

Chemical Composition and *in vitro* Gas Production of Three Local Poaceae in El Djelfa's Region, North-Central Algeria

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Abstract: The nutritive value of some Algerian browse species was studied on the basis of their chemical composition, *in vitro* gas production and fermentation kinetics (gas production technique). The browse species were *Stipagrostis pungens*, *Lygeum spartum* L. and *Stipa tenacissima* L. Generally, there were significant variations $P < 0.05$ between chemical components of all browse species studied herein, except in condensed tannins (CT) content. The organic matter (OM) contents varied with a narrow range from 843.52 to 867.87 g/kg DM. The crude protein (CP) content value of the edible components ranged between 47.06-75.20 g/kg DM, the highest CP value was recorded for *Lygeum spartum* L. The Neutral detergent fiber (NDF) and ADF contents were relatively high $P < 0.05$ in *Stipa tenacissima* L. and low in *Lygeum spartum* L. The total gas production and organic matter digestibility (OMD) ranged from 56.49 to 91.15 (ml/g DM) and 38.15 to 59.03 %, respectively. Generally, high values of gas production and Organic matter digestibility OMD were recorded by *Lygeum spartum* L. followed by *Stipagrostis pungens*. As conclusion, browse species evaluated in the present study show high content of fiber and low crude protein concentration. Nitrogen supplementation and effects of alkaline treatments are then expected to be highly beneficial for *in vitro* organic matter digestibility, as observed in more conventional low quality forages.

Key words: Browse species • Gas production • *In vitro* digestibility • Nutritive value • Rumens fermentation

INTRODUCTION

The Algerian steppe covers more than 30 million ha of land and constitutes a transition area between the green belt in the North and the Sahara desert. The diversity and relative abundance of fodder plants has allowed the steppe to provide animal food for 15% of the Algerian population and constitutes the main source of red meat for the population as a whole [1]. 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 pasture declines, resulting in lower intakes and reduced ruminant productivity. Indigenous browse species are useful

sources of animal feeds, particularly during the dry season, due to remaining green and providing vegetation with better nutritive value when all other annual grass and herbaceous species have died out [2]. The nutritive value of a ruminant feed is determined by the concentrations of its chemical components, as well as their rate and extent of digestion. Determining the digestibility of feeds *in vivo* is laborious, 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 chemical composition and *in vitro* gas production can be used as rapid evaluation tools to assess nutritional quality of feeds [3]. The fodder forage deficit is still pronounced and chronic, as feeding of

livestock is mainly based on grazing and natural fodder resources [4]. Indeed, studies of prospecting collection and evaluation were interested in these local forage species that are already adapted to our climate and local environment [5, 6]. The objective of this work was to evaluate three Poaceae's browse species collected from a semi-arid zone in Algeria, based on the determination of their chemical composition and *in vitro* fermentation measurements, considered as useful indicators for the preliminary evaluation of previously uninvestigated feeding resources.

MATERIALS AND METHODS

Study Area: The study was carried out in semi-arid zone located on the North of Algeria, region of El djelfa (35° 21' 6" N and 3° 21' 39" E), (Fig. 1.) during the period of June 2009, at maturity stage. This region is characterized by altitude level of 845 m, with a climate typically Mediterranean, characterized by wet winters and hot dry summers with a mean annual precipitation of 250 mm year⁻¹ (2000-2010). The average minimum winter and maximum summer temperatures are 5°C in January and 26°C in July, respectively. The rainy season is generally from mid-October [7].

Forage Samples Collection and Processing: Experimental feedstuffs consisted of three autochthonous North African species widely utilized by local farmers. The plants were selected based on herdsman knowledge, that is consumed by, sheep, goats and dromedary. The Poaceae- Gramineae (*Stipagrostis pungens* locally named *Drin* is a perennial grass, growing in the dry regions of North Africa with leaves as tall as one meter.

Whereas its extensive root system prevents sand erosion, leaves are valuable sources of forage and pulp [8], *Lygeum spartum* L. Locally named *Albardin* is one of the most abundant perennial rhizomatous grasses found in Algerian steppes [9]. This species can tolerate extreme conditions of aridity, salinity and high temperatures [10]. Its extensive root system plays a significant role in preventing desertification by stabilizing the sand [11] and *Stipa tenacissima* L. locally named *Alfa* or *Gueddim*, a range coarse bunchgrass which is a characteristic grass of the North African steppes and whose uses are multiple. A fast-growing fibrous plant used as a fiber source for papermaking [12]. All plants were harvested at maturity (June 2009). The edible plant samples (leaves, stems and flowers) were hand plucked and chopped to 2-cm length. The samples were dried at 60°C in forced air oven for 48-h, except samples for tannins determination that were sun dried. The forages were then ground to pass a 1-mm sieve and used for chemical analysis and *in vitro* gas production.

Chemical Analysis: Samples were analyzed for organic matter (OM) by igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen content was measured by the Kjeldahl method [13] and Crude protein (CP) was calculated as N X 6.25. Neutral detergent fiber (NDF) was determined according to Van soest and Wine, [14] and Acid detergent fiber (ADF) content of the samples were determined by the method of Van soest, [15]. Condensed tannin (CT) was determined by butane-HCl method as described by Makkar *et al.* [16]. All chemical analysis was carried out in triplicate.

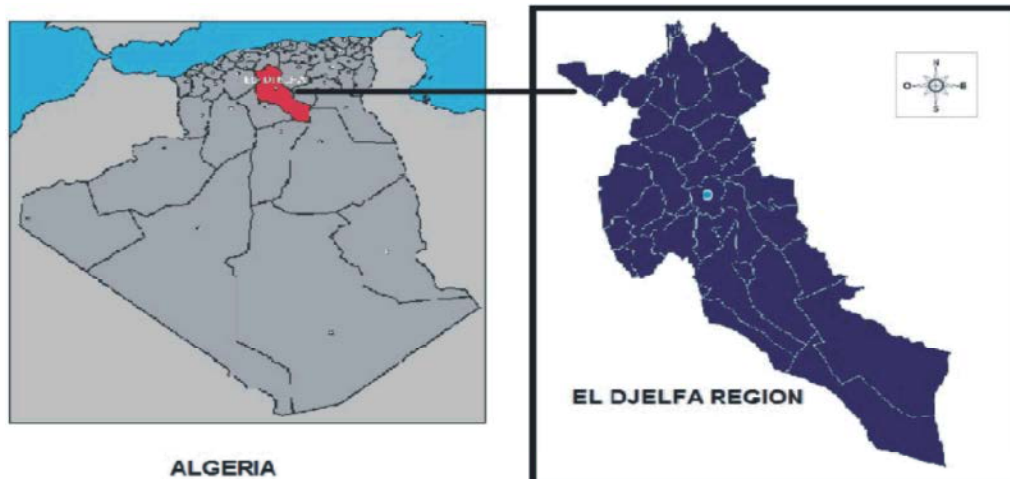


Fig 1: Location of the Study Area

In vitro Study: The tree samples were also milled through a 1 mm sieve and incubated *in vitro* with rumen fluid in 100 ml calibrated glass syringes following the procedures of Menke *et al.* [17]. Rumen fluid was obtained from three fistulated Awassi sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%) with a free access to water and mineral block. Rumen fluid was collected before morning feeding and filtered through four layers of cheesecloth under flushing with CO₂. The rumen fluid was combined with buffered solution in the ratio of 1:2, respectively. Approximately 0.200 gram air dried samples were weighed into calibrated glass syringes which were prewarmed at 39°C. Then 30 ml rumen fluid-buffer mixture was transferred into each syringe. The glass syringes containing samples and rumen fluid-buffer mixture were placed in a water bath at 39°C. Gas production was measured at 3, 6, 12, 24, 48, 72 and 96h. Three syringes containing only diluted rumen fluid were incubated as blanks and used to compensate for gas production in the absence of substrate. The average of the volume of gas produced from the blanks was deducted from the volume of gas produce per sample against the incubation time and from the graph, the gas production characteristics were estimated using the equation $Y = a + b(1 - e^{-ct})$ as described by Orskov and Mcdonald, [18] Where:

Y = volume (ml) of gas produced at time t , a (ml) is amount of gas corresponding to the rapidly degradable fraction, b (ml) is amount of gas corresponding to the slowly degradable fraction and c (ml h^{-1}) is fractional gas production or degradation rate and t (h) incubation time. The estimation of these parameters has been made by the Neway Excel software [19].

ME (MJ/kg DM) content of the samples was calculated using equation of Menke *et al.* [17] as follows: $\text{ME (MJ/kg DM)} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP}$, where $\text{GP} = 24 \text{ h net gas production (ml/200 mg)}$; $\text{CP} = \text{Crude protein Organic matter digestibility (\%)} \text{ of the samples}$

was calculated using equation of Menke *et al.* [17] as follows: $\text{OMD (\%)} = 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.0651 \text{XA}$, where $\text{XA: ash content (\%)}$

Statistical Analysis: All data obtained were subjected to analysis of variance (ANOVA) using the randomized completed block design. Significance between individual means was identified using the Turkey's multiple range tests. Mean differences were considered significant at $P < 0.05$. Analysis of variance (PROC ANOVA) was performed using the SAS software package [20].

RESULTS

Chemical composition of the three browse species is shown in Table 1. Generally, there were significant variations ($p < 0.05$) between chemical components of all browse species studied herein, except in CT content. The OM contents varied with a narrow range from 843.52 to 867.87 g/kg DM. The OM content was highest ($p < 0.05$) in *Stipa tenacissima* L. and *Lygeum spartum* L. followed by *Stipagrostis pungens*. The CP content value of the edible components ranged between 47.06-75.20 g/kg DM, the highest CP value was recorded for *Lygeum spartum* L. while the lowest value was recorded for *Stipagrostis pungens*. The NDF and ADF contents were relatively high ($p < 0.05$) in *Stipa tenacissima* L. and low in *Lygeum spartum* L. with value ranging from 696.96 to 775.20 g/kg DM and 500.16 to 562.06 g/kg DM, respectively. On the other hand, low values of CT were observed in the browse species and range from 13.70-14.30 g/kg DM which could have beneficial effect on protein fermentation.

As shown in Table 2, the browse species had significant effect ($p < 0.05$) on the gas production (both GP24 and total gas production) are affected, ME and OMD of the studied feedstuff. The gas production at 24 h incubation and ME ranged from 22.79 to 45.01 (ml/g DM) and 5.58 to 8.75 (MJ/kg DM), respectively.

Table 1: Chemical composition (g/kg DM) of three browse species

Nutrients (g/kg DM)	Browse species			SEM	Significance
	<i>Stipagrostis pungens</i>	<i>Lygeum spartum</i> L.	<i>Stipa tenacissima</i> L.		
OM	843.52 ^b	869.06 ^a	867.87 ^a	4.254	***
Ash	156.47 ^a	130.94 ^b	132.12 ^b	4.254	***
CP	47.06 ^b	75.20 ^a	50.27 ^b	4.619	***
NDF	746.83 ^b	696.96 ^c	775.20 ^a	11.80	***
ADF	514.71 ^b	500.16 ^b	562.06 ^a	9.721	***
CT	14.13	14.98	13.70	0.2798	NS

a, b, c Row means with common superscripts do not differ ($P < 0.05$); S.E.M.: standard error mean; OM: Organic matter; CP: Crude protein; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; CT: Condensed tannins; NS: Non-significant, *** $P < 0.05$

Table 2: *In vitro* fermentation parameters, GP24h (ml/g DM), Total Gas (ml/g DM), ME (MJ/kg DM), OMD (%), a (ml/g DM), b (ml/g DM) and c (h⁻¹)

Estimate Parameters	Browse species			SEM	Significance
	<i>Stipagrostis pungens</i>	<i>Lygeum spartum</i> L.	<i>Stipa tenacissima</i> L.		
GP 24 (ml/g DM)	34.01 ^{ab}	45.01 ^a	22.79 ^b	6.86	***
Total Gas (ml/g DM)	75.26 ^b	91.15 ^a	56.49 ^c	5.16	***
ME(MJ/kg DM)	7.09 ^{ab}	8.75 ^a	5.58 ^b	0.54	***
OMD (%)	48.13 ^{ab}	59.03 ^a	38.15 ^b	3.55	***
a	7.67	5.80	6.35	0.47	NS
b	92.37 ^a	94.19 ^a	49.15 ^b	7.61	***
c	0.0125 ^b	0.0216 ^a	0.0087 ^c	0.0019	***

a, b, c Row means with common superscripts do not differ ($P < 0.05$); S.E.M.: standard error mean; GP24h: Gas production at 24h; ME: Metabolizable energy, OMD: Organic matter digestibility, a: amount of gas corresponding to the rapidly degradable fraction; b: amount of gas corresponding to the slowly degradable fraction c: degradation rate; NS: Non-significant, *** $P < 0.0$

The total gas production (i.e. 96h incubation) and OMD ranged from 56.49 to 91.15 (ml/g DM) and 38.15 to 59.03 % respectively. Generally, high values ($p < 0.05$) of gas production and OMD were recorded by *Lygeum spartum* L. followed by *Stipagrostis pungens*. The *in vitro* fermentation parameters, *Lygeum spartum* L. had the higher ($p < 0.05$) (b) amount of gas corresponding to slowly degradable fraction 94.19 (ml/g DM) and degradation rate c with 0.0216 (h⁻¹), whereas *Stipa tenacissima* L. recorded low values ($p < 0.05$).

DISCUSSION

Browse species are common feedstuff for animals, particularly for browsing species like goats, sheep and camels [21] and wildlife. Crude protein among the plants was different and typically below nitrogen requirements of ruminal bacteria, except *Lygeum spartum* L. with 75.20 (g/kg DM). In facts, rumen microbe requires minimum crude protein of 70-80 (g/kg DM) to optimize cell wall degradation [22] below which feed intake is reduced [23]. According to Patterson *et al.* [24], feedstuffs with a CP content lower than 70 (mg/g DM) require a supplementation of nitrogen to improve their ingestion and digestion by the ruminants. The CP of the three feedstuff evaluated in this study was lower than some previous reports [4, 5, 25, 26]. Differences among studies may be related to stage of harvesting, leaf: stem ratio or genetic variation [27, 28, 29]. In the present experiment, the OM and cell wall component were particularly high and may be explained partly by the environmental conditions prevailing in the area of collection, as high temperatures and low precipitations tend to increase the cell wall fraction and to decrease the soluble contents of the plants [30]. Our values are similar to those reported by

other authors in previous works [4, 5, 26] and with those recorded for other browse forages [31, 32], with some differences among all studies, probably because of the different proportions of foliage and twigs and the different phenological stage of the feedstuff at sampling [5] (our plants were sampled at a mature stage).

Condensed tannins had an important role in forages depending on the amount. High tannin level (5% of DM) in diets reduce cell wall digestibility by binding bacterial enzymes and (or) forming indigestible complexes with cell wall polysaccharides [33]. It is also possible that tannins made protein and/or minerals unavailable for microbial metabolism [34]. Methods are available for detoxification by complexing the tannins with polyethylene glycol [35] and microbiological solutions are being sought [36]. On the other hand, low tannins contents (2-3% DM), in our experiment (13.70 to 14.98 g/kg DM) may be beneficial to ruminants due to their effect in reducing rumen degradation of forage proteins, which can be digested post ruminally [37].

The production of gas by rumen fluid incubated with feedstuff materials *in vitro* has received much attention as a means of evaluating the nutritional quality of feedstuff. In the present study, gas production parameters suggested differences in nutritional value that were generally closely related to chemical composition [38]. The values are within the range reported earlier for browse forages from Algeria [4, 5].

Chemical composition, *in vitro* fermentation and OMD are largely affected by plants species, plant morphological fraction, environmental factors and stage of maturity [39, 40]. *In vitro* organic matter digestibility of three browse species is very low, equivalent to low quality cereal straws. This result has to be related to

chemical composition of these plants which shows high content of fibre and low crude protein concentration. Effects of alkaline treatments are then expected to be highly beneficial for OMD, as observed in more conventional low quality forages [41, 42].

CONCLUSIONS

The browse species evaluated in the present study show high content of fibre and low crude protein concentration. Nitrogen supplementation and effects of alkaline treatments are then expected to be highly beneficial for *in vitro* organic matter digestibility, as observed in more conventional low quality forages when dealing with sustainability of low-input livestock farming systems. Our study has confirmed that chemical composition and *in vitro* gas production technique could be used in initial screening studies to rank browse species according to their nutritive quality, although the nutritional significance of the differences among browse species reported herein would need to be tested *in vivo* to determine if greater use of the alternative browse species in livestock rations is warranted.

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