Introduction

Ruminants can digest ligno-cellulosic feeds as a major component of their diet to get energy for maintenance and production. They are also able to utilize non-protein nitrogen sources for the synthesis of microbial protein in the rumen. This largest digestive compartment hosts a diverse and unique microbial ecosystem composed of anaerobic bacteria, archaea, protozoa and fungi. Hydrogen is produced in considerable amounts during the anaerobic fermentation of nutrients (IPCC 2006; Sirohi et al. 2009). Methanogenesis is an essential process in microbial fermentation, representing the main disposal of the hydrogen generated when organic substrates are fermented. Methanogenesis is also the process by which methanogen archaea obtain energy autotrophically. However, methane produced during anaerobic fermentation in the rumen represents a loss of 2–12% of the gross energy contained in the feed ingested, and contributes to emissions of greenhouse gases into the environment (Moss 1993; Unger et al. 2010). The main factor influencing the amount of methane produced in the rumen is the diet the animal is fed. The diet determines the balance of the microbes existing in the rumen and therefore the fermentation characteristics, including methane production. Ruminants fed forages rich in structural carbohydrate produce more methane than those fed concentrate diets containing higher levels of non-structural carbohydrates (Sauvant & Giger-Reverdin 2009).

In vitro techniques are useful techniques to study the rumen fermentation processes under controlled conditions (Lopez 2005). Feeds or other substrates are incubated in cultures of mixed rumen microorganisms, fermentation end-products are accumulated in the medium and can be measured after a given incubation time (Rahman et al. 2013). The objective of the study reported herein was to evaluate the ruminal fermentation of 10 browse species largely used by farmers to feed their livestock in the arid and semi-arid regions of Algeria. The evaluation is based on the measurement of fermentation end-products (volatile fatty acids (VFAs) and methane) in in vitro batch cultures.

Materials and methods

Study area

This experiment was conducted using plant samples collected from two Algerian locations: Mila (N 36° 31′ 14″′–E 6° 15′ 40″, 289 m altitude) and M’sila (N 35° 26′ 07″–E 004° 20″ 52″, 398 m altitude) (Figure 1). Mila is in eastern Algeria, a semi-arid region with a continental climate and erratic annual precipitations of 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 Le Houerou (1995), the climate of the area is continental, due in part to the Saharan influence. Summer is hot and dry while winter is very cold, with low and irregular rainfall in the order of 100–250 mm/year.

**Browse species collection and preparation**

Ten plant samples were used in this study: eight dicotyledonous plants namely *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Atriplex canescens* (Pursh) Nutt., *Artemisia herba-alba* Asso, *Astragalus gombo* Bunge, *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E. van Wyk (current accepted name *Spartidium saharae* (Coss. & Durieu) Pomel, formerly *Genista saharae*), *Hedysarum coronarium* L., *Medicago sativa* L. and *Ononis natrix* L., and two monocotyledonous plants namely *Hordeum vulgare* L. (straw) and *Stipa tenacissima* L. Selection of the species was based on the available information on their consumption by grazing small ruminants, and on their relative abundance. Samples were collected in June 2010, when the plants were at a flowering (*A. gombo* and *C. saharae*) or at a mature stage (the rest of the species). Samples were collected from numerous individual plant specimens, and edible parts of the plants (leaf and stems less than 3 mm in diameter) were harvested. The material collected was freeze-dried, ground to pass a 1-mm screen and stored at room temperature (i.e. 20–25°C) in sealed containers until analysis.

**In vitro fermentation**

*In vitro* fermentation incubations and analyses of end-products (methane and short chain fatty acids) were performed at the University of León (Spain). *In vitro* gas production was measured according to the procedure described by Theodorou et al. (1994). Buffer and mineral solutions were prepared, mixed and placed in a water bath at 39°C under continuous flushing with CO₂. Rumen fluid was collected from three mature Merino sheep (body weight 48.5 ± 4.33 kg) fed on lucerne hay once a day and with free access to water and mineral/vitamin licks. Samples of rumen contents were withdrawn from each sheep prior to the morning feeding, transferred into separate thermos flasks (one for each sheep) and taken immediately to the laboratory. Rumen fluid was filtered, flushed with CO₂ and added to the buffered mineral solution (1:4 v/v; 1 l strained rumen fluid + 4 l of incubation medium). Ground samples of each plant species (500 mg) were incubated in 50 ml of diluted rumen fluid in 120 ml serum bottles under a CO₂ atmosphere. 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). Six serum bottles containing only rumen fluid inoculum were incubated as blanks and used to correct for methane and VFA production in the absence of substrate.

After 24 h of incubation, volume of gas accumulated in each bottle headspace was determined using a pressure transducer (Bailey & Mackey Ltd., Birmingham, UK) as described by Theodorou et al. (1994). A sample of the gas was collected and transferred to a 10-ml vacuum tube (Venoject®, Terumo Europe N.V., Leuven, Belgium) for methane analysis. Bottles were swirled in ice to stop fermentation, and then opened to measure pH in the incubation medium. A sample of supernatant (0.8 ml) was added to 0.5 ml of deproteinizing solution (20 g metaphosphoric and 4 g crotonic acid/l 0.5N HCl) for VFAs analysis.

**Methane and VFAs analysis**

Methane content in fermentation gas was determined by gas chromatography (GC) using a Shimadzu GC-14 B GC (Shimadzu, Japan) equipped with a Carboxen TM 1000 (45/60, 2 m x 1/8 in.) column (Supelco, USA) and flame ionization detector (FID). Temperatures were 170°C, 200°C and 200°C in the 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 the samples was calculated by external calibration, using a certified gas mixture with (per l) 100 ml CH₄, 250 ml N₂, 50 ml H₂ and 600 ml CO₂ (Carburos Metalicos, Spain).

The VFA were determined by GC using a Perkin-Elmer Auto-System XL GC (Perkin-Elmer Inc., USA), equipped with a semi-capillary TR-FFAP (30 m x 0.53 mm x 1 m) column (Supelco,
USA), FID and an auto-sampler. Temperatures were 140°C in the column and 250°C both in the injector and the detector, and carrier gas (He) flux was 13 ml/min. Each sample was injected automatically with a split ratio of 1/3. Chromatograms were integrated using software Star Chromatography Workstation 6.2 (Varian Inc., USA).

Statistical analysis

All data were analysed using one-way analysis of variance, with browse species as the only source of variation (fixed effect) and source of inoculum (rumen fluid from each sheep, random effect) as a blocking factor. The Bonferroni test was used for the multiple comparisons of means. Significant differences were declared for \( p < .05 \). All analysis were performed using the SAS software package (SAS 2000).

Results and discussion

Data on pH and VFA production from fermentation of the studied browse species are shown in Table 1. There were differences \( (p < .05) \) in pH and VFA production at 24-hour incubations among feedstuffs, with pH ranging from 6.29 (straw) to 6.73 (H. coronarium), total VFA from 1.16 (S. tenacissima) to 3.59 (A. gombo) mmol/g dry matter (DM) incubated and the acetate:propionate ratio from 3.06 (straw) to 5.81 (O. natrix). Acetate molar proportion ranged among the browse species between 686 (M. sativa) and 786 (O. natrix) mmol/mol VFA, and that of propionate between 137 (O. natrix) and 231 (straw) mmol/mol VFA.

Ruminal pH is one of the main factors affecting bacterial attachment (Miron et al. 2001). In the present experiment, the pH values observed were within the limits allowing high fibre digestion (Sung et al. 2007). As observed in previous studies using continuous culture of mixed ruminal microorganisms (Sláter 1986), in sacco disappearance (Mould & Ørskov 1983) and in vitro batch cultures (Hu et al. 2005), fibre digestion decreases at low pH, especially below pH 6.0. Furthermore, cellulolysis and cellulytic bacterial growth are depressed when pH is below 6.0 (Ørskov & Ryle 1990).

Gas and methane production when the studied substrates were fermented are shown in Table 2. There were differences \( (p < .05) \) among feedstuffs, with gas productions ranging from 40 to 119 mmol/g DM incubated, and methane production from 11.4 to 25.8 mmol/g DM incubated. In both cases the lowest values were for S. tenacissima and the greatest for M. sativa.

Consistent with our results, Bouazza et al. (2014) reported differences in VFA and methane production from the rumen fermentation of Algerian Acacia tree foliage. The most fermentable plant species (A. gombo or M. sativa) led to higher production of both fermentation gas and VFA. Getachew et al. (2002) reported a close association between short chain fatty acids and the in vitro gas production. The genus Astragalus includes about 3000 species (Heywood 1978). In Algeria, this genus is represented by about 40 species, including A. gombo; an endemic perennial plant that grows in sandy arid and desert pastures of Algeria (Quezel & Santa 1963). Legume species require less or no nitrogen fertilizer than other plants because of their capacity to fix atmospheric N in the roots. Leguminous forages are also characterized by their high protein contents. In addition, some legume species contain bioactive compounds that may potentially have beneficial effects on rumen fermentation (Copani et al. 2015).

The lower VFA and gas production from S. tenacissima could be due to its low digestibility and high cell wall contents (Boufennara et al. 2012). This species (locally named Alfa or Gueddi) is a range coarse bunchgrass characteristic of the North African steppes with multiple uses in agro-pastoralism systems (Genin et al. 2007), and regardless of its low nutritive value, it is appreciated as a local forage resource. In Algeria, Alfa grass pulp is used in the manufacture of paper (Ahrens et al. 1998).

Degradation of fibrous or cellulosic materials is likely to produce a higher molar proportion of acetate and a lower proportion of propionate. However, feed with low fibre content would be expected to result in a reduction in the acetate:propionate ratio during rumen fermentation (Moss et al. 2000). Fermentation gas is mainly fermented when feedstuffs are fermented to acetate and butyrate, with propionate yielding gas only due to buffering of acid (Getachew et al. 2004). High levels of acetate usually occur in animals fed rations containing large amounts of roughage, whereas lower levels are associated with concentrate diets (Madrid et al. 2002). The acetate to propionate ratios observed with our plants are within the range of values reported in other in vitro studies (Brown et al. 2002). A

Table 1. Total VFAs production (mmol VFA/g dry matter incubated) and molar proportions (mmol/mol total VFA) of acetate, propionate, butyrate and valerate at 24 h of incubation of Algerian browse species.

| Plant family | Plant species            | pH   | Total VTA | Acetate | Propionate | Butyrate | Valerate | C2:C3  |
|--------------|--------------------------|------|-----------|---------|------------|----------|----------|--------|
| Dicotyledons |                          |      |           |         |            |          |          |        |
| Chenopodiaceae | Anthrochneum macrostachyphon | 6.71 | 1.61<sup>a</sup> | 753<sup>abc</sup> | 187<sup>bc</sup> | 49.0<sup>bc</sup> | 12.3<sup>bc</sup> | 4.04<sup>abcd</sup> |
| Atriplex canescens |                       | 6.60 | 1.94<sup>def</sup> | 726<sup>cd</sup> | 175<sup>c</sup> | 83.1<sup>c</sup> | 12.3<sup>bc</sup> | 4.19<sup>abcd</sup> |
| Asteraceae   | Artemisia herba-alba      | 6.48 | 2.71<sup>bcd</sup> | 775<sup>bc</sup> | 156<sup>a</sup> | 48.6<sup>c</sup> | 14.4<sup>bc</sup> | 4.99<sup>bc</sup> |
| Fabaceae – Leguminosae | Astragalus gombo | 6.55 | 2.37<sup>d</sup> | 745<sup>bc</sup> | 172<sup>bc</sup> | 56.3<sup>bc</sup> | 14.4<sup>bc</sup> | 4.25<sup>abcd</sup> |
| Calobota saharae |                       | 6.59 | 2.97<sup>cde</sup> | 745<sup>bc</sup> | 184<sup>bc</sup> | 50.1<sup>c</sup> | 13.0<sup>bc</sup> | 4.09<sup>cde</sup> |
| Hedysarum coronarium |                   | 6.73 | 2.66<sup>de</sup> | 738<sup>d</sup> | 209<sup>ab</sup> | 39.1<sup>c</sup> | 10.8<sup>bc</sup> | 3.57<sup>de</sup> |
| Medicago sativa |                        | 6.53 | 3.59<sup>de</sup> | 686<sup>d</sup> | 209<sup>abc</sup> | 67.4<sup>b</sup> | 26.8<sup>bc</sup> | 3.36<sup>de</sup> |
| Ononis natrix |                        | 6.61 | 2.24<sup>cde</sup> | 786<sup>c</sup> | 137<sup>d</sup> | 58.4<sup>d</sup> | 17.7<sup>b</sup> | 5.81<sup>c</sup> |
| Monocotyledons | Poaceae – Gramineae |      |           |         |            |          |          |        |
| Hordeum vulgare (straw) |                     | 6.29 | 3.12<sup>abc</sup> | 699<sup>de</sup> | 231<sup>a</sup> | 60.8<sup>bc</sup> | 9.0<sup>c</sup> | 3.06<sup>c</sup> |
| Stipa tenacissima |                    | 6.56 | 1.16<sup>c</sup> | 753<sup>abc</sup> | 169<sup>cd</sup> | 54.9<sup>b</sup> | 17.9<sup>b</sup> | 4.50<sup>bc</sup> |
| SEM | 0.02 | 0.153 | 6.23 | 6.39 | 4.62 | 1.49 | 0.175 |        |
| p value | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 | <.001 |

Notes: C2:C3 = acetate to propionate ratio. SEM = standard error of the mean.

<sup>a,b,c,d,e,f</sup>Means in a column with different superscripts are significantly different \( (p < .05) \).
high acetate:propionate ratio is an indication of fermentation of structural carbohydrates and thus of a more fibrous food (Getachew et al. 2004). In addition, acetate to propionate ratio reduction in the rumen has been described as a common feature of several antimethenogenic compounds, which indicates a concurrent decrease of methane formation and a shift in ruminal fermentation (Abecia et al. 2012). According to Janssen (2010), when propionate is formed in the rumen by the reduction of pyruvate less hydrogen is released and hence methanogenesis is reduced in the rumen.

In recent years, methane production from the livestock, especially those consuming large quantities of fibrous food, has gained considerable attention due to the significant role of methane in global warming (Johnson & Johnson 1995). It is well known that methane production is influenced by quality and quantity of feedstuffs. Therefore, several strategies have been developed through dietary manipulation (Durmic et al. 2014; Rira et al. 2014).

Several tannin-rich plants or their extracts have been evaluated for mitigating methane production in the rumen. Rira et al. (2014) reported that tannin-rich plants, such as Gliricidia sepium, Leucaena leucocephala and Manihot esculenta, have the potential for decreasing methane production in vitro and in vivo in sheep. Similar to this study, Chatterjee et al. (2014) noted that Psidium guajava leaves had a potential to reduce methane production in vitro. Bodas et al. (2008) screened more than 400 plant species for their potential as antimethanogenic feed additives for ruminants, and observed that six of the plants reduced methane production by more than 25%, particularly with Rheum nobile. In addition, other plant species have shown a potential to reduce methane from ruminal fermentation, such as Sesbania sesban and Acacia angustissima (Zeleke et al. 2005), or Sapindus sp., Terminalia chebula, Populus tremuloides, Syzygium aromaticum and P. guajava (Kamra et al. 2005). Tannins are known to reduce enteric methane production through a direct inhibitory effect on methanogens depending upon the chemical structure of tannins (i.e. hydrolysable or condensed tannins) and also indirectly by decreasing fibre degradation (Patra & Saxena 2010). Tannins can form complexes with fibre, reducing its degradation and/or limiting the activity of the ruminal microorganisms responsible for cellulose degradation (McSweeney et al. 2001).

Like tannins, saponins and essential oils have been considered as promising natural substances for mitigating methane emissions from ruminants (Goel et al. 2008a; Bodas et al. 2012). Saponin extracts from Yucca schidigera and Quillaja saponaria have been largely examined and demonstrated their potential on methanogenesis both in vitro (Takahashi et al. 2000; Pen et al. 2007) and in vivo (Holtshausen et al. 2009; Wang et al. 2009). Furthermore, Sesbania sesban leaves and Trigonella foenum-graecum seeds have been shown to inhibit methane production in vitro (Goel et al. 2008b). On the other hand, essential oils, known for their antimicrobial activity, have been documented in several studies to decrease methane production (Macheboeuf et al. 2008; Agarwal et al. 2009; Wang et al. 2009). However, Beauchemin and McGinn (2006) in an in vivo study did not reveal any effect on methanogenesis. In general, the reduction in methane production was often accompanied by a decrease in numbers and activity of protozoa (Ando et al. 2003). Since about 25% of rumen methanogens are associated with protozoa, the antimethanogenic effect of essential oils may be partly due to an antiprotozoal activity (Newbold et al. 1995).

When methane production is expressed as total amount per unit of substrate incubated, a reduced value may be due either to a low fermentability of the substrate incubated resulting in lower gas and VFA production or to a specific inhibitory effect on methanogenic archaea or methanogenesis. From a mitigating point of view, a specific effect is of much interest, because a low fermentable substrate is indicative of a feed with low nutritive value. Expressing the methane production per unit of gas or VFA produced may give an indication of a specific effect of the plants on methane production. Using these units of methane production, the differences among species had shrunk substantially (Table 2). The total amount of methane produced from 1 g of DM incubated was lowest with S. tenacissima, most likely because this monocot species is of low degradability. When methane production from S. tenacissima was expressed per VFA produced, the value was the highest, indicating no antimethanogenic effect. The plants showing some potential to reduce methane production through a specific effect were A. herba-alba, A. gombo and O. natrix, for which methane per mol of VFA produced was the smallest within the group of plants studied. However, the

### Table 2. Total gas and methane production (ml/ g dry matter incubated), percentage of methane in gas and methane production per unit of VFAs production at 24 h of incubation of Algerian browse species.

| Plant family               | Plant species                  | Gas production (ml/g DM) | Methane (ml/g DM) | ml methane/100 ml gas | CH4/VFA |
|---------------------------|--------------------------------|--------------------------|-------------------|-----------------------|---------|
| Dicotyledons              | Anthracnium macrostachyum      | 57.4a                    | 20.3b             | 2.08                  | 0.325b  |
|                           | Amplex canescens               | 58.4a                    | 20.4b             | 2.12                  | 0.313b  |
|                           | Artemisia herba-alba           | 83.8d                    | 21.2b             | 2.69                  | 0.263b  |
| Fabaceae – Leguminosae    | Astragalus galus               | 107.4ab                   | 21.7b             | 2.76                  | 0.269b  |
|                           | Calobota saharae               | 102.7bc                   | 21.0b             | 2.99                  | 0.310b  |
|                           | Hedysarum coronarium           | 92.0d                    | 21.3b             | 3.64                  | 0.364ab |
|                           | Medicago sativa               | 119.3a                   | 21.7b             | 3.35                  | 0.335ab |
|                           | Ononis matrix                  | 67.3a                    | 20.4b             | 2.76                  | 0.276b  |
| Monocotyledons            | Hordeum vulgare (straw)        | 110.9ab                   | 19.6b             | 3.18                  | 0.318b  |
|                           | Stipa tenacissima              | 40.0b                    | 11.4d             | 2.57                  | 0.483d  |
|                           | SEM                            | 2.44                     | 0.83              | 1.26                  | 0.031   |
|                           | p value                        | <.001                     | <.001             | .002                  | .005    |

Notes: CH4VFA = mmol of methane per mmol total VFA produced. SEM = standard error of the mean.

a,b,c,d,e,f Means in a column with different superscripts are significantly different (p < .05).

---

**JOURNAL OF APPLIED ANIMAL RESEARCH**

© 2014, Published by Taylor & Francis Group, LLC. All rights reserved.
values were only significantly different from that observed for *S. tenacissima*, suggesting that the browse species studied herein would show little potential for mitigating methane production in the rumen.

**Conclusion**

Based on the yield of fermentation end-products *in vitro*, the most degradable plant species are *A. gombo* and *M. sativa*, and the less degradable *A. macrostachyum* and *S. tenacissima*. *A. herba-alba*, *A. gombo* and *O. natrix* were the plants causing a greater methane reduction, but our results suggest that the browse species studied herein would show little potential for mitigating methane production in the rumen. This study confirms the importance of leguminous forages in small ruminants’ nutrition particularly in the arid and semi-arid regions.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**References**

Abecia L, Toral PG, Martin-García AI, Martínez G, Tomkins NW, Molina-Alcaide E, Newbold CJ, Yánzê-Ruiz DR. 2012. Effect of bromochloromethane on methane emission, rumen fermentation pattern, milk yield, and fatty acid profile in lactating dairy goats. J Dairy Sci. 95:2027–2036.

Agarwal N, Shekhar C, Kumar R, Chaudhary LC, Kamra DN. 2009. Effect of peppermint (*Mentha piperita*) oil on *in vitro* methanogenesis and fermentation of feed with buffalo rumen liquor. Anim Feed Sci Technol. 148:321–327.

Ahrens FW, Gulya T, Worry GL, Walter WP. 1998 Dec 29. Papermaking fabric, and the less degradable *A. herba-alba* firms the importance of leguminous forages in small ruminants browse species studied herein would show little potential for

Ando S, Nishida T, Ishida M, Hosoda K, Bayaru E. 2003. Effect of peppermint (*Mentha piperita*) on *in vitro* screening of the potential of numerous plant *in vivo* feeding on the digestibility, ruminal fermentation and protozoa. Livest Prod Sci. 82:245–248.

Beauchemin KA, McGinn SM. 2006. Methane emissions from beef cattle: effects of fumaric acid, essential oil, and canola oil. J Anim Sci. 84:1489–1496.

Bodas R, Lopez S, Fernandez M, Garcia-Gonzalez R, Rodriguez AB, Wallace RJ, Gonzalez JS. 2008. *In vitro* screening of the potential of numerous plant species as antimonethanogenic feed additives for ruminants. Anim Feed Sci Technol. 145:245–258.

Bodas R, Prieto N, Garcia-Gonzalez R, Andres S, Giraldez FJ, López S. 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim Feed Sci Technol. 176:78–93.

Bouazza L, Boufenara S, Bodas R, Boussouhau H, Tejido ML, Ammar H, Lopez S. 2014. Methane production from the rumen fermentation of Algerian Acacia tree foliage. Forage resources and ecosystem services provided by mountain and Mediterranean grasslands and rangelands. Options Méditerranéennes Series A. 109:797–800.

Boufenara S, Lopez S, Boussouhau H, Bodas R, Bouazza L. 2012. Chemical composition and digestibility of some browse plant species collected from Algerian arid rangelands. Spanish J Agric Res. 10:88–98.

Brown VE, Rymar C, Agnew RE, Givens DI. 2002. Relationship between *in vitro* gas production profiles of forages and *in vivo* rumen fermentation patterns in beef steers fed those forages. Anim Feed Sci Technol. 98:13–24.

Chatterjee PN, Kamra DN, Agarwal N, Patra AK. 2014. Influence of supplementation of tropical plant feed additives on *in vitro* rumen fermentation and methanogenesis. Anim Prod Sci. 54:1770–1774.

Copani G, Ginane C, Le Morvan A, Niderkorn V. 2015. Patterns of *in vitro* rumen fermentation of silage mixtures including sainfoin and red clover as bioactive legumes. Anim Feed Sci Technol. 208:220–224.

Durmic Z, Moate PJ, Eckard R, Revell DK, Williams R, Vercoe PE. 2014. *In vitro* screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. J Sci Food Agric. 94:1191–1196.

Genin D, Khorchani T, Hammadi M. 2007. Improving nutritive value of a North African range grass (*Stipa tenacissima*): effect of dung ash and urea treatment on digestion by goats. Anim Feed Sci Technol. 136:1–10.

Getachew G, Makkar HPS, Becker K. 2002. Tropical browses: content of phenolic compounds, in *in vitro* gas production and stoichiometric relationship between short chain fatty acids and in *in vitro* gas production. J Agric Sci Camb. 139:341–352.

Getachew G, Robinson PH, DePeters EJ, Taylor SJ. 2004. Relationships between chemical composition, dry matter degradation and in *in vitro* gas production of several ruminant feeds. Anim Feed Sci Technol. 111:57–71.

Goel G, Makkar HPS, Becker K. 2008a. Changes in microbial community structure, methanogenesis and rumen fermentation in response to saponin-rich fractions from different plant materials. J Appl Microbiol. 105:770–777.

Goel G, Makkar HPS, Becker K. 2008b. Effect of *Sebseba sesban* and *Carduus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum*) L seeds and their extracts on partitioning of nutrient from roughage and concentrate based feeds to methane. Anim Feed Sci Technol. 147:72–89.

Heywood VH. 1978. Flowering plants of the world. London: Oxford University Press.

Holtshausen L, Chaves AV, Beauchemin KA, McGinn SM, McAllister TA, Odongo NE, Cheeke PR, Benchaa C. 2009. Feeding saponin-containing *Yucca schidigera* and *Quillaja saponaria* to decrease enteric methane production in dairy cows. J Dairy Sci. 92:2809–2821.

Hu ZH, Tu HQ, Zhu RF. 2005. Influence of particle size and pH on anaerobic degradation of cellulose by rumen microbes. Int Biodeter Biodegr. 55:233–238.

IPCC. 2006. Guidelines for national greenhouse inventories. Agriculture, forestry and other land use, Emissions from livestock and manure management. vol. 4. p. 10.1–10.87.

Janssen PH. 2010. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Technol. 160:1–22.

Johnson KA, Johnson DE. 1995. Methane emission from cattle. J Anim Sci. 73:2483–2492.

Kamra DN, Aragwai N, Chaudhary LC. 2005. Inhibition of ruminal methanogenesis by tropical plants containing secondary compounds. Int Cong Ser. 1293:156–163.

Le Houérou H-N, editor. 1995. Bioclimatologie et biogéographie des steppes terres et désertisation. Montpellier: CIHEAM. (Options Méditerranéennes Série B. 10:1–396)

López S. 2005. *In vitro* and in situ techniques for estimating digestibility. In: Dijkstra J, Forbes JM, France J, editors. Quantitative aspects of ruminant digestion and metabolism. 2nd ed. Wallinford: CAB International; p. 87–121.

Macheboeuf D, Morgavi DP, Papon Y, Mouset JL, Arturo-Schaan M. 2008. Dose-response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. Anim Feed Sci Technol. 145:335–350.

Madrid J, Megías MD, Hernández F. 2002. *In vitro* determination of ruminal dry matter and cell wall degradation, and production of fermentation end-products of various by-products. Anim Res. 51:189–199.

McSweeney CS, Palmer B, McNeill DM, Krause DO. 2001. Microbial inter- actions with tannins: nutritional consequences for ruminants. Anim Feed Sci Technol. 91:83–93.

Miron J, Ben-Ghedalia D, Morrison M. 2001. Invited review: adhesion mechanisms of rumen cellulolytic bacteria. J Dairy Sci. 84:1294–1309.

Moss AR, Jouany JP, Newbold CJ. 2000. Methane production by ruminants: its contribution to global warming. Annu Zoot. 49:231–253.

Mould FL, Erskov ER. 1983. Manipulation of rumen fluid pH and its influence on cellulolysis in sacco, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. Anim Feed Sci Technol. 10:1–14.
Newbold CJ, Lassalas B, Jouany JP. 1995. The importance of methanogens associated with ciliate protozoa in ruminal methane production in vitro. Lett Appl Microbiol. 21:230–234.

Ørskov ER, Ryle M. 1990. Energy nutrition in ruminants. New York, NY: Elsevier Applied Science.

Patra AK, Saxena J. 2010. A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry. 71:1198–1222.

Pen B, Takaura K, Yamaguchi S, Asa R, Takahashi J. 2007. Effects of *Yucca schidigera* and *Quillaja saponaria* with or without b-1, 4 galactooligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. Anim Feed Sci Technol. 138:75–88.

Quezel P, Santa S. 1963. Nouvelle Flore de l’Algérie et des régions désertiques méridionales, vol. 1–2. Paris: CNRS.

Rahman MM, Salleh MAM, Sultana N, Kim MJ, Ra CS. 2013. Estimation of total volatile fatty acid (VFA) from total organic carbons (TOCs) assessment through in vitro fermentation of livestock feeds. Afr J Microbiol Res. 7 (15):1378–1384.

Rira M, Morgavi DP, Archimède H, Marie-Magdeleine C, Popova M, Bousseboua H, Doreau M. 2014. Potential of tannin-rich plants for modulating ruminal microbes and ruminal fermentation in sheep. J Anim Sci. 93:334–347.

SAS. 2000. SAS/STAT® Users Guide, 8.1. 4th ed. Cary, NC: SAS Institute Inc.

Sauvant D, Giger-Reverdin S. 2009. Modélisation des interactions digestives et de la production de méthane. Inra Prod Anim. 22:375–384.

Sirohi SK, Pandey N, Goel N, Singh B, Mohini M, Pandey P, Chaudhry PP. 2009. Microbial activity and ruminal methanogenesis as affected by plant secondary metabolites in different plant extracts. Int J Environ Sci Eng. 1:52–58.

Slyter LL. 1986. The ability of pH-selected mixed ruminal microbial population to digest fiber at various pHs. Appl Environ Microbiol. 52:390–391.

Sung HG, Kobayashi Y, Chang J, Ha A, Hwang IH, Ha JK. 2007. Low ruminal pH reduces dietary fiber digestion via reduced microbial attachment. Asian-Aust J Anim Sci. 20:200–207.

Takahashi J, Miyagawa T, Kojima Y, Umetsu K. 2000. Effects of *Yucca schidigera* extract, probiotics, monensin and L-cysteine on rumen methanogenesis. Asian-Aust J Anim Sci. 13:499–501.

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France J. 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol. 48:185–197.

Unger N, Bond TC, Wang JS, Koch DM, Menon S, Shindell DT, Bauer S. 2010. Attribution of climate forcing to economic sectors. Proc Natl Acad Sci USA. 107:3382–3387.

Wang CJ, Wang SP, Zhou H. 2009. Influences of flavomycin, ropadiar, and saponin on nutrient digestibility, rumen fermentation, and methane emission from sheep. Anim Feed Sci Technol. 148:157–166.

Zeleke AB, Clément C, Hess HD, Kreuzer M, Soliva CR. 2005. Effect of foliage from multi-purpose trees and a leguminous crop residue on in vitro methanogenesis and ruminal N use. Int Cong Ser. 1293:168–171.