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Influence of seasonal variation on chemical composition and methane production of *Calobota saharae* in M'sila, north-central Algeria

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ABSTRACT

The current trial was conducted to study the effect of season on chemical composition, condensed tannin and methane production of *Calobota saharae* shrub harvested at three different seasons, in winter (mid-January), spring (mid-May) and summer (end of July). Leaves, thin twigs, some flower and seeds (when existing) were clipped with scissors from the aerial part of the plants then dried and analysed for chemical composition. Gas and methane production were determined at 24 h incubation time. Season of harvest had a significant effect (p <0.05) on the chemical composition, gas and methane production. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and acid detergent lignin (ADL) of *Calobota saharae* were high during summer (dry season) and low in spring and winter (wet season). The NDF, ADF and ADL contents ranged from 463.05 to 616.82 and 352.27 to 488.21 and 121.19 to151.73 (g/kg DM) respectively. The CP content was lower (p <0.05) in summer (96.84g/kg DM) versus spring (138.85 g/kg DM) and winter (139.42g/kg DM). The gas and methane production at 24 h incubation varied between 65.75 to 77.75 and 6.50 to 10.17 (ml/g DM) respectively and decreased significantly (p <0.05) from spring to summer. In conclusion, season had a significant effect on the nutritive value of *Calobota saharae* shrub. *Calobota saharae* should be grazed or harvested during winter and spring since these seasons provide this shrub with high ME and CP content for ruminant.

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. These steppe areas are used mainly for sheep production with local breeds well adapted to the extreme environmental conditions and showing a particular productive performance. Currently, steppe rangelands are in a process of degradation due to the fragility of the physical environment and changes in the nomadic pastoral traditions (Aidoud, 1994). Feed supplementation during drought is infeasible for livestock producers in resource-poor countries due to high feeding costs (Benavides, 1994). Thus, and alternative approach for overcoming such feed constraints is the propagation of nutrients local fodder shrubs on degraded land. One ligneous species suitable for browse in areas of severe aridity is *Calobota saharae* (Coss. & Durieu) Boatwr. & B-E. van Wyk (formerly *Genista saharae* or *Spartidium saharae*). Calobota have a great ecological significance in Mediterranean countries. They colonize degraded forests and deforested areas that characterize the landscape (Lopez Gonzalez, 2001).

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 (Getachew *et al*., 2004). *In vitro* methods provide less expensive and more rapid alternatives. Thus, i*n vitro* gas production technique has received much attention as a mean of evaluating the nutritional quality of feeds (Williams, 2000). In Algeria, there is limited information on the nutritive value of local shrubs and both *in vitro* and *in vivo* studies are not available. Moreover there is limited information on the impacts of seasonal variations on nutritional values of local most grazed leguminous shrub. A study was therefore carried out to evaluate the effects of season on chemical composition, *in vitro* gas and methane production of indigenous shrub *Calobota Saharae*.

Material and methods

Collection of *Calobota saharae*

The study was carried out during 2009 in a medium-sized shrub-grassland between El Maader and Bousaada district located in the north central Algeria (N35° 26' 07,9''; E004°20'52,8'') (**Fig.1**), at an altitude of about 398 m above sea. The area is an arid high plateau with steppe like plains and extensive barren soils. Its climate is continental, semi-arid, subjected partly to Saharan influences with an annual average temperature of 21C° (average temperature of 34 C° in summer and 10 C° in winter), and a low and irregular rainfall not exceeding 250 mm per year. Representative samples from the aerial parts of plants were randomly hand clipped with scissors along a transect of about 2 Km, in winter (mid-January), spring (mid-May) and summer (end of July) during the year 2009. Leaves, thin twigs (young stem) and some flower and seeds (when existing) were clipped with scissors from the aerial part of the plants, and taken immediately to the laboratory where the samples from different specimens were pooled, oven dried at 50 °C (Makkar , 2003), and subsequently ground to a 1 mm screen.

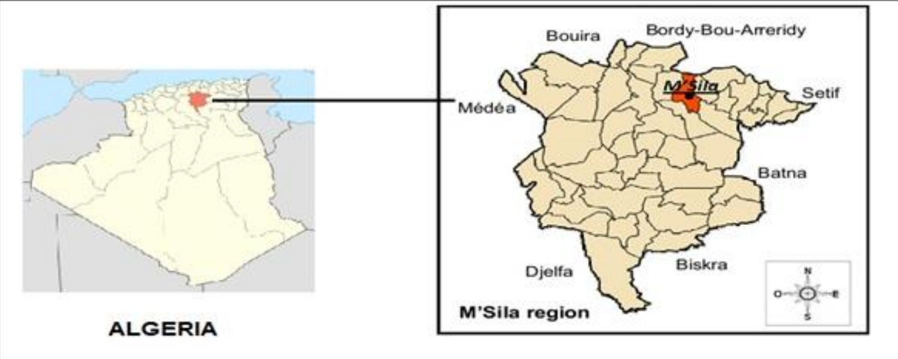

Figure.1. Location of the study area

Chemical composition and condensed tannins

Ash (Ash method ID 942.05), ether extract (EE, method ID 7.045) and crude protein by Kjeldhal (CP, method ID 984.13) in samples were determined by the procedures of the Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were analysed following the methodology described by Van Soest *et al*. (1991) using an ANKOM Model 220 Fibre Analyser (Macedon, NY, USA). Levels of condensed tannins of each sample were determined using the method described by Terrill *et al*. (1992).

***In vitro* gas (GP24h) and methane production**

Four adult and mature Merino sheep (body weight 48.3±3.45 kg) fitted with a permanent ruminal cannula were used for the extraction of rumen fluid to carry out the *in vitro* incubations (gas production and methane) of the browse material. A sample of rumen contents was withdrawn prior to morning feeding. Of each of the animals takes a similar amount of rumen contents, which is 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 CO2 atmosphere. Batch cultures of mixed rumen micro-organisms were used to study the ruminal fermentation, gas and methane production. The experimental procedure was based on Theodorou *et al*.(1994) protocol with some modifications.

Serum bottles of 120 mL were used. In each incubation run triplicate samples (0.5 g dry matter, DM) were placed into the bottle and incubated. Pressure in the bottle headspace and volume of gas produced were measured at 24 inoculations using a Wide Range Pressure Meter (Spec Scientific LTD, Scottsdale, AZ, USA) and a calibrated glass syringe as described by Theodorou *et al*. (1994) respectively. An aliquot of the gas produced was taken in a 10 mL vacuum tube (Venoject®, Terumo Europe N.V., Leuven, Belgium) for CH4 concentration analysis. Fermentation flasks without samples (i.e., blanks) were included to allow correction for gas produced directly from rumen fluid.

Methane content in fermentation gas was determined by gas chromatography (GC) using a Shimadzu GC-14 B GC (Shimadzu, Japan) equipped with CarboxenTM 1000, 45/60, 2m×1/8 in. column (Supelco, USA) and flame ionization detector (FID). Temperatures were 170, 200 and 200 ◦C in column, injector and detector, respectively and carrying gas (He) flux was 24 ml/min. Each gas sample (0.5 ml) was manually injected using Pressure-Lok® syringes A-2 Series of 500_l (Supelco, USA). Methane content in samples was calculated by external calibration, using a certified gases mixture with (per L) 100 ml CH4, 250 ml N2, 50ml H2 and 600 ml CO2 (Carburos Metalicos, Spain).

Statistics

All data obtained were subjected to analysis of variance (ANOVA) using the randomized completed block design. Significance between individual means was identified using the Tukey'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 (SAS, 2002).

Results and Discussion

The changes in chemical composition of *Calobota saharae* at different seasons of harvest are presented in table 1. There were differences between growth seasons in ash, cell wall components, CP, EE and condensed tannins contents (p <0.05). The CP content was lower in summer (96.84 g/kg DM) versus spring (138.85 g/kg DM) and winter (139.42 g/kg DM). In winter, *Calobota saharae* had lower (p <0.05) NDF, ADF, ADL and HCL content than in summer, and intermediate values were observed in spring. In the other hand, high values of ash and EE were observed during spring versus summer and winter with values ranging between 3.92 and 5.44, 20.64 and 23.79 (g/kg DM) respectively.

Table 1: Chemical composition and condensed tannins (g/kg DM) contents of *Calobota saharae* harvested at three different seasons for all chemical parameters.

Nutrients	Seasons			SEM	Significance
	Winter	Spring	Summer		
Ash	3.967^{b}	5.447^{a}	3.923^{b}	0.254	***
NDF	526.24^{b}	463.05^{c}	616.82^{a}	22.52	***
ADF	402.17^{b}	352.27^{c}	488.21^{a}	19.98	***
ADL	128.84^{b}	121.19^{c}	151.73^{a}	4.656	***
HCL	124.10^{a}	110.78^{b}	128.59^{a}	2.922	***
CP	139.42^{a}	138.85^{a}	96.84^{b}	7.212	***
EE	22.18^{ab}	23.79^{a}	20.64^{b}	0.567	***
Free CT	15.30^{a}	13.92^{ab}	12.83^{b}	0.403	***

a, b, c Row means with common superscripts do not differ (P<0.05); **S.E.M.:** standard error mean; **Ash:** Ash %, **NDF:** Neutral detergent fiber, **ADF:** Acid detergent fiber, **ADL:** Acid detergent lignin, **HCL**: Hemicellulose, **CP:** Crude protein, **EE:** Ether extract, **Free CT**: Free condensed tannins, ******* P<0.05

The 24h volume of gas produced (GP24) and methane (CH_4 24h) production in each season are in figure 2. There are marked decreases in gas production and methane from spring to summer.

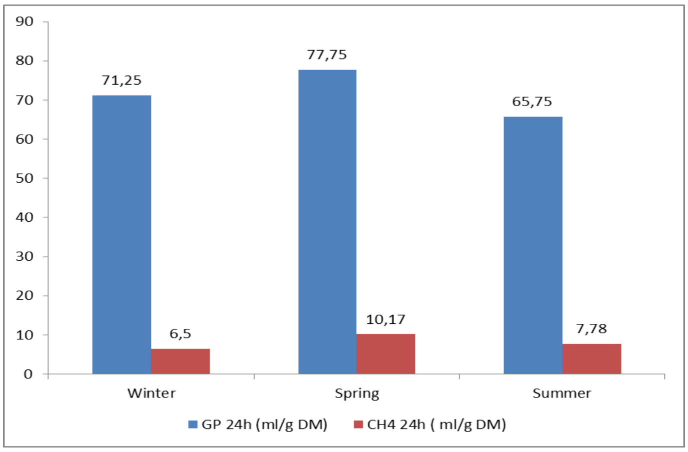

Figure 2: Gas and methane production (ml/g DM)

In the current study, large differences in the nutritive value of *Calobota saharae* were observed, which were primarily the results of changes in maturity of the collected leaves. Reduction (p <0.05) in CP content of *Calobota saharae* in summer versus other seasons, is consistent with other studies, as was the observation that the minimum CP content of fodder tree leaves in the dry season was more than twice that of grasses in the wet season (Evitayani Warly *et al*., 2004). However, the CP content of *Calobota saharae* remained relatively high (96.84 g/kg DM) during summer which is higher than the minimum level of 7-8 % DM required for optimum rumen function and feed intake in ruminants livestock (Van Soest, 1994). The lower CP content during summer may be largely due to moisture stress experienced by *Calobota saharae* during this period and build-up of lignocellulosic fibre structures of the plants, diluting the nitrogen (Anele *et al*., 2009).

The wide variations in NDF, ADF, ADL and HCL contents of *Calobota saharae* in winter and summer are consistent with the report of Onwuka *et al.* (1989) on browse species in the humid lowlands of West Africa and in other parts of the tropics (Dzowela *et al*., 1995). The seasonal differences in cell wall constituents may relate to the differences in elements of the weather between seasons, and their effects on cell wall lignification as well as translocation of nutrient to the different part of the plant (Larbi *et al*., 1998). Moreover, cell wall concentration in shrub fodder is negatively correlated with palatability (Kaitho *et al*., 1997).

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). In addition, condensed tannins in fodder trees and shrubs could also, help in the control of gastro intestinal parasites because they have biological properties that decrease fecal egg count in sheep and goats, and hatch rate and Laval development in feces (Min and Hart , 2003). 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). As can be seen from table 1, the observed condensed tannin levels of *Calobota saharae* harvested at three different seasons were low magnitude. Therefore, low condense tannin of *Calobota saharae* seems to have a potential for beneficial effect when included into ruminant diet as it can increase rumen undegradable CP without decreasing digestibility (Kaplan *et al*., 2014).

Methane is a product of microbial fermentation, particularly in the rumen. At the end of a complex interplay of microbiological and chemical processes, methanogens use hydrogen to reduce carbon CO_2 to CH_4 (Mills *et al*., 2001). Lopez *et al*. (2010) suggested that the methane reduction potential of any feedstuffs can be estimated from the percentage of methane of *in vitro* gas production and the feedstuffs can be arbitrarily divided in three groups, low potential (% methane in gas between >11% and ≤14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore, *Calobota saharae*, shrub had no methane reduction potential since the percentage of methane for all the seasons is between 10 to 13 %. The recent interest in methanogenesis reflects effects to identify mitigation strategies to reduce output of this greenhouse gas from livestock and manure. It also reflects an interest in reducing a source of energy loss that arises when feed is digested by ruminants.

Conclusions

The results presented in this study indicate that the major differences in nutritive value of *Calobota saharae* shrub, in terms of chemical composition and rumen microbial fermentation, are caused by the effect of the growing season. This study has practical implications for the development of agro-forestry technologies. *Calobota saharae* shows potential as a fodder shrub for revegetation projects in degraded ecosystems in arid and semiarid lands.

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