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Chemical Composition, *In Situ* Degradation and Fermentation Kinetics of Some Browse Plant Species Collected from Algerian Arid and Semi-Arid Areas

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Abstract. The chemical composition and digestibility of twelve plant samples (*Arthrocnemum macrostachyum*, *Atriplex canescens*, *Artemesia herba-alba*, *Astragalus gombo*, *Calobota saharae Ceratonia siliqua*, *Gleditsia triacanthos*, *Hedysarum coronarium*, *Medicago sativa*, *Ononis natrix* L, *Hordeum vulgare* and *Stipa tenacissima* L.) grown in arid and semi-arid areas of Algeria were evaluated (in 2010). Feed components were determined by proximate analysis whereas phenolic and tannin compounds were analyzed by colorimetric procedures. Digestibility was assessed by conventional gravimetric *in vitro* and *in situ* methods. In general, crude protein content in dicotyledon (dicots) species was always greater than that in monocotyledon (monocots) grass showing higher Neutral and Acid Detergent Fiber (NDF and ADF) and lower lignin contents than dicots. The tannin concentration varied considerably between species, but in general, the plants investigated in this study had low tannin contents (except for *Ceratonia siliqua*, *Gleditsia triacanthos* and *Hedysarum coronarium*). Monocots showed lower *in vitro* and *in situ*, fermentation rate and cumulative gas production than dicots species. This study indicated that a large reserve of plant species in the local flora is available that could be potentially used for livestock feeding. These feeds, if fully exploited, could assist in increasing the level of production and productivity of the livestock resources in the region.

Key Words: Digestibility, In Situ degradation, Forage, *In vitro* gas production, Plant secondary compounds, Tannins

Introduction

Livestock, especially ruminants, are an important component of many farming systems of the arid and semi-arid regions in Algeria. They are valued for their meat, milk, manure and in many cases, sources of traction power. Traditionally, they graze natural pastures, fallow lands, crop re-growth and residues. However, the major constraint on the performance of grazing ruminants in these regions is the scarcity of high quality pastures. The situation is even worse during the dry season when the quality and quantity of the natural pastures decline, resulting in lower animal intakes of different natural pastures and reduced ruminant productivity (Teferedegne, 2000; Ammar *et al.*, 2004a).

As an example, the crude protein 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 (Medjekal *et al.,* 2015). In addition, uncontrolled and excessive use of increasingly scarce communal grazing areas on dry rangelands has contributed to their degradation, reducing 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 (Boufennara *et al.,* 2012).

Supplementation during the dry season is not a profitable practice due to high feeding costs (Benavides, 1994). A potential strategy for increasing the quality and availability of feeds for smallholder ruminant animals in the dry season may be the use of fodder trees and shrub forages (Pezo, 1991) in drought conditions. Browsing (shrubs and tree foliage) plays a significant role in providing fodder for ruminants in many parts of the World. Most browse species have the advantage of maintaining their greenness and nutritive value throughout the dry season (Bakhashwain *et al.,* 2010).

Despite their potential as feeds, most shrubs also contain large amounts of tannins, which have been evolved by plants as a defense mechanism against being consumed by herbivores. The presence of tannins in nutritionally important forage trees, shrubs, legumes, cereals and grains often limits their utilization as feedstuffs (Kumar and Vaithiyanathan, 1990). The anti-nutritive effects of tannins are associated with their ability to combine with dietary proteins, polymers such as cellulose, hemicellulose and pectin, and minerals retarding their digestion (McSweeney *et al.,* 2001). Tannin-binding agents such as Poly Ethylene Glycol (PEG) offer a viable technique to enhance the nutritive value of tannin-rich trees and shrubs (Nsahlai *et al.,* 2011).

The nutritive value of a ruminant feed is determined by the concentration of its chemical components as well as their rate and extent of digestion. Determining the digestibility of feeds *in vivo* is laborious and expensive, requires large quantities of feed and is largely unsuitable for single feedstuffs thereby making it unsuitable for routine feed evaluation. *In vitro* methods provide less expensive and more rapid alternatives. Both *in vitro* gas production and the ANKOM (Andy Komarek) devices can be used as rapid evaluation tools to assess nutritional quality of feeds (Khanal and Subba, 2001).

The *in situ* rumen dry matter disappearance technique (Orskov and McDonald, 1979; Orskov *et al.,* 1980) evaluates forages for their rate and extent of fermentation in the rumen. The simplicity of the technique and the appeal of giving information without resorting to highly technical and expensive laboratory procedures has led to its widespread use, especially in developing countries (Sileshi *et al.,* 1996).

Detailed investigation of these fodders is very important in order to identify the better ones in terms of nutrient content and

digestibility. This study was conducted with objective to evaluate various browse and shrub species collected from a semi-arid zone in Algeria based on the determination of their chemical composition, *in vitro* digestibility, *in situ* disappearance, and tannins concentration in the edible part of the plants, considered as useful indicators for the preliminary evaluation of some previously uninvestigated feeding resources.

Materials and Methods

Study Area

This experiment was conducted using plant samples collected from two Algerian locations: Mila (36°31′14″N and 06°15′40″ E, 289 m altitude) and Msila (35° 26′ 07 9″ N and 04° 20′ 52, 8″ E 398m altitude). Mila is in eastern Algeria in a semi-arid region with continental climate and erratic annual precipitation as 742 mm/year. M'sila is in north central Algeria in the Saharan Atlas region at the northern edge of Saharan Desert between the Atlas Mountains and the el-Hodna depression and salt lake. According to Köppen classification, the climate of this region is dry desert climate, characterized by high temperature ranging between 24 and 41°C, and scarce and erratic annual precipitations for a total of 100 and 250 mm/year (Le Houérou, 1995). This area is characterized by an ecological diversity represented by two principal ecosystems: steppe and forest ecosystems. M'Sila area presents very interesting natural vegetation: high altitude formations include *Cedrus atlantica* and others like *Pinus halepensis* and *Juniperus phoenicea* and low altitude formations contain *Artemisea herba-alba*. Of vocation primarily agro-pastoral, the principal activity of the population of rural areas is breeding sheep and caprine (Rebbas and Bounar, 2014).

Feed samples

Twelve plant samples were used in this study: ten dicots plants namely *Arthrocnemum macrostachyum* (*A. macrostachyum*), *Atriplex canescens* (*A. canescens*), *Artemisia herba-alba* Asso (*A. herba-alba*), *Astragalus gombo* Bunge (*A. gombo*), *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E.van Wyk (*C. saharae*), *Ceratonia siliqua* (*C. siliqua*), *Gleditsia triacanthos* (*G. triacanthos*), *Hedysarum coronarium* (*H. coronarium*), *Medicago sativa* (*M. sativa*) and *Ononis natrix* L (*O. natrix*), and two monocots plants namely *Hordeum vulgare* (*H. vulgare*) and *Stipa tenacissima* L. (*S. tenacissima*). Selection of the species was based on the available information (Boufennara *et al.*, 2012; Bouazza *et al.*, 2012) on their consumption by grazing small ruminants, and on their relative abundance. Samples were collected in June 2010 when plants were at flowering (*A. gombo* and *C. saharae*) or at a mature stage (final stage of biological function for the rest of the species). Sampling was conducted during the dry season because this is the time of the year when these plants may be more important for grazing. Pods (*C. siliqua* and *G. triacanthos* trees) were picked in November 2010 from the ground. Branches and twigs of several specimens of each species were clipped with scissors (total apical parts) and immediately taken to the laboratory where leaves, flowers and fruits (when available) were manually separated. Then, the twelve samples were immediately freeze-dried and milled in a hammer mill using a 1 mm sieve.

Chemical analysis

The freezing dried samples were ground in a Willey Mill to pass through 1mm sieve for the determination of chemical composition. Feed samples were analyzed for Dry Matter (DM) and following the method of AOAC (2000). Nitrogen was determined using the micro-Kjeldahl method (AOAC, 2000). Crude Protein (CP) was calculated as N x 6.25. The Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were analyzed according to

Van Soest *et al.* (1994) using the ANKOM Fiber Analyzer (ANKOM Technology, Fairport, NY). Both fiber fractions were expressed including residual ash.

Samples were also analyzed for phenolic compounds following the procedures described by Makkar (2003). For extraction and quantification of tannins, 10 mL of each solvent was inserted into a test tube containing 500 mg of the sample, then put into a beaker glass that was filled with water. The tube was then placed in an ultrasonic water bath (Barnstead/Lab Line Aqua Wave 9377, E60H, Germany) and extracted for 20 min at room temperature. Each sample was centrifuged at 4°C for 10 min; this procedure was repeated twice and the supernatants were combined. Total Extractable Phenols (TEP) were determined using the Folin-Ciolateau reagent and tannic acid as the 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, 2003). The concentration of TET was calculated by difference as:

$$\mathrm{TET} = \mathrm{TEP} - \text{non} - \text{precipitable phenols}$$

Free Condensed Tannins (FCT) were measured in the extract using the butanol-HCl assay with the modifications of Makkar (2003) and using purified quebracho tannins as the standard. The Bound Condensed Tannins (BCT) were measured in the solid residue remaining after extraction of phenolic compounds. The concentration of Total Condensed Tannins (TCT) was calculated as $\mathrm{TCT} = \mathrm{FCT} + \mathrm{BCT}$. The concentrations of phenols and tannins were expressed in g tannic acid equivalent/kg DM whereas the concentration of condensed tannins was expressed in g quebracho equivalent/kg DM. All chemical analyses were performed in triplicate.

In vitro studies

a) Fistulated donor animals and rumen fluid

Rumen fluid was obtained from four mature Merino sheep (body weight 49.04±4.23 kg) fitted with permanent rumen fistula (60 mm diameter) maintained in cages and fed lucerne hay *ad libitum* (CP 167 g, NDF 502 g, ADF 355 g and ADL 71 g/kg DM) and had free access to water and a mineral/vitamin block. A sample of rumen contents was withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory. Rumen fluid from the four sheep was mixed, strained through various layers of cheesecloth and kept at 39°C under a CO_2 atmosphere (Ammar *et al.*, 2004a).

b) Inoculum preparation

The rumen fluid was diluted (1:4 v/v) with a culture medium containing macro- and micro-mineral solutions, resazurin and a bicarbonate buffer solution and prepared as described by Menke and Steingass (1988). The medium was kept at 39°C 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 Van Soest *et al.* (1966).

c) *In vitro* gas production kinetics

The method used for gas production measurements was described by Theodorou *et al.* (1994). About 500 mg of each sample were incubated in 50 ml of diluted rumen fluid (10 ml mixed rumen fluid + 40 ml medium according to Menke and Steingass (1988) prepared under a CO_2 constant flow) in 120 ml serum bottles pre-warmed at 39°C and flushed with CO_2. Six bottles containing only diluted rumen fluid were incubated as blanks and used to compensate for gas production in the absence of substrate. All the bottles were crimped with aluminum caps and placed in the incubator at 39°C, being shaken at regular times. Volume of gas produced in

each bottle was recorded at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h after inoculation time using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona). 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). 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):

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

Where:

G (mLg^{-1}) denotes the cumulative gas production at time t;

A (mLg^{-1}) is the asymptotic gas production;

c ($/h^{-1}$) is the fractional rate of gas production and

L (h) is the lag time.

ME [MJ/kg DM] content of all samples was calculated using equation of Menke et al, (1979) as follows:

$$\text{ME [MJ/kg DM]} = 2.20 + 0.136\ \text{GP} + 0.057\ \text{CP}$$

Where:

GP = 24 h net gas production [ml/200 mg];

CP = Crude protein.

d) Polyethylene glycol (PEG) bioassay for the assessment of tannins activity

Tannin-binding agents such as polyethylene glycol (PEG) offer a viable technique to enhance the nutritive value of tannin-rich trees and shrubs. Polyethylene glycol is an inert unabsorbed molecule that can form stable complexes with tannins, preventing the binding between tannins and proteins and can even displace protein from a pre-formed tannin-protein complex (Makkar, 2003; Brown and Ng'ambi, 2017). Additional 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 sample with each inoculum source 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. 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.

e) *In vitro* Dry Matter Digestibility

The procedure followed was the *in vitro* filter bag method in ANKOM Daisy incubators (Ammar *et al.*, 1999). 400 mg were weighed into ANKOM F57 polyester/polyethylene bags (size 5 cm × 5 cm; pore size 25 μm) which were sealed with a heater and placed in incubation jars. Each jar was a 5-L glass recipient with a plastic lid provided with a single-way valve which avoids the accumulation of fermentation gases. A culture medium containing macro- and micro-mineral solutions, a bicarbonate buffer solution and resazurin was prepared as described by Menke and Steingass (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). 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 (ivDM loss). Then, bags used to measure *in vitro* digestibility following the original method of Tilley and 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 (IVD-TT). On the other hand, the other batches 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 can be considered as a determination of the True *In Vitro* Digestibility (TIVD). 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.

f) *In situ* Dry Matter Disappearance

The procedure to measure *in situ* disappearance has been described in detail by López *et al.* (1991). *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 (7.5 ×15 cm and 45 µm pore size) were inserted into the rumen of the sheep prior to the morning feeding. Bags were incubated in duplicate for 12, 24, and 96 h. At each incubation time, bags were removed from the rumen, immediately rinsed in cold 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. Zero-time washing losses were estimated by soaking 2 bags/ sample in water for 10 min, then washed following the same procedure without being previously incubated in the rumen and dried as before.

Statistical analysis

One-way analysis of variance 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 (r) were determined pairwise between the variables studied. Analysis of variance (PROC MIXED) and correlation (PROC CORR) were performed using the SAS software package (SAS, 2000).

Results

The crude protein content of the plant species samples varied widely, being particularly high for *A. gombo* and low for the grasses *S. tenacissima* and *H. vulgare*. Protein content in dicots species ranged from 68.4 to 222.4 g kg^{-1} DM and was always greater than that in monocotyledon grasses. In general, monocots had higher NDF and ADF and lower lignin contents than dicots. The lowest ADL content (43.6 g kg^{-1} DM) was found in *A. macrostachyum* and the highest (177.9 g kg^{-1} DM) in *C. siliqua*. Based on their chemical composition, these feedstuffs could be classified as highly fibrous, as all forages showed high fiber (NDF and ADF) and lignin contents, particularly the grasses (Table 1).

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

Plant family	Plant species	Organic matter	Crude protein	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin
Dicotyledons						
Chenopodiaceae	*A. macrostachyum*	787 ± 2.56	68.4 ± 0.55	297 ± 2.10	148 ± 2.30	43.6 ± 2.11
	A. canescens	836 ± 0.29	192.5 ± 0.62	314 ± 7.58	141 ± 4.47	57.3 ± 7.37
Asteraceae	*A. herba-alba*	809 ± 0.44	135.2 ± 0.94	457 ± 5.75	322 ± 2.84	101.1 ± 2.41
Fabaceae	*A. gombo*	817 ± 0.68	222.4 ± 0.46	414 ± 5.44	300 ± 12.13	82.2 ± 4.59
	C. saharae	840 ± 2.29	114.8 ± 0.19	555 ± 12.14	432 ± 6.86	139.3 ± 3.36
	C. siliqua	834 ± 2.05	71.9 ± 0.55	317 ± 2.31	284 ± 7.79	177.9 ± 0.81
	G. triacanthos	821 ± 1.04	116.6 ± 0.57	346 ± 8.09	190 ± 3.55	62.1 ± 1.83
	H. coronarium	820 ± 0.36	142 ± 0.58	539 ± 11.42	407 ± 8.09	154.9 ± 4.39
	M. Sativa	814 ± 1.24	154.8 ± 0.32	438 ± 22.85	301 ± 1.39	75.3 ± 4.41
	O. natrix L.	824 ± 1.85	99.9 ± 0.66	534 ± 13.01	430 ± 10.62	112.3 ± 0.38
Monocotyledons						
Poaceae	*H. vulgare*	834 ± 1.31	51.5 ± 0.24	667 ± 10.92	361 ± 7.29	49.9 ± 2.19
	S. tenacissima L.	813 ± 0.83	45.9 ± 0.13	780 ± 16.23	506 ± 15.54	73.9 ± 2.91

(Samples were collected in June 2010, when plants were at flowering (*A. gombo* and *C. saharae*) or at a mature stage (final stage of biological function for the rest of the species). Pods from (*C. siliqua* and *G. triacanthos* trees) were picked in November (2010) from the ground and *H. vulgare* was used as straw).

The Total extractable phenols (TEP) content varied widely from 3.02 g kg^{-1} DM g in *H. vulgare* to 85.30 g kg^{-1} DM in *G. triacanthos* whereas the content of Total extractable tannins (TET) ranged from 12.80 g kg^{-1} DM in *C. saharae* to 69.92 g kg^{-1} DM in *A. macrostachyum*. The highest contents of TEP and TET were observed in the Fabaceae-Leguminousae family (*G. triacanthos* and *O. natrix*) whereas grasses, *A. herba-alba* and Chenopodiaceae and Poaceae plants showed lower concentrations. The Free condensed tannins (FCT) varied widely from 4.6 g kg^{-1} DM g.kg^{-1} DM in *A. herba-alba* to 525.0 g kg^{-1} DM in *C. siliqua*. total condensed tannins (TCT) varied widely among species, being the highest in *C. siliqua* and the lowest in *H. vulgare* (Table 2).

Based on the results observed with the PEG bioassay, the species with the highest tannin biological activity on gas production would be *C. siliqua, O. natrix, G. triacanthos*, and to a lesser extent, *A. gombo* and *C. saharae* (p <0.01) and it did not exist for the other species (Table 2).

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

Plant family	Plant species	Total extractable phenols (TEP)	Total extractable tannins (TET)	Free condensed tannins (FCT)	Total condensed tannins (TCT)	Tannin biological activity [a] at incubation times: 6h	12h	24h	48h
Dicotyledons									
Chenopodiaceae	*A.macrostachyum*	8.34±0.34	69.92±0.38	7.6±0.25	28.6±1.12	0.98±0.01	1.14±0.05	0.91±0.01	0.92±0.02
	A. canescens	8.26±0.35	60.21±0.36	6.4±0.38	38.05±2.13	0.88±0.03	0.95±0.01	1.07±0.01	0.99±0.00
Asteraceae	*A. herba-alba*	3.46±0.11	17.54±0.07	4.6±0.19	44.6±2.03	1.04±0.01	1.18±0.16	1.02±0.08	0.89±0.05
Fabaceae	*A. gombo*	10.64±0.36	67.41±0.36	7.6±0.22	36.8±1.78	1.07±0.03	1.03±0.02	0.99±0.03	0.90±0.02
	C. saharae	6.03±0.41	12.80±0.21	6.8±0.36	28.0±0.75	1.01±0.03	1.00±0.06	1.04±0.03	0.99±0.02
	C. siliqua	64.16±12.28	34.52±12.15	525±10.25	723.4±11.35	1.36±0.13	1.18±0.07	0.84±0.05	0.74±0.04
	G.triacanthos	85.30±5.82	60.92±5.78	251.8±9.38	455.5±10.80	1.23±0.16	1.26±0.09	1.07±0.07	0.91±0.06
	H.coronarium	50.81±4.09	27.08±4.06	258.3±7.75	512.7±12.36	1.20±0.02	1.16±0.03	0.98±0.02	0.86±0.01
	M. Sativa	4.72±0.32	33.24±0.35	9.4±0.46	25.7±0.85	0.96±0.00	0.99±0.02	0.88±0.04	1.00±0.04
	O. natrix L.	12.89±0.23	88.06±0.18	10.8±0.55	45.3±0.90	1.27±0.13	1.62±0.08	0.95±0.06	1.01±0.01
Monocotyledons									
Poaceae	*H. vulgare*	3.02±0.12	19.34±0.15	6.6±0.87	25.0±0.75	0.96±0.04	0.99±0.03	1.06±0.03	1.01±0.02
	S. tenacissim	62.44±1.81	37.60±1.82	7±0.60	29.7±0.43	0.98±0.04	1.16±0.04	0.97±0.02	0.97±0.02

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

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 digestibility were observed in monocos (being particularly low for *S. tenacissima*) whereas dicots had significantly higher values except for *O. natrix*. The most digestible forages were *A. canescens* and *G. triacanthos*. Intermediate values were found for *A. herba-alba* and *M. sativa* and the lowest *in vitro* digestibility values were for *S. tenacissima*.

As it can be seen from Table 3, there were significant (p<0.05) differences among samples in terms of ME. The ME feedstuff ranged from 3.27 to 6.38 MJ/kg DM. The ME of Dicots was significantly (p<0.05) higher than the Monocots due to low cell wall contents and high CP. The ME content of feedstuff was consistent with the findings of Medjekal *et al.* (2015), Medjekal *et al.* (2016), and Kamalak *et al.* (2005) but lower than that obtained by Karabulut *et al.* (2007).

There was a positive and significant (r=0.50, p=0.002) correlation between *in situ* DM disappearance and *in vitro* gas production data suggest that either method could be used to estimate nutritive value of browse species.

Table 3: ME (MJ/kg DM) *In vitro* dry matter (g/g DM) digestibility and *in situ* dry matter disappearance (g/g DM) at different incubation time

Plant family	Plant species	ME	ivDM loss[1]	TIVD[2]	IVD-TT[3]	*In situ* DM disappearance after incubation times, h			
						0	12	24	96
Dicotyledons									
Chenopodiaceae	*A.macrostachyum*	3.78^{gh}	0.781^{b}	0.785^{b}	0.782^{a}	0.585^{a}	0.706^{a}	0.735^{a}	0.775^{c}
	A. canescens	5.49^{bc}	0.847^{a}	0.828^{a}	0.802^{a}	0.512^{bc}	0.749^{a}	0.789^{a}	0.837^{a}
Asteraceae	*A. herba-alba*	4.99^{d}	0.688^{d}	0.698^{c}	0.595^{cd}	0.324^{e}	0.548^{d}	0.577^{c}	0.663^{f}
Fabaceae	*A. gombo*	6.38^{a}	0.741^{c}	0.724^{c}	0.701^{b}	0.370^{d}	0.617^{bc}	0.658^{b}	0.788^{bc}
	C. saharae	4.88^{de}	0.574^{e}	0.586^{e}	0.581^{d}	0.193^{g}	0.323^{f}	0.331^{f}	0.408^{i}
	C. siliqua	5.11^{bcd}	0.704^{d}	0.716^{c}	0.695^{b}	0.537^{b}	0.719^{a}	0.750^{a}	0.804^{b}
	G. triacanthos	5.55^{b}	0.787^{b}	0.784^{b}	0.794^{a}	0.488^{c}	0.640^{b}	0.679^{b}	0.738^{d}
	H. coronarium	5.14^{bcd}	0.560^{e}	0.636^{d}	0.579^{d}	0.275^{f}	0.417^{e}	0.450^{d}	0.541^{h}
	M. Sativa	5.65^{b}	0.689^{d}	0.720^{c}	0.678^{b}	0.320^{e}	0.587^{cd}	0.655^{b}	0.702^{e}
	O. natrix L.	4.36^{ef}	0.435^{f}	0.451^{f}	0.461^{e}	0.264^{f}	0.437^{e}	0.470^{d}	0.573^{g}
Monocotyledons									
Poaceae	*H. vulgare*	4.14^{fg}	0.688^{d}	0.622^{de}	0.632^{c}	0.221^{g}	0.275^{f}	0.406^{e}	0.685^{ef}
	S. tenacissima L.	3.27^{h}	0.259^{g}	0.250^{g}	0.217^{f}	0.105^{h}	0.158^{g}	0.192^{g}	0.292^{j}
Standard error mean		0.035	0.027	0.022	0.023	0.030	0.031	0.030	0.027

[1] ivDMloss: *in vitro* dry matter loss; [2] TIVD: true *in vitro* digestibility; [3] IVD-TT: *In Vitro* Digestibility of Tilley & Terry; [4] Means in a column with different superscripts are significantly different (p < 0.05).

There were significant (P<0.05) differences among samples studied herein in asymptotic gas production. A significant difference was observed in constant rate (C) and lag time among different forages. The lowest rate of gas production was observed in *S. tenacissima* and the highest in *C. siliqua*. The lowest *in vitro* and *in situ* DM digestibility were observed in monocots (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 monocots 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 average fermentation rate for grasses than for dicot species.

Table 4: *In vitro* fermentation kinetics (estimated from gas production curves) of browse plant species

Plant family	Plant species	A^1 (ml/g)	C^2 h	L^3 h	$G24^4$ ml/g	fermentation rate (ml/g per h)
Dicotyledons						
Chenopodiaceae	*A. macrostachyum*	151.42^{f}	0.0539^{bcd}	0.77^{bc}	108.17^{h}	11.16^{ef}
	A. canescens	209.02^{dc}	0.0479^{cd}	1.97^{a}	135.05^{g}	12.56^{e}
Asteraceae	*A. herba-alba*	225.95^{c}	0.0560^{bcd}	1.28^{ab}	162.02^{ef}	16.54^{ed}
Fabaceae	*A. gombo*	249.80^{b}	0.0698^{ab}	0.82^{bc}	199.30^{cd}	23.17^{cb}
	C. saharae	222.63^{c}	0.0654^{bc}	0.64^{bc}	172.39^{ef}	19.58^{cd}
	C. siliqua	302.18^{a}	0.0868^{a}	0.46^{bc}	261.45^{a}	35.66^{a}
	G. triacanthos	302.72^{a}	0.0623^{bc}	0.34^{bc}	231.86^{b}	26.22^{b}
	H. coronarium	201.24^{ed}	0.0604^{bcd}	0.70^{bc}	150.67^{gf}	16.44^{ed}
	M. Sativa	255.22^{b}	0.0844^{a}	1.04^{ab}	217.69^{cb}	27.38^{b}
	O. natrix L.	185.46^{e}	0.0507^{cd}	0.00^{c}	130.24^{gh}	13.61^{e}
Monocotyledons						
Poaceae	*H. vulgare*	286.32^{a}	0.0432^{d}	1.17^{ab}	176.55^{ed}	16.47^{ed}
	S. tenacissima L.	223.63^{c}	0.0178^{e}	0.00^{c}	77.99^{i}	5.76^{f}
Standard error of mean		5.41	0.0023	0.083	6.17	0.40

[1] A: asymptotic gas production, [2]C: fractional rate of fermentation; [3] Lag time; [4] G24: gas production at 24 h of incubation; Means in a column with different superscripts are significantly different ($p < 0.05$).

Discussion

The chemical composition of forages affects digestibility of nutrients. The CP of the browse species evaluated in this study was lower than some previous reports (Bouazza *et al.*, 2012; Boufennara *et al.*, 2012). Protein requirements in ruminants include protein and/or nitrogen requirements of the ruminal microbial population. Generally, microbial requirements are met at 6-8% crude protein in the diet. Animal requirements range from 7-20% in the diet depending upon species, sex and physiologic state (Huston and Pinchak, 1991). 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 (Boufennara *et al.*, 2012). Differences among studies may be related to stage of harvesting, leaf/stem ratio or genetic variation. It is well known that the nutritive value of grasses is generally high during early growth, but declines rapidly with maturity whereas shrubs generally have high levels of crude protein, phosphorous and calcium throughout the year (Benjamin *et al.*, 1995; El-Shatnawi and Abdullah, 2003). In addition, seasonal distribution of rainfall and soil conditions impose a direct influence on the amount and quality of forage available during the year and indirectly affect animal performance (FAO, 1996; Medjekal *et al.*, 2015).

In the present experiment, the CP content of all leguminous forages is higher than the minimum level of 7-8 % DM required for optimum rumen function and feed intake in ruminant livestock (Van Soest, 1994) showing an interesting potential as fodder resources for small ruminants during this time of the year. Indeed, shrubs such as *A. gombo*, *A. canescens* or forage *M. sativa* contained more than 15% CP on DM basis that is usually required to support growth and lactation (Norton, 1982).

Leguminous shrubs and trees such as *Calluna vulgaris* (heather), *Sarothamnus scoparius* (broom), *Ulex europaeus* (gorse) and *Chamaecytisus palmensis* (tagasaste) have been used as feedstuffs in many regions of the word mainly because of their high protein content throughout the year (Tolera *et al.*, 1997) that can be attributed to the ability of these plants to fix atmospheric nitrogen. Similarly, Khanal and Subba (2001) reported high CP content (>140 g/kg DM) in leaves of many leguminous shrubs. Thus, these plants can be considered as good protein supplements to low quality roughages. However, CP content *per se* should not be the sole criterion of judging the relative importance of a particular feedstuff (Ammar *et al.*, 2004b). There are some references that

should not be less than 7%. According to Paterson *et al.* (1996), feedstuff for grazing animals with CP content lower than 70 mg g^{-1} DM requires a supplementation of nitrogen to improve their ingestion and digestion by the ruminant. The low CP content in *H. vulgare*, *S. tenacissima*, *A. macrostachyum* and *C. siliqua* can be probably due to high proportions of mature leaves and twigs in the samples. It is well known that the CP content declined through the growing season as a response to tissue ageing, particularly during the autumn when nutrient are transferred to perennial tissues before abscission (Ammar *et al.*, 2004b).

The significant variations among browse forages in the cell-wall components may be due to some inherent anatomical or morphological differences related to cell-wall rigidity (Wilson, 1994) and leaf/twig ratio in the samples used in the chemical analysis. Half of the browse species considered in this study contained below 45% NDF on DM basis and this qualifies them as good quality roughages (Singh and Oosting, 1992). The others containing relatively higher ADF and NDF may have low digestibility and intake since digestibility of feeds and ADF content are negatively correlated (McDonalds *et al.*, 2002). Like NDF, ADF is a good indicator of feed quality; higher values within a feed suggest lower-quality feed. The main difference between ADF and NDF is the inclusion of hemicellulose in the calculation of ADF and NDF. Both calculations include cellulose and lignin present in plant material. The two fiber calculations are used in conjunction to determine the amount and energy that will be contained in a feed. Fiber that has low cellulose, lignin and hemicellulose will typically take up less space in the stomach and are able to provide larger amounts of energy to the animal. Higher fiber in these materials takes up more space and produces less energy for the animal to use (Michael, 2017). Our values are similar to those reported for other browse forages (Ammar *et al.*, 2005; Salem *et al.*, 2006; Boufennara *et al.*, 2012).

The concentration of phenolic compounds in the collected material showed considerable variation among species. The analysis of specific tannins gives an indication of the presence of some anti-nutritive factors in browse. Except for some few species (*C. siliqua*, *G. triacanthos* and *H. coronarium*), the plants material investigated in this study had low tannin contents, particularly in *M. sativa*, *A. macrostachyum* and *C. saharae* which would be of little significance in their effects on digestion of nutrients by ruminants, consistently with result pointed out in the literature (Cabiddu *et al.*, 2000; Frutos *et al.*, 2002) with woody leguminous shrubs. Condensed tannin had an important role in forages depending on the amount. Low level tannin (2-3% of DM) may have beneficial effect since the level of tannin in diets prevents the CP from extensive degradation through formation of protein-tannin complexes (Barry, 1987). On the other hand, high tannin level (5% of DM) in diets may result in the increased indigested CP due to excessive formation of tannin-protein complexes (Kumar and Singh, 1984). Thus, low tannin contents may be beneficial to ruminants due to their effect on reducing rumen degradation of forage proteins, which can be digested post-ruminally (Barry, 1989). However, high tannin contents in nutritionally important forage trees, shrubs, legumes, cereals and grains often limit their utilization as feedstuffs (Kumar and Vaithiyanathan, 1990). The high levels of condensed tannins in the three mentioned substrate are consistent with other studies pointed out in the literature (Priolo *et al.*, 2005; Silanikove *et al.*, 2006; Kamalak *et al.*, 2012).

According to Mc Sweeny *et al.* (2001), condensed tannins reduce cell-wall digestibility by binding bacterial enzymes and (or) forming indigestible complexes with cell-wall polysaccharides. It is also

possible that tannins made protein and/or minerals unavailable for microbial metabolism (Mc Mahon *et al.,* 2000). Aharoni *et al.* (1998) determined that tannins affect microbial nutrients use by reducing degradation rates, lengthening lag time and by binding enzymes and substrates especially protein making it unavailable.

In the present study, addition of PEG to most tannin containing forages increased volume of gas production at different incubation times and the response to PEG treatment increased with increased concentration of phenolic compounds in the browse plants. This shows that the fermentation and digestibility of feedstuff high in phenolic compounds are improved by treatment with PEG, resulted from binding of phenolic compounds to PEG. The negative effects of tannins on nutrient intake could be reversed by supplementing tannin-binding agents such as PEG. Supplementation level of about 20 g of PEG 4000 per goat per day optimized DM, OM, NDF and ADF intakes. This level of supplementation is recommended if intake is the parameter of interest moreover, crude protein digestibility was optimized at a PEG 4000 supplementation level of 15.78 g per goat per day (Brown and Ng'ambi, 2017). Makkar (2003) suggested that tannin-complexing agents such as PEG can be used both by farmers as well as the industry to overcome anti-nutritive effects of tannins. It is well established that the incorporation of PEG in the diet has beneficial effects, particularly for tannin rich feeds having between 5-10 % of condensed tannins (Silanikove *et al.,* 1997; Ben salem *et al.,* 2002). The PEG inactivation of tannins increases voluntary feed intake, availability of nutrients and decreases microbial inhibition in degrading the tannin rich feeds, which in turn increases the performance of animals (Bhat *et al.,* 2013). Farmers can feed PEG to animals through water by mixing in a small amount of concentrate or by spraying on tannin-rich feedstuffs. Industry can incorporate PEG in a pelleted diet composed of ingredients including tannin-rich by-products.

Digestibility of the forage species was determined by two conventional and extensively used *in vitro* techniques (Tilley and Terry, 1963; Van Soest, 1994), and also assessed from DM disappearance when samples were incubated *in situ* in the rumen. With all the digestibility measures, a large variability was seen among species with a clear differentiation between the most digestible species (*A. canescens*, *G. triacanthos*, *A. macrostachyum*, and *A. gombo*) and those showing the lowest digestibility coefficients (grass species).

The positive and significant (r=0.50, p=0.002) correlation between *in situ* DM disappearance and *in vitro* gas production data suggest that either method could be used to estimate nutritive value of browse species. According to Khazaal *et al.* (1994), the *in situ* method should be used with caution when estimating the nutritive value of high phenolic feeds. The potential negative effect of phenolic compounds on rumen microbial fermentation is unlikely to be detected by the *in situ* method. In this respect, *in vitro* methods are more reliable in detecting inhibitory compounds in feeds. The *in vitro* gas production technique is a closed system with limited supply of rumen liquor; if there is any anti-nutritive compound, it is likely to affect the activity of rumen microbes. On the contrary, the *in situ* method is associated with a dilution effect, resulted from open system with wider rumen environment and copious supply of rumen fluid to nylon bag contents (Apori *et al.,* 1998).

The *in vitro* gas production technique has received much attention as a means of evaluating the nutritional quality of feedstuff (Williams, 2000). In the present study, the main aim of this technique was to detect differences between fermentative activity in rumen fluid of sheep when browse forages with different chemical composition and tannin contents were incubated. Furthermore, this technique is

considered to be more sensitive to detect such differences than other gravimetric techniques (Williams, 2000). *In vitro* gas production at 24h, *A. herba-alba* and C. *saharae* were higher than those reported by Boufennara *et al.* (2012), which could be due to differences in the chemical composition of feeds in relation to climatic conditions and maturity stage. According to Dann and Low (1988), soil type, fertility and water supply affect tannin concentrations in plants. Additionally, seasonal variations in response to climatic and physiological changes in browse species induce changes in chemical composition and in concentrations of secondary compounds such as tannins. These differences determine the value of browse plant foliage as forage resources for ruminants (Salem, 2005). Moreover, the differences may partly be attributed to other factors not measured in the present study, for instance, the configuration of cell-wall polysaccharides and their effect on rumen microbial attachment and colonization of digest particles (Cheng *et al.*, 1984).

Conclusion

Although chemical composition analysis is essential for understanding the nutritional potential of plant species, it is not sufficient especially in ligneous plants. *In situ* digestion can help, but cannot predict the potency of antimicrobial or other anti-nutritional factors. The anti-nutritive compounds in feedstuff reduce forage digestion rates *in vitro* which, in turn, would affect nutrient availability to the animal and animal performance. In addition, this study indicated that a large reserve of plant species in the local flora like *A. macrostachyum; C. saharae;* and *O. natrix*are could be potentially used for livestock feeding. These feeds, if fully exploited, could assist in increasing the level of production and productivity of the livestock resources in the region. Finally, all of this information may be used to define strategies for rational utilization of steppe grasslands, in particular to make decisions about the optimum time to use these fodder trees as a feed resource or as a supplement for Grazing Animals.

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بررسی ترکیب شیمیایی، در شرایط تخریب و تخمیر سینتیک برخی از گونه‌های گیاهی جمع‌آوری شده از مناطق خشک و نیمه خشک الجزایر به روش درون تنی

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چکیده. در این تحقیق ترکیب شیمیایی و قابلیت هضم دوازده نمونه از گیاهان (*Arthrocnemum macrostachyum*، *Atriplex canescens*، *Artemesia herba-alba*، *Astragalus gombo*، *Calobota saharae*، *Ceratonia siliqua*، *Gleditsia triacanthos*، *Hedysarum coronarium*، *Medicago satarea*، *Lisson Tasona*، *Onison*) که در مناطق خشک و نیمه خشک الجزایر رشد می‌کنند در سال ۱۳۹۰ بررسی گردید. اجزای علوفه با روش تجزیه و تحلیل تقریبی و ترکیبات فنلی و تانن با روش‌های رنگ سنجی مورد بررسی قرار گرفت. قابلیت هضم به روش قراردادی در شرایط درون تنی و برون تنی بررسی شد. به طور کلی، محتوای پروتئین خام در گونه‌های علف دولپه‌ای(dicots) همیشه بیشتر از مقدار موجود در تک لپه‌ای(monocots) بود که حاوی فیبر شوینده خنثی و اسیدی بالاتر (NDF و ADF) و مقدار کمتری لیگنین نسبت به دولپه‌ها بود. غلظت تانن بین گونه‌ها بسیار متفاوت است، اما به‌طور کلی تانن در گیاهان مورد بررسی به میزان کم(به جز *Ceratonia siliqua*، *Gleditsia triacanthos* و *Hedysarum coronarium*) وجود دارد. تک لپه‌ها در شرایط برون‌تنی و درون‌تنی، میزان تخمیر و تولید گاز تجمعی کمتری را نسبت به گونه‌های دولپه‌ای نشان داد. این مطالعه نشان داد که تنوع خوبی از گونه‌های گیاهی بومی وجود دارد که می‌توانند به طور بالقوه برای تغذیه دام استفاده شوند. این گونه‌های گیاهی در صورت بهره‌برداری کامل می‌توانند به افزایش سطح تولید و بهره-وری منابع دام در منطقه کمک کنند.

کلمات کلیدی: قابلیت هضم، تخریب درون‌تنی، علوفه، تولید گاز در آزمایشگاه، ترکیبات ثانوی گیاه، تانن