotome villosa*, *Cistus salvifolius*, *Phyllirea angustifolia*, *Erica arborea*, *Myrtus communis*, *Pistacia lentiscus* et *Quercus suber*. Les échantillons des feuilles et brindilles sont prélevés en mars. Trois boucs fistulés du rumen conduits sur maquis sont utilisés pour les incubations des sachets de nylon et les fermentations dans les seringues pendant 120 h. Les arbustes se caractérisent par des teneurs moyennes en matières azotées totales (MAT) (7 à 11% de la MS) mais élevées en fibres (31,22 à 53,13% de la MS). La dégradabilité in sacco varie de 33 à 69% et la production de gaz varie de 43 à 68 ml/300 mg de MS. L'arbuste le moins dégradé et le moins fermentescible est *Quercus suber*. L'espèce la plus digestible et la plus fermentescible est *M. communis*. Il n'existe pas de relation entre la teneur en MAT et la dégradabilité in sacco ou le volume de gaz produit. Par contre, une relation négative ($P < 0,05$) est notée avec les fibres. Les résultats de la méthode des sachets de nylon et celle des seringues sont concordants.

Mots-clés : Espèces ligneuses, dégradation in sacco, production de gaz, caprins.

Introduction

Shrubs and trees are an important feed resource for ruminants in the North-West of Tunisia but there has been limited research on their nutritive value. Chemical analysis, particularly in combination with *in situ* degradability and *in vitro* fermentation can help in the preliminary evaluation of the likely nutritive value of previously. This study investigated: (i) chemical composition; (ii) *in situ* disappearance; and (iii) *in vitro* fermentation of eight shrubs available in the North-West of Tunisia and consumed by goats during spring.

Materials and methods

Plant material

Eight indigenous browse samples (leaves, petioles and thin twigs of less than 4 mm in diameter) were collected from tree and shrub species browsed by goats in the North-West of Tunisia. These species were: *Arbutus unedo*, *Calicotome villosa*, *Cistus salvifolius*, *Erica arborea*, *Phyllerea angustifolia*, *Pistacia lentiscus*, *Myrtus communis* and *Quercus suber*. The samples were hand-harvested and dried in a ventilated oven at 40°C to minimize changes in tannin content and activity (Makkar and Singh, 1991).

Chemical analysis

Browse samples were ground to pass through a 1-mm screen, then were analysed for dry matter (DM) and ash by the methods of the Association of Official Analytical Chemists (1975). The nitrogen (N) was determined by Kjeldahl digestion and crude protein (CP) was calculated by multiplying N by 6.25. Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) were determined by the methods of van Soest *et al.* (1991).

In situ degradation

The nylon bag technique (Ørskov *et al.*, 1980) was used to study the ruminal degradation of eight browse species. DM, organic matter (OM) and CP degradation of each shrub were determined on three goats fitted with ruminal cannula conducted on forest. Duplicate 3 g of samples were placed in nylon bags (9 × 17 cm, pore size 50 µm) and suspended in the rumen of animals before their departure to the forest. The bags were removed at 24 h for CP analysis or at 120 h for DM and OM determination. After withdrawal, bags were immediately washed until water was clear and immersed in ice water to stop microbial activity and transported to the laboratory. Bags were dried at 60°C until constant weight and the remaining DM was determined. Residue was removed from bags and analysed for ash and CP. This procedure was repeated within each month during spring season and a total of 3 runs were carried out.

In vitro gas production

The eight ground (1-mm screen) shrub samples (300 mg) were incubated in 100 ml glass syringes containing 30 ml of rumen fluid plus buffer (10:20) as described by Menke and Steingass (1988). Rumen fluid collected from the same goats used for *in situ* incubations was strained through four layers of cheesecloth and mixed with artificial saliva (1:2). The syringe was incubated at 39°C in a water bath for 120 h. Three syringes containing rumen fluid inoculum were incubated as controls. These controls were used to compensate for gas production in the absence of substrate. The syringes were hand shaken frequently and the volume of gas produced was recorded at 0, 3, 6, 9, 12, 24, 36, 48, 72, 96, 120 h.

Statistical analysis

The mean of *in situ* disappearance or gas production from the various plants were subjected to analysis of variance. A correlation matrix of all chemical components of the samples, gas volume or DM and OM degradation was obtained using SAS (1985).

Results and discussion

Chemical composition of shrubs

Total ash, CP and NDF of browse forages are shown in Table 1. Except of *C. villosa*, remaining

forages had ash and CP lower than 11%. Ash content is inversely related to the total organic matter in the plant. All studied species had a low content of ash which means that OM content and gross energy value are high in browse species. CP estimated as Kjeldahl N is absolutely essential for any forage evaluation. However, this analysis, does not indicate the extent of the break down of protein in the rumen and could not evaluate the potential to supply by-pass protein. As a first screening, CP content will give a useful guide but in a second screening, protein solubility may help to describe the quality of protein in shrubs, although the CP content is very similar.

Table 1. Concentrations (% DM) of ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in shrubs species

Plant species	Ash	CP	NDF	ADF	ADL
<i>A. unedo</i>	5.3	5.9	31.2	24.0	12.8
<i>C. villosa</i>	5.2	22.0	36.0	20.0	11.2
<i>C. salvifolius</i>	7.5	9.0	32.5	23.0	9.0
<i>E. arborea</i>	3.5	11.0	50.0	39.8	30.9
<i>P. angustifolia</i>	4.7	6.8	35.4	26.4	13.8
<i>P. lentiscus</i>	5.1	6.7	33.2	25.1	24.0
<i>M. communis</i>	4.3	8.7	38.5	20.4	11.0
<i>Q. suber</i>	4.1	7.3	53.1	35.8	36.0
Vetch oat hay	5.6	7.7	–	44.7	7.6

Clear differences existed in NDF, ADF and ADL contents. The NDF varied from 31.2% in *A. unedo* to 53.1% in *Q. suber*. The ADF was lowest in *C. villosa* and highest in *Q. suber* (35.8%) and *E. arborea* (39.8%). Lignification was higher in *E. arborea* and *Q. suber* (30.9 and 36.3% respectively). These values were much higher than those reported by Khazaal and Ørskov (1994). In this study, while the content of neutral detergent fibre is low, which may indicate a high content of highly digestible cell content, the lignin contents are high. The latter may be interpreted as an indication of heavily lignified and very indigestible cell wall. However, the values for NDF may underestimate the total content of cell wall since certain constituents such as pectin are soluble in the detergent solution. Furthermore, the ADL fraction may not be an appropriate measure of lignin in browse due to the formation of artefacts arising from reactions of tannins with protein and carbohydrates (Mueller-Harvey and Mc Allan, 1992).

To evaluate forages, several considerations are necessary. They include: (i) chemical analysis for minerals such as sulphur, phosphorus, calcium and trace mineral to support minimum requirements, i.e. stated in NRC; (ii) chemical analysis for nitrogen content, potential fermentable nitrogen, bypass protein and intestinal digestibility; and (iii) chemical analysis for secondary components such as tannins which may limit the intake, and phenolics which interfere with protein.

In situ degradation

In situ DM and OM disappearance (Table 2) were variable among the species examined (ranging from 33.9 to 69.6% for DM and from 33.2 to 70.9% for OM) while the disappearance of hay was only 44.7% for DM and 37% for OM. CP degradation was very low (from 4.7 through 48.4%) except of *C. villosa* which had the highest level. Because of microbial contamination (Lindberg, 1981), analysis of the digestion residues for nitrogen yielded little information. In their review, Michalet-Doreau and Ould Bah (1992) stressed the necessity of measuring microbial colonisation to avoid underestimation of *in situ* nitrogen degradation.

The levels of lignin (ADL) negatively affected *in situ* degradation of DM and OM ($r = -0.79$; $P < 0.05$).

Table 2. *In situ* degradation (%) of dry matter (DM) and organic matter (OM) after 120 h, of crude protein (CP) after 24 h, and volume of gas (ml) produced in syringe after 120 h

Plant species	DM	OM	CP	Gas
<i>A. unedo</i>	66.7 ^a	69.9 ^a	10.5 ^f	52 ^c
<i>C. villosa</i>	55.1 ^b	54.7 ^c	75.8 ^a	64 ^b
<i>C. salvifolius</i>	64.7 ^a	67.0 ^a	27.2 ^d	59 ^b
<i>E. arborea</i>	38.8 ^e	37.9 ^e	17.0 ^e	48 ^d
<i>P. angustifolia</i>	45.2 ^c	51.4 ^d	39.6 ^c	60 ^b
<i>P. lentiscus</i>	56.9 ^b	58.9 ^b	28.0 ^d	46 ^e
<i>M. communis</i>	69.6 ^a	70.9 ^a	48.4 ^b	68 ^a
<i>Q. suber</i>	33.9 ^e	33.2 ^f	4.7 ^g	43 ^e
Vetch oat hay	44.7 ^c	37.0 ^e	–	58 ^b

a,b,c,d,e,f,g Values in columns with the same letter do not differ significantly (P < 0.05).

In vitro gas production

The gas produced in syringe after 120 h reflected the differences across the species (ranging from 41.3 to 68 ml/300 mg DM) while the hay produced 58 ml/300 mg DM. The volume of gas was not correlated to CP content but was negatively correlated to ADF ($r = -0.75$, $P < 0.05$) and ADL contents ($r = -0.86$; $P < 0.05$).

One of the most frequent question about gas experiments is how gas data can be compared to those obtained in *in vitro* or *in situ* studies. To validate this comparison, an assumption must be made: in the rumen, gas produced is formed through the transformation of nutrients by microbes. Thus, for each amount of gas produced, an amount of substrate is degraded. In this study, the lowest DM disappearance and gas production were recorded with *Q. suber* and *E. arborea*. However, *in situ* degradation and *in vitro* fermentation were not strongly correlated.

The eight woody species examined showed a great variation in chemical composition, *in situ* degradation and *in vitro* fermentation. Similar trends have been reported in studies of Mediterranean browse plants (Khazaal *et al.*, 1993; Khazaal and Ørskov, 1994; Silanikove *et al.*, 1996; Decandia *et al.*, 2000).

Conclusions

Compared to vetch oat hay, it may be concluded that these woody species have potential as livestock fodder. However, *in vivo* feeding trials should be emphasised to validate this conclusion.

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