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Seasonal variations in the chemical composition and *in vitro* dry matter digestibility of leaves and stems of two Spanish browse legumes

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RESUME – *"Variations saisonnières de la composition chimique et de la digestibilité* in vitro *de la matière sèche des feuilles et des tiges de deux arbustes légumineux espagnols". On a étudié la composition chimique et la digestibilité* in vitro *de matière sèche (MS) (déterminée par deux techniques différentes) des feuilles et des tiges de deux arbustes légumineuses (*Cytisus scoparius *et* Genista florida*) collectées en différents stades de maturité de la montagne de León (Nord Ouest de l´Espagne). En comparaison avec les tiges, les feuilles ont montré des contenus en protéine brute (PB) et une digestibilité de MS plus élevés, et des niveaux plus bas en paroi cellulaire. Chez les deux espèces et en fonction de la saison, tant le contenu en PB comme la digestibilité de la MS ont diminué, alors que le contenu en paroi cellulaire a augmenté. Ces changements saisonnières ont été plus accusés au niveau des tiges que des feuilles. Il a été conclu que le contenu en PB et la digestibilité de MS des feuilles et des tiges des deux espèces sont comparables à ceux du foin de bonne qualité, et par la suite peuvent être considérées un patrimoine fourrager très important dans l´alimentation des ruminants en pâturage, en particulier durant les périodes de sècheresse.*

Mots clés: *Plantes arbustives, valeur nutritive, digestibilité, stade de maturité.*

Introduction

In the Mediterranean regions with a severe dry season, shrubby vegetation represents an integral component of the silvo-pastoral systems, and sometimes their grazeable material is considered important for the nutrition of range animals (Papachristou *et al.*, 1999). However, quite often these feed resources have been disregarded or undervalued mainly because of insufficient knowledge about their potential feeding value. The nutritive value of forage is, in principle, affected by its chemical composition and digestibility which depend largely on plant species, botanical fraction of the plant, seasonally and maturity, etc. (Lambert *et al.*, 1989b; Papachristou and Papanastis, 1994). The main objective of the present study was to investigate changes in chemical composition and *in vitro* digestibility of leaves and stems of two browse species, which were harvested at different stages of maturity.

Material and methods

Source of shrubby samples: Leaves and stems from the leguminous species *Cytisus scoparius* and *Genista florida* were collected from the province of León (Norwest of Spain) and taken at different growing seasons: Spring, Summer and Autumn of 1998. The sampling area was located in uplands with an altitude of 750 m above sea level. The climate is Mediterranean (mean annual rainfall and temperature is 564 mm and 10.6º C, respectively). Branches of 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 manually separated from the original samples, then immediately freeze-dried and milled in a hammer mill using a 1-mm sieve for later analysis.

Chemical analysis: Nitrogen content (N) was measured using the Kjeldahl method (AOAC, 1995). Crude protein (CP) was calculated by multiplying N x 6.25. Neutral detergent fibre (NDF) was determined according to the technique proposed by Van Soest *et al.* (1991); while the analysis 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. *In vitro* dry matter digestibility: For the determination of the *in vitro* dry matter digestibility two different techniques were followed, the one proposed by Tilley and Terry (1963) and that suggested by

Goering and Van Soest (1970). Both techniques were carried out separately. Four Merino sheep housed in individual cages, fitted with a rumen fistula and fed 1 kg alfalfa hay daily provided rumen fluid for *in vitro* incubations. A sample of rumen contents 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 a CO_2 atmosphere. A culture medium containing macro- and micro-mineral solutions, bicarbonate buffer solution and resazurin 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 (250 mg) were weighed out into polyester bags (two bags per sample), which were sealed and placed in incubation jars (one bag of each sample in each jar), 5 l glass recipients with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases. Then the buffered rumen fluid was anaerobically transferred into the incubation jars (2 l per jar). The jars were placed in a revolving incubator (DAISY, ANKOM) at 39ºC, with continuous rotation to facilitate the effective immersion of the bags in the rumen fluid. For the determination of the *in vitro* digestibility according to Tilley and Terry (1963), the incubation in buffered rumen fluid for 48 h was followed by a 48 h pepsin-HCl digestion after which bags were washed in distilled water, dried and weighed out. For the other technique, the 48 h incubation in diluted rumen fluid was followed by an extraction in a neutral detergent solution at 100ºC for 1 h as described by Goering and Van Soest (1970). *In vitro* digestibility by the Tilley and Terry (1963) can be considered a measure of apparent *in vitro* digestibility (AIVD), whereas values obtained with the other technique would represent true *in vitro* digestibility (TIVD).

Incubations of samples were performed in two (Tilley and Terry, 1963) and three (Goering and Van Soest, 1970) runs carried out in different weeks.

In order to detect seasonal effects on the *in vitro* dry matter digestibility, data obtained in both techniques were submitted to analysis of variance (Steel and Torrie, 1981).

Results and discussion

The fibre composition and CP contents varied widely between morphological parts (leaves or stems) and with maturity stage (Table 1). For both species, the CP content was higher in leaves than in stems and the difference increased with maturity stage, what is in agreement with results reported by Lambert *et al*. (1989b). The highest CP concentration was in spring, during initial leaf growth and stem elongation. At these initial stages of development, growth is accompanied by a high mitotic activity due to cellular growth and a strong demand for nutrients, in particular N (Ryan and Bormann, 1982). Thereafter, the quantity of this element decreased, particularly during the autumn. A similar pattern of variation in CP content in relation to the season was also reported by other researchers (Papachristou and Papanastis, 1994; Gonzalez-Andrés and Ortiz, 1996). Irrespective of the seasonal variations and the morphological part, the levels of CP observed for all samples and growing seasons were high, similar to those normally detected in grazing species. However, it is noteworthy that the utilisation of N from browse species may be not as high as expected (Shayo and Udén, 1999).

As expected, NDF, ADF and lignin contents showed an opposite trend to that observed by CP (Table 1). Similar results were reported by many researchers (Papachristou *et al*., 1999; Khanal and Subba, 2001) as usually the progress in maturity is reflected in a pronounced development of the supporting tissues and a slower relative growth rate of the metabolic tissues, resulting in increased contents of all the cell wall components with a decrease of the CP content. The negative relationship observed between cell wall and crude protein contents of many browse species was reported previously by Ammar (2002). For both species, the large increase in cell wall contents was more pronounced in the stems than in the leaves. Similar results were reported by Lambert *et al*. (1989b). The lignin content was relatively high especially in the stems and for the last maturity stages (>12%). The lignin content tended to be greater in *G. florida* than in *C. scoparius*. Moreover, the lignin content increased with maturity at a faster rate than other cell wall fractions. It is well established that a low content of poorly digestible cell wall components (ADF and ADL) and a high CP content are indicators of a good forage quality (Van Soest, 1994). Therefore, at the light of our results, leaves have a higher nutritive value than stems. The decline in nutritive value with maturity occurs at a faster rate in stems than in leaves.

Table 1. Seasonal variation of the chemical composition (g/kg of DM) of leaves and stems of both legume species

Species		Leaves			Stems		
		Spring	Summer	Autumn	Spring	Summer	Autumn
C. scoparius	CP	277	221	171	242	191	145
	NDF	220	280	288	267	487	561
	ADF	150	147	145	159	324	375
	ADL	28	53	54	39	98	133
G. florida	CP	282	258	172	232	153	126
	NDF	464	504	413	442	639	655
	ADF	250	254	260	289	436	475
	ADL	85	124	128	112	157	181

As expected, TIVD was always higher than AIVD, because the neutral detergent is able to extract most of the microbial matter from the incubation residue. *In vitro* dry matter digestibility (both AIVD and TIVD) was highest at early maturity stages and tended to decrease as growing season progressed (Table 2). This trend was consistent for leaves and stems. The decline in digestibility observed from spring to autumn was linear, except for leaves of *C. scoparius*. This is expected because with advancing maturity the proportion of structural carbohydrates in the plant increases, while the cell contents and digestibility decrease. Again the decline in digestibility along the growing season was more pronounced in stems than in leaves of both legume species. In the case of TIVD, this decrease accounted for up to 24% in stems of both species and up to 1% and 12% for leaves of *C. scoparius* and *G. florida*, respectively.

Table 2. Seasonal variation of the *in vitro* dry matter digestibility (g/kg DM) of shrub leaves and stems species

Sample	Season	AIVD (g/kg DM)		TIVD (g/kg DM)	
		C. scoparius	*G. florida*	*C. scoparius*	*G. florida*
Leaves	Spring	836	858 a	883	889 a
	Summer	845	789 b	867	843 b
	Autumn	819	729 c	876	786 c
	sed	11.3	17.3	13.5	13.8
Stems	Spring	863 a	746 a	901 a	778 a
	Summer	729 b	640 b	767 b	665 b
	Autumn	609 c	589 c	688 c	591 c
	sed	18.6	14.3	27.2	25.9

For the same parameter and within the same column, values with different letters are statistically different ($P<0.05$).

Irrespective of the maturity stage, leaves were always more digestible than stems, in agreement with Lambert *et al.* (1989a). It is well accepted that forage degradation in the rumen is mainly affected by the cell wall content and its lignification, as lignin is an indigestible fraction and acts as a barrier limiting the access of microbial enzymes to the structural polysaccharides of the cell wall. Ammar (2002) reported that NDF, ADF and lignin were significant and negatively correlated with *in vitro* digestibility.

Conclusions

The chemical composition of these species should not be the only point to assess their relative importance as ruminant feeds, and other parameters, in particular palatability, need to be considered. Anyway, the foliage of both legume shrubs showed high CP contents, sufficient to be considered as high protein forages that can be used as supplements for low quality roughage's. Both browse species could also have a satisfactory energy value, considering their high DM digestibility. The CP content and digestibility decline with maturity, especially in the stems. As compared with stems, leaves had a higher nutritive value and were less affected by seasonal variations.

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