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Chemical composition and digestibility of some browse plant species collected from Algerian arid rangelands

S. Boufennara[1, 2], S. Lopez[1, 3, *], H. Bousseboua[1], R. Bodas[3] and L. Bouazza[1, 2]

[1] *Departamento de Producción Animal, Universidad de Léon, E-24007 León, Spain*
[2] *Département de Microbiologie Appliquée, BP 360. Route de Ain El-Bey, Université Mentouri de Constantine, Algeria*
[3] *Instituto de Ganadería de Montaña (CSIC-ULE), E-24346 Grulleros (León), Spain*

Abstract

Many wild browse and bush species are undervalued mainly because of insufficient knowledge about their potential feeding value. The objective was to evaluate some nutritional attributes of various Algerian browse and shub species (*Atriplex halimus, Artemisia campestris, Artemisia herba-alba, Astragalus gombiformis, Calobota saharae, Retama raetam, Stipagrostis pungens, Lygeum spartum and Stipa tenacissima*). Chemical composition, phenols and tannins concentration, *in vitro* digestibility, *in vitro* gas production kinetics and *in vitro* bio-assay for assessment of tannins using buffered rumen fluid, and *in situ* disappearence of the edible parts of the plants (leaves, thin twigs and flowers) were determined. In general, protein content in dicotyledon species was always greater than in monocotyledon grasses, these showing higher neutral and acid detergent fibre and lower lignin contents than dicots. The tannin concentrations varied considerably between species, but in general the plants investigated in this study had low tannin contents (except for *Artemisia* spp. and *S. tenacissima*). Monocots showed lower *in vitro* and *in situ* digestibilities, fermentation rate, cumulative gas production and extent of degradation than dicot species. The plants were clustered by principal components analysis in two groups: poor-quality grasses and the most digestible dicot species. Chemical composition (neutral detergent fibre and protein) and digestibility were the main influential variables determining the ranking. In conclusion, *A. halimus, A. campestris, A. herba-alba* and *A. gombiformis* can be considered of greater nutritional value than the highly fibrous and low digestible grasses (*S. pungens, L. spartum* and *S. tenacissima*) that should be considered emergency roughages.

Additional key words: chemical composition; forage; gas production; *in vitro* digestibility; tannins.

Resumen

Composición química y digestibilidad de varias especies arbustivas características de pastizales en zonas áridas de Argelia

El objetivo de este trabajo fue el de evaluar varias especies arbustivas de Argelia (*Atriplex halimus, Artemisia campestris, Artemisia herba-alba, Astragalus gombiformis, Calobota saharae, Retama raetam, Stipagrostis pungens, Lygeum spartum* y *Stipa tenacissima*). Se determinó la composición química, la concentración de fenoles y taninos, la producción de gas y digestibilidad *in vitro* y la degradabilidad *in situ* de la parte comestible del pasto arbustivo (hojas, tallos finos y flores). Los contenidos en proteína y lignina fueron superiores en las dicotiledóneas que en las monocotiledóneas, mientras que los contenidos en fibra fueron más elevados en las monocotiledóneas. La concentración en taninos fue variable entre especies y, excepto para *Artemisia* spp. y *S. tenacissima,* los contenidos de estos compuestos fueron exiguos en la mayoría de las especies. Las monocotiledóneas fueron menos digestibles, con menores valores de ritmo de fermentación, producción de gas y degradabilidad ruminal. A partir de un análisis de componentes principales se observaron dos agrupaciones de las plantas: en un grupo las monocotiledóneas de baja calidad nutritiva y en otro grupo las dicotiledóneas más digestibles. Este agrupamiento fue determinado fundamentalmente por la composición química (fibra y proteína) y la digestibilidad. En conclusión, *A. halimus, A. campestris, A. herba-alba* y *A. gombiformis* pueden ser consideradas de mejor calidad (considerando su composición y digestibilidad), mientras que *S. pungens, L. spartum* y *S. tenacissima* podrían considerarse como recursos de baja calidad que sólo serían utilizados cuando no hay disponibilidad de otros alimentos.

Palabras clave adicionales: composición química; digestibilidad *in vitro;* forrajes; producción de gas; taninos.

* Corresponding author: s.lopez@unileon.es
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Introduction

The problem of animal feed supply and quality is aggravated in arid, semi-arid and tropical regions with scarce and erratic rainfall that limits the growth of herbaceous species and biomass yield in rangelands. Thus, livestock in such regions have to survive on recurrent shortage of feed resources of insufficient nutritional value for most part of the year (Robles *et al*., 2008). As an example, the crude protein (CP) content of herbaceous rangeland vegetation declines drastically during the dry season in semi arid regions, leading to prolonged periods of under nutrition of livestock raised under such adverse environmental conditions (Yayneshet *et al.,* 2009). In addition, uncontrolled and excessive use of increasingly scarce communal grazing areas on dry rangelands has contributed to their degradation, reducing further the availability of livestock feed resources. Considering all these aspects related to livestock feeding in dry areas, there is an increasing interest in the rational utilization of potential livestock feed resources such as browse species that are adapted to these environments (Robles *et al*., 2008). Indigenous browse species are useful sources of animal feeds, as these plants remain green during the dry season and provide vegetation with better nutritive value than other annual grass and herbaceous species that become withered (Aregawi *et al.,* 2008). Indigenous shrub and bush species have also a potential to prevent desertification, mitigating the effects of droughts, allowing soil fixation and enhancing the restoration of the vegetation and the recuperation of rangelands (Robles *et al*., 2008).

Rangelands in Algeria represent two-thirds of the total land widespread, mainly in the arid regions, where browse species can be decisive for grazing ruminants in periods of feed scarcity. However, in spite of their abundance, many wild browse and bush species have been generally undervalued mainly because of insufficient knowledge about their potential feeding value. Some of the ligneous species studied herein are consumed to a certain degree by small ruminants grazing in Mediterranean rangelands (Kababya *et al.*, 1998). These forage species may be complementary to the annual herbaceous vegetation as fodder from these species is available at different times of the year, considering their phenology and growth season (Delillis & Fontanella, 1992). Although these resources gain increasing significance as the nutritional value of grass drops, they never reach a prominent place in the diet, because their low CP content and high fibre contents and low digestibility (Wilson, 1977; Cabiddu *et al*., 2000). Moreover, some of these plants contain anti-nutritional secondary compounds (phenolics, tannins) with potential adverse effects such as inhibition of rumen microbial fermentation, as well as decreased feed digestibility and animal performance (Min *et al*., 2003; Waghorn & McNabb, 2003; Mueller-Harvey, 2006). In spite of their limited nutritional value, these forage resources are indispensable as feeds for herbivores when production systems are based on grazing rangelands (Papanastasis *et al.*, 2008).

The objective of this work was to evaluate various browse and shrub species collected from an arid zone in Algeria, based on the determination of their chemical composition, *in vitro* digestibility, *in situ* disappearance and tannin concentration in the edible part of the plants, considered as useful indicators for the preliminary evaluation of previously uninvestigated feeding resources.

Material and methods

Description of the study site

The study was conducted in Bou Saâda district, north central Algeria (35° 15.768' N, 04° 13.885' E), in the Saharan Atlas region, at the northern edge of the Sahara Desert between the Atlas Mountains and the el-Hodna depression and salt lake. The area is an arid high plateau (496-981 m altitude) with steppe like plains and extensive barren soils. According to the Köppen classification, the climate of this region is *BWh* (dry desert climate), characterized by high temperatures ranging between 24 and 41°C, and scarce and erratic annual precipitations for a total of 350-700 mm. Under these environmental conditions, the plant species studied show a slow vegetative growth and phenological

Abbreviations used: A (asymptotic gas production); ADF (acid detergent fibre expressed with residual ash); ADL (acid detergent lignin); *c (*fractional rate of gas production); CP (crude protein); DM (dry matter); D144 (dry matter disappearance after 144 h of incubation); ED (extent of degradation); G24 (cumulative gas production at 24 h); IVD-TT (*in vitro* digestibility of Tilley & Terry); ivDMloss (*in vitro* dry matter loss); *L* (lag time); NDF (neutral detergent fibre expressed with residual ash); PCA (principal components analysis); PEG (polyethylene glycol); TIVD (true *in vitro* digestibility); TCT (total condensed tannins); TEP (total extractable phenols); TET (total extractable tannins).

development throughout most of the year, often lagged in response to the infrequent major rainfalls.

Forage and roughage material

Nine browse plant species were used in this study: six dicotyledon plants namely *Atriplex halimus* L., *Artemisia campestris* L., *Artemisia herba-alba* Asso, *Astragalus gombiformis* Pomel, *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E. van Wyk (formerly *Genista saharae* or *Spartidium saharae*), and *Retama raetam* (Forssk.) Webb & Berthel, and three monocotyledon plants namely *Stipagrostis pungens* (Desf.) De Winter (formerly *Aristida pungens*), *Lygeum spartum* Loefl. ex L. and *Stipa tenacissima* L. Selection of the species was based on the available information on their consumption by grazing small ruminants, and on their relative abundance in the area of study. Samples were collected in June 2009, when plants were at a flowering (*A. halimus*, *A. gombiformis*, *R. raetam* and *L. spartum*) or at a mature stage (the rest of species). Sampling was during the dry season, because this is the time of the year when these plants may be more important for grazing. Between six and ten specimens of each plant species were sampled to obtain a representative aliquot of the edible biomass. Leaves, thin twigs (young stems) and some flowers (when existing) were clipped with scissors from the aerial part of the plants, and taken inmediately to the laboratory where the samples from the different specimens were pooled, oven-dried at 50°C (Makkar, 2003), and subsequently ground to pass a 1 mm screen.

Chemical analysis

Dry matter (DM, method ID 934.01), ash (method ID 942.05) and CP (method ID 954.01) contents were determined following the methods of AOAC (2000). Neutral and acid detergent fibre (NDF and ADF, respectively) and sulphuric acid detergent lignin (ADL) were determined with the ANKOM fibre analyser as described by Van Soest *et al*. (1991). Sodium sulphite, but not α-amylase, was added to the solution for the NDF determination. Both fibre fractions were expressed including residual ash.

Phenolic compounds were extracted following the procedures described by Makkar (2003). Total extractable phenols (TEP) were determined according to the method of Julkunen-Tiitto (1985) using the Folin-Ciolateau reagent and tannic acid as standard. Total extractable tannins (TET) were estimated indirectly after adsorption of TEP to insoluble polyvinylpyrrolidone, and measuring the remaining total phenols (or non-precipitable phenols) in the supernatant (Makkar *et al*., 1993). Concentration of TET was calculated through subtraction as follows TET = TEP − non-precipitable phenols. Free condensed tannins were measured in the extract using the butanol-HCl assay (Porter *et al*., 1986), with the modifications of Makkar (2003) and using purified quebracho tannin as standard. The bound condensed tannins were measured in the solid residue remaining after extraction of phenolic compounds. Concentration of total condensed tannins (TCT) was calculated as follows: TCT = free condensed tannins + bound condensed tannins. Concentration of phenols and tannins were expressed in g tannic acid equivalent kg^{-1} DM, whereas the concentration of condensed tannins was expressed in g quebracho equivalent kg^{-1} DM. All chemical analyses were performed in triplicate.

Animals and rumen fluid extraction for *in vitro* and *in situ* studies

Four mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula (60 mm diameter) were used for the extraction of rumen fluid or for *in situ* incubation of nylon bags. Animals were fed with lucerne hay *ad libitum* (167 g CP, 502 g NDF, 355 g ADF and 71 g ADL kg^{-1} DM) and had free access to water and mineral/vitamin block. Samples of rumen contents were withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory, where rumen fluid was strained through various layers of cheesecloth and kept at 39°C under a constant flow of CO_2.

In vitro digestibility

In vitro DM digestibility was determined using the ANKOM-DAISY procedure (Ammar *et al.*, 1999) following two different approaches, the proposed by Tilley & Terry (1963), and the one described by Van Soest *et al*. (1966). Both techniques were carried out separately in different incubations.

A culture medium containing macro- and micromineral solutions, a bicarbonate buffer solution and resazurin was prepared as described by Menke & Ste-

ingass (1988). The medium was maintained at 39°C and saturated with CO_2. Oxygen in the medium was removed by the addition of a reducing solution containing cysteine-HCl and sodium sulphide, as described by Van Soest *et al*. (1966). Rumen fluid was then added to the medium in the proportion 1:4 (v/v). Samples of plant material (400 mg) were weighed into artificial fibre bags (size 5 cm × 5 cm, pore size 20 µm) which were heat-sealed and placed in incubation jars (5 L glass recipients with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases). Each incubation jar was filled with 2 L of the buffered rumen fluid transferred anaerobically, and closed with the lid, mixing the contents thoroughly. The jars were then placed in a revolving incubator (Ankom Daisy II digestion system, ANKOM Technology Corp., Fairport, NY, USA) at 39°C, with continuous rotation to ensure the effective immersion of the bags in the rumen fluid. After 48 h of incubation in buffered rumen fluid, samples were dried to estimate *in vitro* DM loss after 48 h incubation. Then, bags used to measure *in vitro* digestibility following the original method of Tilley & Terry (1963) were subjected to a 48 h acid pepsin-HCl digestion, and the dry residue remaining in the bag was considered as the apparently indigestible DM to estimate *in vitro* digestibility (IVDTT). On the other hand, the other batch of bags were gently rinsed in cold water followed by an extraction with a neutral detergent solution at 100°C during 1 h as described by Van Soest *et al*. (1966). According to Van Soest (1994), the extraction with the neutral detergent removes bacterial cell walls and other endogenous products, and therefore can be considered as a determination of the true *in vitro* digestibility. With each procedure, each browse sample was incubated in tetraplicate, with one bag per sample incubated in each jar, and rumen fluid from each of the four sheep being incubated separately in each of the four jars.

In vitro gas production kinetics

Gas production profiles were obtained using an adaptation of the technique described by Theodorou *et al.* (1994). Ground samples (500 mg) were incubated in 50 mL of diluted rumen fluid (10 mL mixed rumen fluid + 40 mL medium prepared under a CO_2 constant flow) in 120 mL serum bottles. Six bottles containing only diluted rumen fluid were incubated as blanks and used to compensate for gas production in the absence of substrate. Once filled up, all the bottles were closed with rubber stoppers, crimped with aluminium seals, shaken and placed in an incubator to controlled temperature 39°C. Volume of gas produced was recorded at several incubation times (3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 72, 96, 120 and 144 h after inoculation time) using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). At the end of the incubation (after 144 h), the contents of each serum bottle were filtered using sintered glass crucibles (coarse porosity no. 1, pore size 100-160 µm) under vacuum. Then the residue was washed out with a neutral detergent solution at 100°C during 1 h and oven-dried at 100°C for 48 h to estimate the potential DM disappearance (g g^{-1} DM). Incubations were performed using three different inocula (rumen fluid from three sheep used separately) with two bottles per rumen fluid inoculum (for a total of six observations —three replicates— per sample). In order to estimate the fermentation kinetic parameters, gas production data were fitted using the exponential model proposed by France *et al.* (2000):

$$G = A\left[1 - e^{-c(t-L)}\right] \text{ for } t \geq L,$$

where G (mL g^{-1}) denotes the cumulative gas production at time t; A (mL g^{-1}) is the asymptotic gas production; c (h^{-1}) is the fractional rate of substrate fermentation and L (h) is the lag time. Volume of gas (mL g^{-1} DM) produced after 24 h of incubation was used as an index of digestibility and energy feed value, as suggested by Menke & Steingass (1988). According to France *et al.* (2000), the extent of degradation in the rumen (ED, g g^{-1}DM) for a given rate of passage (k, h^{-1}) was estimated as:

$$ED = \frac{c \times D144}{c + k} e^{-kL},$$

where $D144$ is the DM disappearance after 144 hours of incubation. To calculate ED, a rate of passage of 0.03 h^{-1} (characteristic for sheep fed with forage diet at maintenance level) was used.

Polyethilenglycol (PEG) bio-assay for the assessment of tannins

The gas production technique described above was also used for this biological assay. Incubations were carried out in serum bottles with or without the addition of 500 mg PEG. Ground samples (300 mg) were weighed out into serum bottles, kept at approximately

39°C and flushed with CO_2 before use. Two bottles were used for each substrate with each inoculum source (rumen fluid from three sheep was used separately as three different inocula giving three replicates per treatment), one for each treatment (with or without PEG). Bottles were tightly closed and placed in the incubator at 39°C, being shaken at regular times. The volume of gas produced in each bottle was recorded at 6, 12, 24 and 48 h after inoculation time, using a pressure transducer. Gas production was corrected by subtracting the volume of gas produced from blank cultures. Tannin activity was calculated as the ratio between cumulative gas measured in the PEG bottle and that recorded in the control (no PEG) bottle, for each sample and inoculum. For each sample, values from the three replicates (inoculum sources) were averaged.

In situ degradability

The procedure to measure *in situ* disappearance has been described in detail by López *et al*. (1991, 1999). *In situ* DM degradability in the rumen of each browse species was determined as the DM disappearance when samples (3 g DM) weighed in nylon bags (45 µm pore size and 7.5 × 15 cm size) were incubated for 24 and 96 h in the rumen of three fistulated Merino sheep fed with alfalfa hay (3 bags per sample and incubation time, one in each sheep). At the end of incubation, bags were removed from the rumen, rinsed with cold tap water and washed in a washing machine with cold water for 3 cycles of 3 min each. The washed bags were dried in a forced draft oven at 100°C for 48 h, and the residual DM used to calculate DM disappearance at each incubation time. Two bags per sample were washed following the same procedure without being previously incubated in the rumen to estimate DM disappearance at 0 h (estimate of DM solubility and particle loss from the bag).

Statistical analysis

One way analysis of variance (Steel & Torrie, 1980) was performed on *in vitro* digestibility, gas production fermentation kinetics and *in situ* degradability data, with browse species as the only source of variation (fixed effect) and source of inoculum (random effect) as a blocking factor. Tukey's multiple comparison test was used to determine which means differed from the rest ($p < 0.05$). Pearson linear correlation coefficients were determined pair-wise between the variables studied. Clustering of browse species from a multivariate analysis of all data recorded (chemical composition, tannin content and biological activity, *in vitro* digestibility, gas production fermentation kinetics and *in situ* degradability data), and assessment of the relative influence of each variable on that clustering were established by principal components analysis (PCA). Analysis of variance, correlation and PCA were performed using the procedures MIXED, CORR and FACTOR of the SAS software package (SAS Institute, 2008), respectively.

Results

Chemical composition of the plant material collected from the different species is shown in Table 1. The CP content of the plant species samples varied widely, being

Table 1. Chemical composition (g kg^{-1} dry matter) of Algerian forages

Plant family	Plant species	Organic matter	Crude protein	Neutral detergent fibre	Acid detergent fibre	Acid detergent lignin
Dicotyledons						
Chenopodiaceae	*Atriplex halimus*	805	153.6	360	181	59.9
Asteraceae	*Artemisia campestris*	898	115.0	330	212	97.5
	Artemisia herba-alba	920	123.9	378	258	101.1
Fabaceae - Leguminosae	*Astragalus gombiformis*	871	223.4	340	218	46.7
	Calobota saharae	955	109.8	574	427	135.2
	Retama raetam	956	108.7	623	445	199.5
Monocotyledons						
Poaceae - Gramineae	*Stipagrostis pungens*	945	95.2	771	425	58.3
	Lygeum spartum	936	72.7	801	535	62.5
	Stipa tenacissima	964	74.6	793	476	73.2

particularly high for *A. gombiformis* (223 g kg^{-1} DM) and low for the grasses *L. spartum* and *S. tenacissima* (73 and 75 g kg^{-1} DM, respectively). Protein content in dicotyledon species ranged widely from 109 to 223 g kg^{-1} DM and was always greater than in monocotyledon grasses. In general, monocots had higher NDF and ADF and lower lignin contents than dicots, although material collected from *C. saharae* and *R. raetam* had high NDF and lignin contents, whereas that from *A. halimus* and *A. gombiformis* showed low lignin contents.

Tannin composition of the plant species is presented in Table 2. The highest contents of TEP and TET were observed in the Asteraceae family (*Artemisia* spp.), whereas grasses, *A. halimus* and leguminous plants showed lower concentrations. TCT varied widely among species, being highest in *S. tenacissima* and lowest for *A. halimus*. Based on the results observed with the PEG bioassay, the species with highest tannin biological activity on gas production would be *A. campestris* and, to a lesser extent, *S. tenacissima* ($p < 0.001$), negligible for *S. pungens*, and it did not exist for the other species. Tannin values observed with the different techniques were significantly correlated, and TET were positively correlated with tannin biological activity ($r = 0.75$, $p = 0.020$ at 48 h incubation). There were no incubation time effect ($p = 0.455$) or a significant interaction ($p = 0.070$) between incubation time and plant species, thus effects of PEG on gas production (indicative of the presence of tannins) were similar at all incubation times.

In vitro digestibility and *in situ* DM disappearance were variable ($p < 0.05$) across the examined forages (Table 3). The lowest *in vitro* and *in situ* DM digestibilities were observed in monocotyledons (being particularly low for *S. tenacissima*), whereas dicots had significantly higher values. Similar trends were observed for the *in vitro* fermentation kinetics estimated form the gas production curves (Table 4). Although the monocotyledons showed higher asymptotic gas (parameter A) than dicots ($p < 0.05$), their fermentation rate (parameter c) was significantly lower ($p < 0.05$), resulting in lower gas production (at 24 h incubation) and ED for grasses than for dicot species.

The PCA based on data recorded on chemical and tannin composition, *in vitro* and *in situ* DM digestibility and fermentation kinetics, grouped the studied species in two clusters (Fig. 1). One of them included the grasses and the other one some of the dicots (*A. gombiformis* and *Artemisia* spp), with the leguminosae *C. saharae* and *R. raetam* in an intermediate position. The same analysis was used to examine the most influential variables on the observed clustering (Fig. 2). All variables, with the exception of lignin, were highly influential. Chemical composition (NDF and CP) and DM digestibility were the main variables determining the ranking of species on factor 1 (58.2% of variance accounted for), whereas the second factor (19.4% of variance accounted for) would be explained mainly by the content and activity (PEG bioassay) of tannins.

Table 2. Phenolic compounds (g kg^{-1} DM, standard equivalent) and tannin biological activity[a] of Algerian forages

Plant family	Plant species	Total extractable phenols	Total extractable tannins	Free condensed tannins	Total condensed tannins	Tannin biological activity[a] at incubation times:			
						6 h	12 h	24 h	48 h
Dicotyledons									
Chenopodiaceae	*Atriplex halimus*	16.1	8.4	42.1	69.1	0.85	0.93	0.97	0.97
Asteraceae	*Artemisia campestris*	84.3	57.1	62.7	114.3	1.40	1.36	1.27	1.23
	Artemisia herba-alba	63.7	36.4	80.6	118.8	0.84	0.92	0.97	0.99
Fabaceae - Leguminosae	*Astragalus gombiformis*	13.5	3.0	51.6	78.3	0.93	0.95	0.94	0.95
	Calobota saharae	29.8	9.6	76.4	109.7	0.90	0.94	0.95	0.96
	Retama raetam	8.5	2.0	40.5	77.9	0.92	0.99	1.01	1.01
Monocotyledons									
Poaceae - Gramineae	*Stipagrostis pungens*	10.2	4.8	46.5	78.7	1.09	1.08	1.05	1.03
	Lygeum spartum	35.6	11.1	77.2	102.4	1.00	1.00	1.01	1.01
	Stipa tenacissima	12.2	3.8	165.5	213.9	1.16	1.13	1.07	1.04

[a] Tannin biological activity as the ratio between gas production measured at different incubation times adding PEG *vs.* control (*i.e.*, gas PEG / gas control).

Table 3. *In vitro* dry matter (g g^{-1} DM) digestibility and *in situ* dry matter disappearance (g g^{-1} DM) at different incubation times of Algerian forages

Plant family	Plant species	ivDMloss[1]	IVD-TT[2]	TIVD[3]	*In situ* DM disappearance after incubation times:		
					0 h	24 h	96 h
Dicotyledons							
Chenopodiaceae	*Atriplex halimus*	0.542^{ab}	0.756^{a}	0.755^{a}	0.404^{b}	0.716^{a}	0.804^{a}
Asteraceae	*Artemisia campestris*	0.550^{a}	0.741^{a}	0.731^{a}	0.366^{c}	0.633^{b}	0.789^{a}
	Artemisia herba-alba	0.539^{ab}	0.660^{b}	0.686^{a}	0.331^{d}	0.505^{c}	0.634^{b}
Fabaceae - Leguminosae	*Astragalus gombiformis*	0.519^{b}	0.742^{a}	0.755^{a}	0.495^{a}	0.675^{ab}	0.849^{a}
	Calobota saharae	0.444^{c}	0.560^{c}	0.550^{b}	0.265^{e}	0.479^{c}	0.523^{cd}
	Retama raetam	0.517^{b}	0.580^{c}	0.595^{b}	0.195^{f}	0.369^{d}	0.442^{d}
Monocotyledons							
Poaceae - Gramineae	*Stipagrostis pungens*	0.349^{d}	0.458^{d}	0.455^{c}	0.135^{g}	0.305^{d}	0.528^{c}
	Lygeum spartum	0.359^{d}	0.449^{d}	0.451^{c}	0.265^{e}	0.506^{c}	0.614^{b}
	Stipa tenacissima	0.252^{e}	0.269^{e}	0.341^{d}	0.117^{g}	0.198^{e}	0.328^{e}
SEM[4]		0.0152	0.0153	0.0154	0.0063	0.0142	0.0170

[1] ivDMloss: *in vitro* dry matter loss; [2] IVD-TT: *in vitro* digestibility of Tilley & Terry; [3] TIVD: true *in vitro* digestibility; [4] Standard error of the mean [a, b, c, d, e, f, g] Means in a column with different superscripts are significantly different ($p < 0.05$).

Discussion

In these arid areas small domestic ruminants have to resort more and more to natural standing shrubs, forbs and ligneous grasses as the only forage resources available during the dry season. The CP content of the browse species studied herein is higher than the minimum level of 7-8% DM required for optimum rumen function and feed intake in ruminant livestock (Van Soest, 1994). According to Paterson *et al.* (1996), feedstuffs with a CP content lower than 70 mg g^{-1} DM require a supplementation of nitrogen to improve their ingestion and digestion by the ruminants. Our CP values are similar to those reported for other Mediterranean shrubs (Cabiddu *et al.*, 2000; Frutos *et al.*, 2002; Ammar *et al.*, 2004a,b; Arhab *et al.*, 2009). The low CP contents in *L. spartum* and *S. tenacissima* can be probably due to high proportions of mature leaves and twigs in the samples. Protein was lower in these grasses than in the dicots. Leguminous forages and trees

Table 4. *In vitro* fermentation kinetics (estimated from gas production curves) of Algerian forages

Plant family	Plant species	A[1] (mL g^{-1} DM)	*c*[2] (h^{-1})	G24[3] (mL g^{-1} DM)	D144[4] (g g^{-1} DM)	ED[5] (g g^{-1} DM)
Dicotyledons						
Chenopodiaceae	*Atriplex halimus*	174^{c}	0.0412^{b}	103.6^{c}	0.835^{bc}	0.452^{b}
Asteraceae	*Artemisia campestris*	226^{abc}	0.0784^{a}	188.2^{a}	0.894^{a}	0.623^{a}
	Artemisia herba-alba	208^{bc}	0.0818^{a}	175.4^{a}	0.822^{c}	0.578^{a}
Fabaceae - Leguminosae	*Astragalus gombiformis*	206^{bc}	0.0760^{a}	171.6^{a}	0.874^{ab}	0.620^{a}
	Calobota saharae	207^{bc}	0.0472^{b}	139.1^{b}	0.666^{de}	0.401^{b}
	Retama raetam	226^{abc}	0.0391^{b}	132.9^{b}	0.707^{d}	0.385^{b}
Monocotyledons						
Poaceae - Gramineae	*Stipagrostis pungens*	295^{a}	0.0169^{c}	76.5^{d}	0.634^{e}	0.190^{c}
	Lygeum spartum	277^{ab}	0.0154^{c}	74.7^{d}	0.550^{f}	0.172^{c}
	Stipa tenacissima	253^{abc}	0.0118^{c}	56.1^{d}	0.469^{g}	0.126^{c}
SEM[6]		16.8	0.00247	5.24	0.0087	0.0135

[1] A: asymptotic gas production, [2] c: fractional rate of fermentation; [3] G24: gas production at 24 h of incubation; [4] D144: DM disappearance after 144 h of incubation; [5] ED: extent of degradation for a fractional passage rate of 0.03 h^{-1}; [6] Standard error of the mean[: a, b, c, d, e, f, g] Means in a column with different superscripts are significantly different ($p < 0.05$).

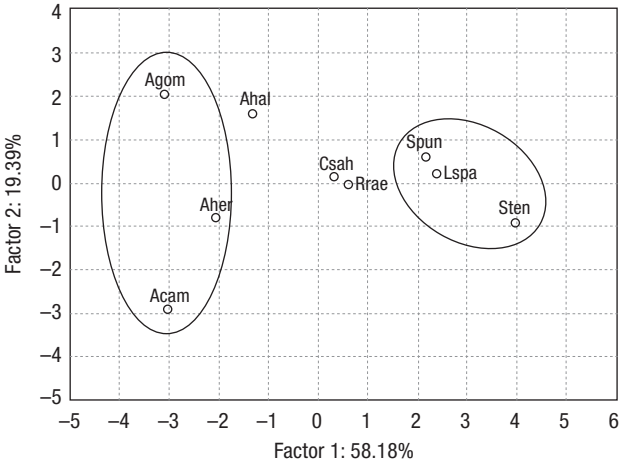

Figure 1. Plant species discrimination on the basis of principal component analysis performed on data recorded on chemical and tannin composition, *in vitro* and *in situ* digestibility and fermentation kinetics (Ahal = *Atriplex halimus*, Acam = *Artemisia campestris*, Aher = *Artemisia herba-alba*, Agom = *Astragalus gombiformis*, Csah = *Calobota saharae*, Rrae = *Retama raetam*, Spun = *Stipagrostis pungens*, Lspa = *Lygeum spartum* and Sten = *Stipa tenacissima)*.

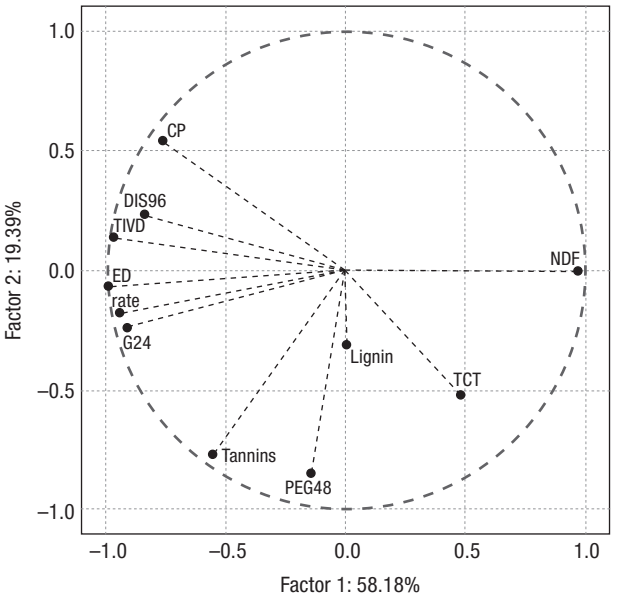

Figure 2. Discrimination of the most influential variables on the observed clustering on the basis of principal component analysis performed on data recorded on chemical and tannin composition, *in vitro* and *in situ* digestibility and fermentation kinetics (CP = crude protein; DIS 96 = *in situ* DM disappearance after 96 h incubation; ED = extent of degradation; G24 = cumulative gas production at 24 h; Lignin = acid detergent lignin; NDF = neutral detergent fibre; PEG48 = tannin biological activity at 48 h incubation; rate = fractional rate of gas production; TIVD = true *in vitro* digestibility; TCT = total condensed tannins; Tannins = total extractable tannins).

have been used as a basic feed animal supply in many regions of the world, mainly because of their high protein contents (Norton, 1994; Tolera *et al.*, 1997; Ammar *et al.*, 2004b) throughout the year.

Based on their chemical composition, these feedstuffs could be classified as highly fibrous, as all forages showed high fibre (NDF and ADF) and lignin contents, particularly the grasses. The high level of fibre content in some of the forage species could be explained partly by the environmental conditions prevailing in the area of Bou Saâda, as high temperatures and low precipitations tend to increase the cell wall fraction and to decrease the soluble contents of the plants (Pascual *et al.*, 2000). Our values are similar to those reported for other browse forages (Larbi *et al.*, 1998; Ammar *et al.*, 2005; Gasmi-Boubaker *et al.*, 2005; Salem *et al.*, 2006), with some differences among all studies, probably because of the different proportions of foliage and twigs in the samples and the different phenological stage of the plants at sampling (our plants were collected at a mature stage).

There was a considerable variation between species in the tannin concentrations in the material collected. The analysis of specific tannins gives an indication of the presence of some anti-nutritive factors. Except for some few species (*Artemisia* spp. and *S. tenacissima*), the plants investigated in this study had low tannin contents (in particular *R. raetam, A. halimus* and *S. pungens*), which would be of little significance in their effects on digestion of nutrients by ruminants. With high protein content and low fibre and tannin contents, some of the leguminous species could be regarded as with a potentially high nutritive value. It is pertinent to mention that a high proportion of TCT was recovered as free condensed tannins that may be responsible for the possible detrimental effects of condensed tannins on microbial fermentation of nutrients in the rumen. There was also a considerable variation between the different methods of analysis in the ranking of plant species according to their tannin concentrations. This variation is expected since the chemical properties that are involved in the activity of polyphenols determined by the different methods are widely different. The colorimetric methods should be used with caution as a quantitative assay. Nevertheless, the strong correlations between the different methods of analysis and the values observed with the PEG bioassay support the idea that *in vitro* gas production technique coupled with the use of PEG is a useful tool for screening the potential effects of tannins in shrub species due to its simplicity

and accuracy. The percentage increase in gas production as result of the blocking effect of PEG on tannins would be an indicator of the biological activity of tannins on rumen microbial fermentation. Indeed, a significant increase in *in vitro* gas production when material was incubated in the presence of PEG has been reported for different tanniferous substrates (Barber *et al.,* 1990; Canbolat *et al.*, 2005; Rubanza *et al.,* 2005; Singh *et al.,* 2005). Based on this analysis, the species with a highest tannin anti-nutritional activity would be *A. campestris* followed by *S. tenacissima*. The addition of PEG was not always accompanied by an increase in gas volume. The extent of positive or negative effect of tannin in the shrubs would vary depending not only on the level of tannins in plants but also on their type and their biological activity (Hagerman *et al*., 1992; Barry & McNabb, 1999).

Digestibility of the forages browse samples was determined by two conventional and extensively used *in vitro* techniques (Tilley & Terry, 1963; Van Soest *et al.*, 1966), and also assessed from the *in situ* DM disappearance when samples were incubated in the rumen. The ED and degradation rate were estimated from gas production profiles derived from measurement of fermentation gas produced when the material was incubated *in vitro* (Theodorou *et al.*, 1994). With some differences among techniques in the values estimated, all techniques resulted in similar ranking and grouping of forages. With all the digestibility measures it was observed a large variability among species, with a clear differentiation between the most digestible species (*A. halimus*, *A. campestris*, and *A. gombiformis*) and those showing the lowest digestibility coefficients (grass species). These differences among browse species in digestibility may be partly attributed to the variations in chemical composition (mainly cell wall content and composition). *In vitro* DM loss showed significant negative correlations with NDF ($r = -0.92$; $p < 0.001$) and ADF ($r = -0.81$; $p = 0.008$), whereas free condensed tannins was negatively correlated with IVD-TT ($r = -0.71$; $p = 0.033$). The rate of degradation c (h^{-1}) was correlated with NDF ($r = -0.91$; $p < 0.001$) and ADF ($r = -0.79$; $p = 0.012$).

The PCA clustered the studied species in two groups considering jointly all the measures of chemical composition and digestibility: one including the poor-quality grasses and another one including the most digestible dicot species (*A. gombiformis* and *Artemisia* spp.). Grasses examined in the present study were, at the time plants were sampled, highly fibrous and with low CP content and digestibility. On the contrary, *A. gombiformis* and *Artemisia* spp. were the most digestible species and with highest CP content, showing an interesting potential as fodder resources for small ruminants during this time of the year. The leguminosae species *C. saharae* and *R. raetam* were intermediate between both clusters, with higher NDF and lower digestibility than the dicot species included in the high-quality group. The shrub *A. halimus* was close to the group of most digestible dicots, but differed slightly probably due to the lower gas production volumes observed with this plant species. With high digestibility and low gas production, fermentation efficiency (mg digestible DM mL^{-1} gas) would be greater with this shrub species than with any other species. Therefore, *A. halimus* should be considered of high potential for range ruminants browsing shrubs and trees when grazing herbaceous material becomes insufficient to match their nutritional requirements. According to the position of the variables in the factors derived from the PCA, which explained a total of 77.6% of the variance, chemical composition (NDF and CP) and digestibility were the main variables determining the ranking on species on factor 1. The position of the variables on one edge of this axis confirms that all digestibility variables were strongly and positively correlated to each other and also to CP, with high and negative correlations of all these variables with NDF (positioned on the opposite end of the axis). The second factor would be defined mainly by the content and activity (PEG bioassay) in tannins, having a considerable impact on the classification of browse species according to their nutritional quality.

As final conclusions, all the chemical, *in vitro* and *in situ* measurements are useful tools in initial screening studies to rank the forages according to their nutritive quality. A significant variation in *in vitro, in situ* digestibility and fermentations kinetics were observed among the samples. Part of this variation was associated with crude protein and cell wall contents. The moderate to high CP and low fibre content, along with high *in vitro* digestibility and *in situ* DM disappearance found in *A. halimus***,** *A. campestris***,** *A. herba-alba* and *A. gombiformis* suggest that these plants could have a greater nutritional value than the highly fibrous and low digestible grasses (*S. pungens*, *L. spartum* and *S. tenacissima*) that should be considered emergency roughages of poor quality, to be used by the ruminants only when drought decreases grazing herbaceous biomass yield.

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