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Effect of Season on Potential Nutritive Value, Methane Production and Condensed Tannin Content of Fourwing Saltbush *(Atrilplex canescens)*

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Abstract: The current trail was conducted to study the effect of season on the potential nutritive value, methane production and condensed tannin of *Atriplex canescens* sampled at three different seasons (winter, spring and summer). Gas and methane productions of Atriplex canescens were determined at 24 h incubation time. Season of sampling had a significant effect ($p < 0.05$) on the chemical composition, gas production, methane production, metabolizable energy and *in vitro* dry matter digestibility of Telly and Terry. The CP content was lower in spring (167.68 g/kg DM) and summer (171.08 g/kg DM) versus winter (200.89 g/kg DM). In winter and spring, *Atrilplex canescens* had higher ($p < 0.05$) NDF, ADF, ADL and HCL contents than in summer. In all the seasons, condensed tannins and EE content were generally low, whereas the ash content was extremely high ($p < 0.05$) with (243.8 g/kg DM) in spring and (197.3 g/kg DM) in winter. Sampling season had a significant effect on the nutritive value of *Atrilplex canescens*. Its nutritive value decreased in spring and increase in summer and winter. *Atrilplex canescens* should be grazed or harvested during winter and summer since these seasons provide this shrub with high ME and CP content for ruminant.

Key words: *Atriplex canescens* · Nutritive Value · Condensed Tannin · *in vitro* Gas Production · Methane Production

INTRODUCTION

The Mediterranean region encompasses large areas of arid and semi-arid zones. These zones may be defined as areas where rainfall, relative to the level of evapotranspiration, is inadequate to sustain reliable crop production. Most of the arid and semi-arid zones of the Mediterranean region are rangelands and characterized by wide variability in rainfall and temperature [1]. In Algeria, the steppes cover more than 20 million hectares, harbor a human population estimated at 7.2 million among which numerous shepherds. Steppes are grazed by 15 million sheep (data from the Algerian Ministry for Agriculture and Rural development, [2]. The increase in the number of livestock and establishment of settled farms have contributed to overgrazing and deterioration of these areas, currently, steppe rangelands are in a continued process of degradation [3,4]. Land degradation and desertification are among the most serious challenges facing the sustainable development of society and human well-being. Drought tolerant plants are widely used in desertification control and degraded land recovery. Four-wing saltbush (*Atriplex canescens*), a C4 shrub native to North America, was chosen and massively planted since 1994 due to its drought and cold winter temperature resistance in Algerian steppe and its ability to bridge the food deficit of livestock during long dry season [5-7]. However, there is limited information on about the potential nutritive value of this shrub in such environmental conditions.

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Chemical composition alone is of limited use to evaluate the nutritive value of plants, especially those containing secondary compounds [8]. Recently, the *in vitro* gas production technique, which provides empirical equations to estimate digestibility and the metabolizable energy (ME) content of animal feed [9], has gained wide acceptance in research on the nutritional evaluation of animal feeds [10]. In addition, *in vitro* gas production was used to screen the feedstuffs in terms of their methane reduction potential [11,12]. Methane production during rumen fermentation is one of important contributors to global warming [13]. Plant development is the major factor affecting forage quality and as plant change from vegetative to reproductive stages, forage quality generally decreases. Our study was undertaken to follow the evolution in the chemical composition, *in vitro* digestibility and *in vitro* gas production of saltbush four-wing (*Atriplex canescens*) through its vegetative cycle.

MATERIALS AND METHODS

Area of Sample Collection: This 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"), at an altitude of about 398 m above sea. The area is an arid high plateau with steppe like plains and extensive barren soils. It has a continental climate with hot dry summers and very cold winters, with irregular rainfall of between 100 and 250 mm/ year. Under these environmental conditions, the plant species studied show a slow vegetative growth and phonological development throughout most of the year, often lagged in response to the infrequent major rainfalls.

Plant Sampling and Preparation: Representative samples from the aerial parts of plants were randomly hand along a transect of about 2 Km, at the distinct times during the year 2009, in winter (mid-January), spring (mid-May) and summer (end of July). 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 [14] and subsequently ground to a 1 mm screen.

Chemical Analysis: 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 [15]. 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*. [16] using an ANKOM Model 220 Fibre Analyser (Macedon, NY, USA).

Levels of condensed tannins (free condensed tannins, protein and fibre-bound condense tannins) of each sample were determined using the method described by Terrill *et al*. [17]. Free tannins are estimated by HCL-butanol, after the extraction of this fraction with diethyl ether to remove lipids and interfering pigments. The residue from the acetone extraction is treated with sodium dodecyl sulfate (SDS) and the extract obtained reacts again with HCL-butanol assay protein bound tannins. After SDS extraction the remaining residue is also treated with HCL-butanol to assay fibre-bound condensed tannins [18]. Solution of purified quebracho tannin (1 mg/ml aqueous acetone, 700 ml/l) was the standard. Absorbance was measured against a blank at 550 nm. The CH_4 concentration was determined by gas chromatography (GC) using a HP Hewlett 5890, Packard Series II gas chromatograph (Waldbronn, Germany). A sample of 0.5 ml of gas was injected using a 1 ml Sample-Lock® syringe (Hamilton, Nevada, USA).

Animals and Rumen Fluid Extraction: 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 (digestibility and gas production) of the browse material. Animals were fed twice a day (0900h and 1600h) a diet that consisted of alfalfa hay and grain oats in a proportion 60:40 at approximately and had free access to water and mineral/vitamin licks. A sample of rumen contents was withdrawn prior to morning feeding, Extraction of rumen contents was performed through the rumen cannula and with the aid of a PVC tube 1.5 cm in diameter, whose distal end is placed in the bag ventral rumen and which applies a slight suction. 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 CO_2 atmosphere. Time required from rumen content collection to the inoculation of bottles was less than 30 minutes.

***In vitro* Dry Matter Digestibility of Tilley and Terry (IVD-TT) and Metabolizable Energy (ME) MJ/kg:** Analysis of *in vitro* dry matter (DM) digestibility (IVD-TT) followed the method of Tilley and Terry [19]. A culture medium containing macro- and micro-mineral solutions, resazurin and a bicarbonate buffer solution was prepared as described by Van Soest *et al*. [20]. 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–HCl and Na_2S, as described by Van Soest *et al*. [20]. Rumen fluid was then diluted into the medium in the proportion 1:5 (v/v). Samples (400 mg) were weighed out into artificial fibre bags (size 5 cm × 5 cm, pore size 20m) which were sealed with heat and placed in incubation jars. Each jar is a 5 L glass recipient 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 facilitate the effective immersion of the bags in the rumen fluid. After 48 h of incubation in buffered rumen fluid, samples were subject to a 48 h pepsin-HCl digestion as described by Tilley and Terry [19].

ME (MJ/kg DM) content of *Atriplex canescens* samples was calculated using equation of Menke *et al.* [21] as follows:

ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP,
where
GP = 24 h net gas production (ml/200 mg);
CP = Crude protein.

***In vitro* Gas Production:** 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*. [22] protocol with some modifications. Three identical 48 h incubation runs were carried out in three consecutive weeks. Rumen content from each sheep was obtained before the morning feeding, immediately transported to the laboratory into thermal bottles, mixed and strained through four layers of cheesecloth into a warmed Erlenmeyer flask with an O_2-free headspace. The buffer solution of Goering and Van Soest [23] was previously prepared into an Erlenmeyer flask under a CO_2 stream and kept one hour with an O_2-free headspace after the resazurine colour turnover showed an O_2-free solution. Particle-free ruminal fluid was mixed with the buffer solution in a proportion 1:4 (vol/vol) at 39°C under continuous flushing with CO_2. Buffered ruminal fluid (50 mL) was added into each bottle under CO_2 flushing. Bottles were sealed with butyl rubber stoppers and aluminium caps and placed in a water bath at 39 °C. Serum bottles of 120 mL (Laboratorios Ovejero S.A.,León, Spain) 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 2, 4, 6, 8, 12, 24 and 48 h after inoculation using a Wide Range Pressure Meter (Spec Scientific LTD, Scottsdale, AZ, USA) and a calibrated glass syringe as described by Theodorou *et al.* [22], respectively. After 24 and 48 h of incubation an aliquot of the gas produced was taken in a 10 mL vacuum tube (Venoject®, Terumo Europe N.V., Leuven, Belgium) for CH_4 concentration analysis. Fermentation flasks without samples (i.e., blanks) were included to allow correction for gas produced directly from rumen fluid. Volume of gas (ml/g DM) produced after 24 h of incubation (GP24) was used as an index of energy feed value.

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 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 [24].

RESULTS

The chemical composition and tannins content of *Atriplex canescens* (ATCA) are in table 1. As expected, there were differences ($p <0.05$) between growth seasons in all chemical component of ATCA except in free condensed tannins. The CP content was lower ($p <0.05$) in spring (167.68 g/kg DM) and summer (171.08 g/kg DM) versus winter (200.89 g/kg DM). In winter and spring, ATCA had higher ($p <0.05$) NDF, ADF, ADL and HCL contents than in summer. In all the seasons, condensed tannins and EE content were generally low,

Table 1: Chemical composition and condensed tannins (g/kg DM) contents of *Atriplex canescens* harvested at three different seasons

	Seasons				
Nutrients	Winter	Spring	Summer	SEM	Significance
CP	200.89[a]	167.68[b]	171.08[b]	5.553	***
NDF	400.75[a]	352.49[b]	282.19[c]	18.01	***
ADF	172.70[a]	152.17[a]	101.19[b]	11.28	***
ADL	64.06[a]	63.75[a]	47.05[b]	2.834	***
HCL	228.05[a]	229.28[a]	181.00[b]	11.01	***
Ash	197.3c	243.8a	212.0b	6.859	***
EE	12.65[c]	16.64[a]	14.30[b]	0,589	***
Free CT	12.56	12.18	12.42	0,119	NS
PCT	2.14[ab]	4.69[a]	1.71[b]	0,625	***
FCT	20.46[a]	12.23[b]	12.91[b]	1,669	***
TCT	35.16[a]	29.10[b]	27.04[b]	1,511	***

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

Table 2: Gas production (GP24 (ml/g DM) methane 24h (ml/g) *in vitro* digestibility (%) and metabolic energy (MJ/KG DM)

	Seasons				
Estimate parameters	Winter	Spring	Summer	SEM	Significance
GP24h (ml/g)	60.50[a]	47.00[b]	67.66[a]	3,336	***
CH_4 24h (ml/g)	8.70[a]	4.67[b]	7.94[a]	0,6631	***
IVD-TT (%)	73.05[b]	74.05[b]	79.34[a]	0,9246	***
ME (MJ/KG DM)	11.58[a]	9.65[b]	12.38[a]	0,4643	***

a, b, c Row means with common superscripts do not differ (P<0.05); S.E.M.: standard error mean; GP24: gas production 24hour (ml/g), CH_4: methane production 24 hour (ml/g), IVD-TT: *in vitro* digestibility of Tilley and Terry (%), ME: Metabolisable energy (MJ/KG DM)

whereas the ash content was extremely high (p <0.05) with (243.8 g/kg DM) in spring and (197.3 g/kg DM) in winter. As shown in table 2. The season had a significant effect on gas production, methane production, IVD.TT and ME. The gas and methane production at 24 h incubation ranged from 47 to 67.66 ml and 4.67 to 8.70 ml respectively and decreased (p <0.05) significantly during spring. The IVD.TT and ME of ATCA ranged from 73.05 and 79.34 % and 9.65 and 12.38 MJ/kg DM respectively.

DISCUSSION

Reduction (p <0.05) in CP content of ATCA in summer versus in winter, is consistent with other studies. For example, the level of CP, EE and NFE decreased whereas CF, ash and DM contents increased on passing from the wet season to the dry season [25]. However, the CP content of ATCA remained relatively high (171.08 g/kg DM) in summer, suggesting the possibility that ATCA may be used as a dry season fodder and /or as feed supplement to low quality diets. In addition, the values of CP content obtained are above the 7% CP requirement for ruminants that should provide ammonia required by rumen microorganisms to support optimum microbial growth. CP contents obtained in the current study are comparable with finding of Van Niekerk *et al*. [26] who reported that CP ranged from 187 g/kg DM in ATCA (Santa Rita) located in Mier to 206 g/kg DM Field reserve 1) in Mier. However, Mellado *et al.* [27] reported values of 141, 172 and 146g/kg/ DM respectively for spring, summer and fall. The increase in CP levels measured during winter is most likely due to the increase soil moisture levels following the small rain [28].

The marked decrease in ADF, NDF, ADL and HCL from winter to summer may be explained by changing in maturity stage of ATCA; in which flowering generally occurs between May and September. This period can vary, however, with genotype and location [29]. The high level of fiber content in ATCA could be explained partly by the environmental conditions prevailing in the area of

Bousaada, as high temperatures and low precipitations tend to increase the cell wall fraction and to decrease the soluble content of the plants [30]. Our values are similar to those reported for other browse forages [31-33], with some differences among all studies, probably because of the different proportions of foliage and twigs in the samples and the different phonological stage of the plants at sampling. Cell wall in concentration in shrubs fodder is negatively correlated with palatability, voluntary dry matter intake and potential dry matter degradability [34,35].

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 [36]. 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 [37]. As can be seen from table 1, the observed total condensed tannin levels of ATCA harvested at three different seasons were low magnitude. Hence, low condensed tannin of Fourwing saltbush seems to have a potential for beneficial effect when included into ruminant diets as it can increase rumen indegradable CP without decreasing digestibility. Furthermore, the lack of accurate laboratory techniques and reliable compounds to be used as standards are major difficulties into condensed tannin analysis. Colorimetric methods should be used with caution as a quantitative assay. The differences between our tannin values and other reported in the literature [32,33], could be due to the nature of the assays used, nature of tannin in different fodder species, standards used for the quantification, plant growth stage and the influence of soil and climatic conditions [38].

The marked decrease in gas production, methane production, ME and IVD-TT occurred in spring were closely associated with increase in less digestible cell contents (NDF and ADF) and decrease in CP of ATCA. Gas production after 24h was higher ($p < 0.05$) in summer versus spring and this was in agreement with cell wall concentration. The gas production is closely associated with the amount of fermentable substrate in diets. In accordance with our result, Haddi *et al*. [39] reported significant differences in gas 24h among five halophytes shrubs including *Atriplex halimus*, *Salsola vermiculata* and *Sueada molis.*

Methane is one of the potent greenhouse gasses, contributing significantly to the environment pollution. The livestock contribute about 20% (gut microbial and animal waste fermentation). Lopez *et al.* [40] suggested that the methane reduction potential of any feedstuff can be estimated from the percentage of methane *in vitro* gas production and the feedstuff can be arbitrary 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 ATCA had low potential since the percentage of methane for all three seasons is between 11 and 14 %. The differences in ATCA among season reflect the observed differences in ADF, NDF, HCL and ADL concentrations. It could also relate to differences in concentrations of secondary compounds such as tannins in the fodder [41-43] as well as differences in the configuration of cell wall polysaccharides [44]. As conclusion, ATCA is a valuable plant that provides livestock and wildlife habitat and food. Season had a significant effect on the nutritive value of ATCA. Its nutritive value decreased in spring and increase in summer and winter. ATCA should be grazed or harvested during winter and summer since these seasons provide this shrub with high ME and CP content for ruminant. Furthermore, ATCA can be an effective fodder component in mixed diets for livestock mainly during winter and summer due to its droughts resistance and salt and freezing tolerance.

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Conflict of Interest: All of the authors have declared that no competing interests exist.

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