CHEMICAL COMPOSITION AND *IN VITRO* RUMEN BIOGAS PRODUCTION OF SOME PERENNIAL PLANTS SPECIES

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ABSTRACT

The nutritive value of some Algerian perennial plants species were examined on the premise of their *in vitro* gas production, chemical composition and fermentation kinetics. The plant species were *Aristida pungens*, *Artemesia herba-alba, Artemesia vulgarus, Atriplex halimus, Genista saharae*, *Lygeum spartum, Retama retam, Stipa tenacissima.* L and oat hay as a standard. Importants variation have been registered (P <0.05) regarding all the chemical components of the perennial plants species. The crude protein (CP) content value ranged between 5.02 and 16.15 (% DM), the highest CP value was recorded for *Atriplex halimus* (*A. halimus*). Acid detergent fiber (ADF) and Neutral detergent fiber (NDF) were generally high in *Stipa tenacissima* and low in *Atriplex halimus* (*P* <0.05). The organic matter digestibility (OMD) and the total gas production vary from 31.74 to 59.1 % and 15.46 to 39.52 ($ml.g^{-1}$ DM), respectively. In general, high values of Organic matter digestibility (OMD) and gas production were noted for *A. halimus* succeeded by *Genista saharae* (*G. saharae*). This necessitates prudent nutritional interventions such as supplementation with barley and wheat bran. Additionally, in order to improve fibrous feeds digestibility, some exogenous fibrolytic enzymes are of growing interest as additives to ruminant nutrition. *A. halimus* is a valuable plant that provides wildlife habitat and food for livestock. It is a very effective fodder element in mixed diets and as a supplement for livestock, mainly during the dry season in arid and semi-arid regions.

Key Words: digestibility, *in vitro* gas production, perennial plants,

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INTRODUCTION

There are two types of flora: spontaneous annual herb cover, Acheb, which appears within a few days of the first rain, and can last for about a month if the rain is sufficient, and perennials, which form the grassland of the permanent flora of the grassland (Nedjraoui, 1981; Aidoud, 1989; Le Houérou, 2001a). Perennial plants species plays an important role and used for ruminant grazing because they are adaptable, drought resistant, provide reliable forage production, have extensive root systems, increase soil fertility, and require low input costs. Moreover, perennial plants-based phytoremediation can play a key role in the restoration of contaminated sites and the maintenance of ecosystem services (Awasthi *et al.,* 2017; Pandey and Singh, 2020). Advantageously, these characteristics make them ideal for inclusion in balanced and sustainable grazing systems, especially in areas with little rainfall. Additionally, numerous perennial plants are ideal for surviving the lack of moisture and heat typical of many areas of south Algeria (Arid and Semi-Arid regions) (Singh *et al.,* 2018; Medjekal *et al.,* 2020). However and despite their potential as feeds, most of these perennial species contain large amounts of tannins, which plants have used as a defense mechanism against consumption by herbivores. The presence of these molecules in some forage cereals, legumes, grains, shrubs, and trees, usually restrict their utilization as feeding stuffs (Kumar and Vaithiyanathan, 1990). In the context of pasture ecology and sustainable use, long-term monitoring and management also require information about the nutritional value of these perennial plant species (Tefera *et al.,* 2009). Nutritive value evaluation of ruminant feeds should involve both the determination of nutrient concentration as well as the digestibility potential of forage types. The most interesting approach to this issue has been proposed by Menke et al 1979, as the *in vitro* gas production technique associated with chemical composition to evaluate the potential nutritive value of previously uninvestigated forages since *in vitro* gas production technique is quick, cheap and less time consuming. The use of chemical composition in combination with *in vitro* digestibility and degradability are useful tools in the evaluation of the nutritive value of forage resources from rangelands (Rittner and Reed, 1992). The present study aims to evaluate the chemical composition and *in vitro* cumulative gas production of some perennial plant species in the semi-arid area of Djelfa, which is a typical pattern of the North African grassland due to its huge delay when the animals on the grassland had to build reserves to save the long dry season.

MATERIALS AND METHODS

Study Area: This experiment was conducted in a semi-arid area located in the Djelfa region (35°21`6"N and 3°21`39"E) in northern Algeria **(Fig. 01)**, during the period of March 2020. Djelfa is located a 300 km South far away from the capital of Algeria, in the middle of Algerian steppe which makes a band between the humid fertile North and the huge Algerian desert (Boubakeur, 2018). Djelfa 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 (2000-2010) (Nedjimi, 2012). The average minimum temperature in winter and the maximum temperature in summer are 5°C in January and 26°C in July (Medjekal *et al*., 2015). By far the warmest months are: July, August and June in Djelfa. The coldest month is January with temperature 12℃ and with an average 14 mm of precipitation. Other cold months are: December 13℃, February 14℃ and November 17℃. The rainy season is generally from mid-October to May (Le Houérou, 2001b).

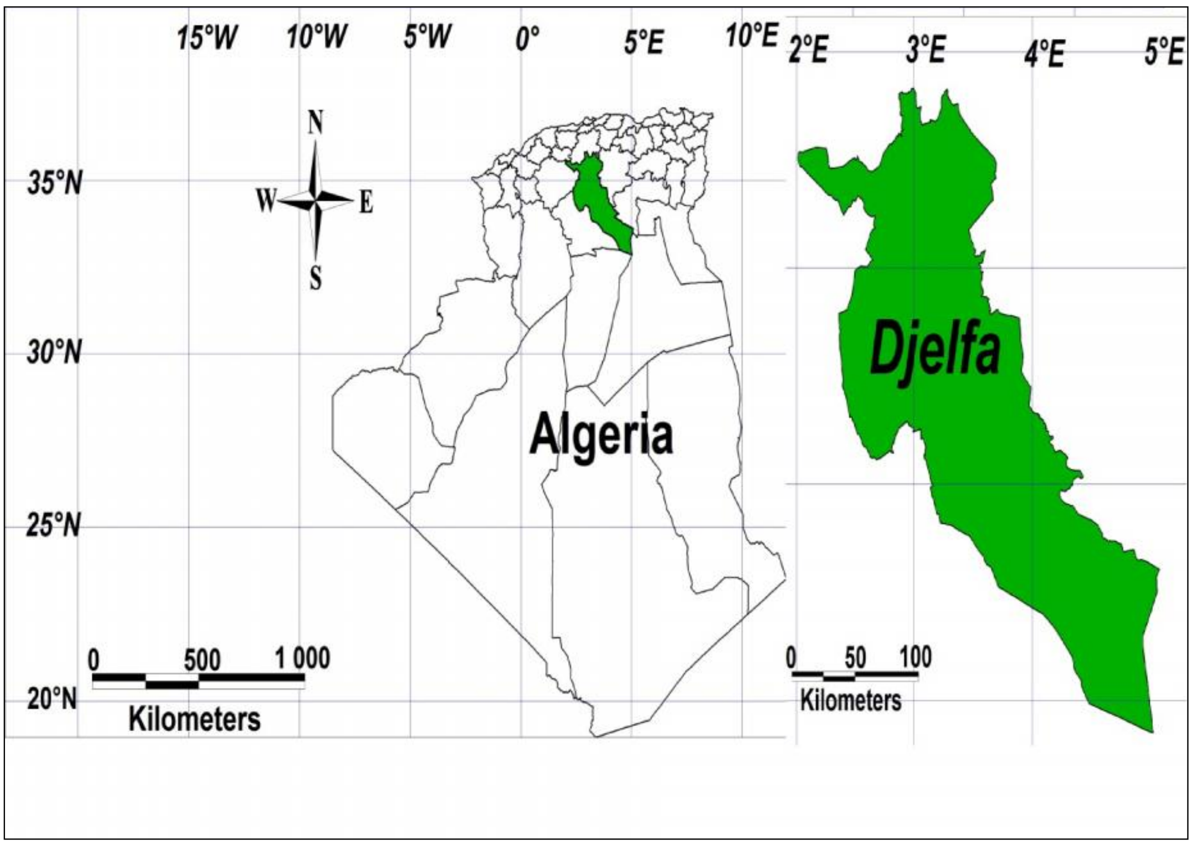

Fig. 1: The study area (MapInfo Professional 8.0)

Plants species: Eight perennial plant species were used in this study: *Aristida pungens (A.pungens)*, *Artemesia herba-alba (A.herba-alba), Artemesia vulgarus (A.vulgaris), Atriplex halimus (Ahalimus), Genista saharae (G.saharae), Lygeum spartum.*L *(L.spartum), Retama retam (R.retam), Stipa tenacissima.* L *(S.tenacissima)* and oat hay as a standard. Selection of the species was based on the available information (Boufennara *et al.,* 2012; Medjekal *et al.,* 2020) on their relative abundance and on their consumption by grazing small ruminants. Samples were collected in July 2020 when plants were at flowering (*A.pungens, A.herba-alba, A.vulgaris, A.halimus and G.saharae*) or at a mature stage (final stage of biological function for the rest of the species). Sampling was carried out during the dry season, because this is the most important grazing time of the year for these plants. The branches and twigs of several specimens of each perennial plants species were cut with scissors (total top part) and immediately taken to the laboratory, where the leaves, flowers and fruits (if any) were manually separated. Then to twelve samples of the eight substances were then immediately freeze-dried and ground using a 1 mm sieve in a hammer mill.

Chemical analysis: The dry matter (DM), the neutral detergent fiber (NDF), the acid detergent fiber (ADF), and the crude protein (CP) of ground perennial plants species are analyzed, and the content is determined following the methods of AOAC (2000). As described by Makkar *et al.* (1995), condensed tannins are determined by the butanol HCl method. All chemical analyzes were performed in triplicate.

***In vitro* gas production:** Menke *et al.* (1979) describe methods for measuring gas production. 200 mg of each sample is incubated in 50 ml of diluted ruminal fluid (10 ml of mixed ruminal fluid + 40 ml of medium prepared according to Menke and Steingass (1988) under a constant flow of CO_2). A 100 ml glass syringe is preheated to 39 ° C and flushed with CO_2. Rumen liquid is diluted (1:4 v/v) with medium containing a macro-and micro-mineral solution, resazurin, and a bicarbonate buffer solution, and is prepared as described by Menke and Steingass (1988). Rumen fluid was obtained from three mature Ouled Djellal sheep (body weight 45.06±3.46 kg) equipped with permanent rumen cannula (60 mm diameter) kept in cages and fed alfalfa hay *ad libitum* (CP 175 g, ADF 332 g, NDF 480 g and ADL 92 g/kg DM) with free access to water and a mineral/vitamin block. Rumen fluid from the three sheep was mixed, strained through various layers of cheesecloth and kept at 39°C under a CO_2 atmosphere. The medium was maintained at 39°C and flushed with CO_2. As described by Van Soest *et al.* (1966), a solution containing cysteine hydrochloride and Na_2S was added to reduce oxygen in the medium. Production of gas was measured at, 3, 6, 12, 24, 48, 72 and 96 h after incubation according to the technique of Menke and Steingass (1988) by reading plunger position and adjusted for blank. With three replicates we had six observations per sample. We used three different inocula to perform incubations (three sheep used separately to give rumen fluid) with two glass syringe per rumen fluid inoculum.

The equation of Menke *et al.* (1979) was used to determine ME (MJ/kg DM) content of the perennial species:
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.

The OMD (%) of the studied samples was determined using equation of Menke *et al*. (1979) as follows:
OMD (%) = 14.88 + 0.889GP + 0.45CP +0.0651 XA,
GP = 24 h net gas production.
where XA: ash content (%).

Cumulative gas production results were fitted to the exponential model $P = A + B\ (1 - e^{-ct})$ of Ørskov and McDonald (1979), with: P (ml): gas production at time t, A (ml): gas produced from soluble fraction, B (ml); gas produced from insoluble but fermentable fraction, A + B (ml): potential of gas production and C: the fractional rate of gas production per hour (ml/h).

Statistical analysis: All data of chemical composition, gas production, fermentation kinetics parameters, metabolizable energy and organic matter digestibility were analyzed with one way analysis of variance (Steel et al., 1997). A multiple comparisons of means Tukey's test was used ($P<0.05$). SAS software package (SAS, 2000) was used to perform analysis of variance between different variables.

RESULTS

The overall chemical composition of perennial plants species measurement results are summarized in **Table 01**. There was a significant variation ($P < 0.05$) in the content of all measured nutrients, DM, Ash, ADF, NDF, CP and CT among the perennial plant species.

A. halimus showed the highest Ash content (28.72% DM), whereas the lowest concentrations were observed in *S. tenacissima* (13.21% DM). *S. tenacissima* and *A. pungens* were highest in NDF (77.52 and 74.68% DM, respectively). The same trend was observed for ADF contents (61.05 and 51.05 % DM, respectively in *S. tenacissima* and Oat hay). The highest content of CP was recorded for *A. halimus* (16.15) (% DM) and the lowest ($P < 0.05$) content of CP was recorded for *A. pungens* (4.70). However, *R. retam*, *A. vulgarus* and *A. herba alba* had registered CP content between 9 and 13 % DM, which can support moderate level of productivity in ruminants. Low CT contents were recorded for all the samples studied herein, less than 2 % DM which will be beneficial with no anti-nutritional effect on feed's protein.

Table 01: Chemical composition (% DM) of the whole perennial plants species.

Plants species	*DM*	*Ash*	*ADF*	*NDF*	*CP*	*CT*
Aristida pungens	84.35[b]	15.64[d]	51.05[b]	74.68[ab]	4.70[f]	1.41[ab]
Artemesia herba -alba	76.44[d]	23.24[b]	38.57[cd]	46.17[e]	9.08[cd]	1.66[a]
Artemesia vulgarus	81.44[c]	18.56[c]	33.66[d]	35.20[f]	9.62[c]	1.33[ab]
Atriplex halimus	71.22[e]	28.72[a]	17.28[e]	32.67[f]	16.15[a]	1.44[ab]
Genista saharae	87.77[a]	12.22[e]	46.75[bc]	52.56[d]	8.88[cd]	1.30[b]
*Lygeum spartum.*L	86.90[a]	13.09[e]	50.01[b]	69.69[c]	7.52[de]	1.49[ab]
Retama retam	86.97[a]	13.03[e]	45.49[bc]	51.92[d]	13.69[b]	1.46[ab]
Stipa tenacissima. L	87.66[a]	13.21[e]	61.05[a]	77.52[a]	5.02[f]	1.36[ab]
Oat hay	83.60[bc]	16.39[d]	53.68[ab]	73.90[b]	6.09[ef]	1.46[ab]
S.E.M	0.6733	0.3697	9.664	1.5304	0.3237	0.0143
Significance	***	***	***	***	***	***

*a, b, c, d, e, f, Means of column with different letters are significantly different (p<0.05); **S.E.M**: standard error mean; **DM**: Dry matter %, **CP**: Crude protein, **NDF**: Neutral detergent fiber, **ADF**: Acid detergent fiber, **CT**: Condensed tannin, *** P<0.001*

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Fig. 02 exhibits the cumulative gas production curve of the nine different samples grown in buffered ruminal fluid. For the different samples studied in this experiment, the profile of fermentation of the differents substrates lengthens before becoming stable at 90 hours. This profile is precisely a characteristic of the fibrous substrates which express a more or less high latency time and spend a long period of stay in the rumen. The highest asymptotic gas production was observed in *A. halimus* and *G. saharae* (39.52 and 38.69 mL.g^{-1}(%DM), respectively), while *S. tenacissima* recorded the lowest value with (15.46 mL. g^{-1} DM). As may be seen in **Table 02**, the gas production values after 96 hours incubation varied (P <0.05) between plants species. The highest total gas production (TGP) values were recorded for *A. halimus*, *G. saharae* and *R. retam* than for the standard and *S. tenacissima*. L. The same trend was observed for ME (8.49, 8.15 and 7.96 MJ/Kg DM, respectively in *A. halimus*, *R. retam* and *G. saharae*).

The most digestible plants species were *A. halimus*, *R.retam* and *G. saharae*. Intermediate values were recorded for of *L. spartum.* and *A. vulgarus*, and the lowest OMD (% DM) values were for *S. tenacessima*. L. Fermentation kinetics parameters are also presented in Table 02. Asymptotic gas production (A) and TGP (ml) were lowest for *S. tenacissima*.L and greatest for *G. saharae.*

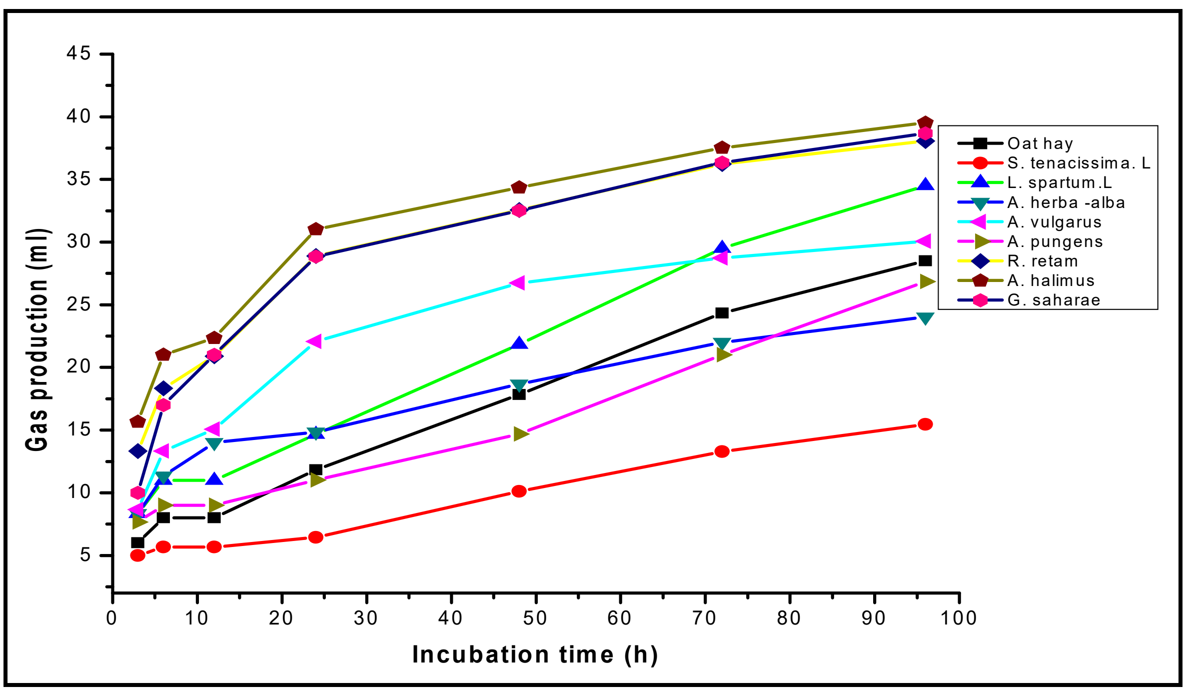

Fig. 2: Cumulative gas production profiles (OriginPro 2008)

Table 02 shows fermentation kinetics parameters. The asymptotic gas production (A) and TGP (ml) were low for *S. tenacissima*.L and high for *G. saharae*.

Table 02: Gas production kinetics, metabolisable energy and organic matter digestibility of the perennial plants species.

Species	***A (ml)***	***B (ml)***	***C (ml/h)***	***TGP (ml)***	***ME (MJ /Kg DM)***	***OMD* (%)**
Aristida pungens	7.58^{cd}	21.6^{d}	0.0091^{c}	26.85^{bcd}	6.12^{de}	41.88^{de}
Artemesia herba -alba	8.26^{bc}	25.57^{bcd}	0.0225^{bc}	24.01^{cd}	5.98^{de}	41.83^{de}
Artemesia vulgarus	6.57^{cde}	23.97^{cd}	0.0410^{b}	30.07^{abc}	6.83^{bcd}	47.15^{bcd}
Atriplex halimus	13.73^{a}	21.34^{d}	0.0407^{b}	39.52^{a}	8.49^{a}	59.15^{a}
Genista saharae	8.95^{bc}	31.06^{bc}	0.3614^{a}	38.69^{a}	7.96^{abc}	54.06^{bcd}
Lygeum spartum.L	7.73^{bc}	31.88^{bc}	0.0044^{c}	34.51^{abc}	7.32^{abcd}	49.80^{abcd}
Retama retam	11.17^{ab}	30.75^{bc}	0.0402^{b}	38.07^{ab}	8.15^{ab}	55.74^{ab}
Stipa tenacissima. L	3.08^{e}	32.22^{b}	0.0034^{c}	15.46^{d}	4.59^{e}	31.74^{e}
Oat hay	4.09^{de}	45.54^{a}	0.0033^{c}	28.51^{abc}	6.42^{cd}	44.04^{cd}
S.E.M	1.54	7.84	0.0001	16.84	0.32	13.80
Significance	***	***	***	***	***	***

*a, b, c, d, e, Means of column with different letters are significantly different ($p<0.05$); A (ml): initial gas produced from soluble fraction; B (ml) gas produced from insoluble but fermentable fraction; C: the fractional rate of gas production per hour (ml/h).S.E.M.: standard error mean; NS: Non-significant, TGP: gas production rate (ml); ME: Metabolisable energy (MJ/kg DM); OMD: Organic matter digestibility %, *** $P<0.001$*

DISCUSSION

The high fiber (NDF and ADF) content recorded by the perennial plants species studied in this experiment and the low protein are usual of steppique and arid region grasses as reported by, Arhab *et al.* (2009), Boufennara *et al.* (2012) and Medjekal *et al.* (2020). Despite mature perennial plants species were largely utilized in livestock production overall the long months dry season, their nutritive value is commonly poor because of low protein content and the excessive fiber resulting in low ruminants livestock production. This necessitates prudent nutritional interventions such as supplementation with barley and wheat bran. The significant differences through perennial forages in the NDF and ADF content may be explained by some internal morphological or anatomical differences in relation with the cell wall structure and inflexibility (Wilson, 1994) and leaves to twigs proportion in the used samples in our aexperiment. Crude protein contents in *A. halimus* was similar to those described by Bouazza *et al.* (2012) and Boufennara *et al.* (2012), however the NDF and ADF values were higher. This reveals the capacity of Atriplex spp. to produce annual ruminants feed and their far-more salt acceptance than many other grassland species (Azam *et al.,* 2012).

A. herba-alba is largely used during spring by small ruminant's producers. In fact, *A. herba-alba* is particularly the shrublands most abundant plant species of the North African's steppe (Le Houérou, 1989). In comparison with CP content of *A. herba-alba* collected from Tunisian rangelands (Ben Salem *et al.,* 1994), our samples shows slightly higher values. However, samples for the same plant species from Morocco recorded significantly high CP contents (El Aich (1992). Moreover, in this experiment, low tannin concentrations and intermediate digestibility were recorded for all the perennial plants species.

Condensed tannins (CT) are secondary plant polyphenol molecules that have been considered as anti-nutritional due to their capacity to link protein in feeds, microbial cells, and enzymes, hence, disrupting microbial digestion and retarding ruminal protein and dry matter digestion (McSweeney, 2001). The analysis method could affect condensed tannins compounds in the plants material, because the chemicals used may have different reactivity against the type and the amount of condensed tannins present in each sample. The absence of precise laboratory methods and adequate compounds used as standards are the main difficulties of CT analysis. According to Bouazza *et al.* (2012) colorimetry should be used with caution as quantitative analysis methods. Hence, the difference between our CT value and other values reported in the literature may be due to the growth stages of the plants, the nature of the common measurements, the standards quantification, the nature of the tannins in the different food species and the influence of soil and climatic factors (Rubanza *et al.,* 2005).

Forages nutritive value rely on how appropriately they are solubilized and well degraded inside the digestive system and then, how many nutrients and energy can be supplied to rumen microbiota and ruminant (Longo *et al.,* 2012). One of the big advantages of the *in vitro* gas production technique was the prevalent correlation between the *in vitro* gas production and feed degradability, in relation with the chemical composition of feed (Menke and Steingass, 1988; López, 2005).. The *in vitro* gas production method was esteemed to be hypersensitive in detection of suchlike variations more than other *in vitro* gravimetric technique (Williams, 2000). Bouazza *et al.* (2012) announced a high OMD values for *A. halimus* rather than the values in our experiment, specifying no relation with the high gas production potential (parameter B); these authors considered the higher content of CP of *A. halimus* as a factor that contributed to its elevated OMD values.

In addition, the same authors recorded higher levels ($P<0.01$) of gas production and ME in *A. halimus* in comparison with some foliage from fodder trees and shrubs, which is in agreement with the findings of the present study. Gas production after 96 hours of incubation was higher in *A. halimus* and *G. saharae* ($p <0.05$), compared to the standard and all other samples which is in agreement with cell wall concentration. Indeed, gas production is closely associated with the amount of fermentable substrate in the diets. Consistent with our result, Haddi *et al*, (2003) reported significant differences in gas production at 24 hours between the five halophytic shrubs, including *Atriplex halimus*, *Salsola vermiculata* and *Sueada mollis*. Similar observations to our study were also noted by Kamalak et al, (2005) for the so-called hay, *Gundelia tournifortii*, and by Kaplan et al, (2014) for the minor hay Sanguisorba. According to Fagg and Stewart (1994), legumes native to arid regions, such as *C. saharae*, because of their symbiotic nitrogen fixation with legume-nodding bacteria (collectively called rhizobia) contribute in their majority to soil fertilization by increasing soil nitrogen and organic matter content. They also provide high quality forage, prevent erosion, and contribute to soil stabilization and restoration of arid and semi-arid ecosystems.

Conclusion: Perennial plants species evaluated in the present study show high content of fibre and low crude protein concentration. This necessitates prudent nutritional interventions such as supplementation with barley and wheat bran. Additionally, as additives to ruminant nutrition, exogenous fibrolytic enzymes are of growing interest as a way of improving digestibility of fibrous feeds. *A. halimus* should continue to be used in soil protection and low intensity farming systems of semi-arid and arid zones where, together with harvest residues and rough grazing, it can form part of a balanced diet for livestock. In all cases, *A. halimus* is interesting as a perennial species because it is relatively requiring little in terms of management and is able to thrive on degraded soils.

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