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To cite this article: Samir Medjekal, Mouloud Ghadbane, Raúl Bodas, Hacène Bousseboua & Secundino López (2018) Volatile fatty acids and methane production from browse species of Algerian arid and semi-arid areas, Journal of Applied Animal Research, 46:1, 44-49, DOI: 10.1080/09712119.2016.1257432

To link to this article: https://doi.org/10.1080/09712119.2016.1257432

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Published online: 18 Nov 2016.

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**Volatile fatty acids and methane production from browse species of Algerian arid and semi-arid areas**

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**ABSTRACT**

The objective of the study was to determine the in vitro rumen fermentation end-products of 10 browse species. Serum bottles containing 500 mg of substrate, 10 ml of sheep rumen fluid and 40 ml of buffered medium were incubated for 24 h. After incubation, pH, methane and volatile fatty acid (VFA) productions were recorded. There were differences among feedstuffs (p < .05) in pH and VFA production. Astragalus gombo resulted in the highest and Stipa tenacissima in the lowest VFA production. Gas production was highest for Medicago sativa and lowest for S. tenacissima. Methane production (ml/g DM incubated) varied greatly. The lowest methane production was for S. tenacissima and Arthrocnemum macrostachyum (11.4 and 11.5 ml/g DM, respectively) and the highest for M. sativa and A. gombo (25.8 and 22.7 ml/g DM, respectively). The differences among species shrank when methane was expressed per ml of total gas produced or per mol of VFA produced. This indicates that a lower methane production would be due to a low fermentability of the substrate incubated, rather than to a specific inhibitory effect on methanogenesis. Hence, the 10 browse species studied herein would show little potential for mitigating ruminal methane production.

**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 (López 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 thermost 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 acid/l 0.5N HCl) for VFA 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 × 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 Autosystem XL GC (Perkin-Elmer Inc., USA), equipped with a semicapillary TR-FFAP (30 m × 0.53 mm × 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 2. 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:proionate 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 (Slyter 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 cellylolytic bacteria 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, Bouaza 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 Gueddim) 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 produced mainly 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).

<table>
<thead>
<tr>
<th>Plant family</th>
<th>Plant species</th>
<th>pH</th>
<th>Total VFA</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Valerate</th>
<th>C2:C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicotyledons</td>
<td>Arthrocneum macrostachyum</td>
<td>6.71</td>
<td>1.61</td>
<td>75.8</td>
<td>18.5</td>
<td>49.9</td>
<td>12.3</td>
<td>4.04</td>
</tr>
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<td>Atriplex canescens</td>
<td>6.69</td>
<td>1.94</td>
<td>72.6</td>
<td>17.6</td>
<td>83.1</td>
<td>12.5</td>
<td>4.19</td>
</tr>
<tr>
<td></td>
<td>Artemisia herba-alba</td>
<td>6.48</td>
<td>2.71</td>
<td>77.5</td>
<td>15.6</td>
<td>48.8</td>
<td>14.4</td>
<td>4.99</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>Astragalus gombo</td>
<td>6.55</td>
<td>3.71</td>
<td>742</td>
<td>17.5</td>
<td>56.3</td>
<td>14.5</td>
<td>4.31</td>
</tr>
<tr>
<td>Fabaceae – Leguminosae</td>
<td>Calobota saharae</td>
<td>6.59</td>
<td>2.92</td>
<td>742</td>
<td>18.5</td>
<td>50.1</td>
<td>13.8</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td>Hedysarum coronarium</td>
<td>6.73</td>
<td>2.66</td>
<td>73.8</td>
<td>19.9</td>
<td>39.1</td>
<td>10.5</td>
<td>3.57</td>
</tr>
<tr>
<td></td>
<td>Medicago sativa</td>
<td>6.53</td>
<td>3.59</td>
<td>68.6</td>
<td>19.5</td>
<td>68.4</td>
<td>25.8</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>Ononis natrix</td>
<td>6.61</td>
<td>2.24</td>
<td>78.6</td>
<td>13.7</td>
<td>58.4</td>
<td>17.8</td>
<td>5.81</td>
</tr>
<tr>
<td>Poaceae – Gramineae</td>
<td>Hordeum vulgare (straw)</td>
<td>6.29</td>
<td>3.12</td>
<td>69.9</td>
<td>23.1</td>
<td>60.8</td>
<td>9.0</td>
<td>3.06</td>
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<tr>
<td></td>
<td>Stipa tenacissima</td>
<td>6.56</td>
<td>1.16</td>
<td>75.3</td>
<td>16.9</td>
<td>54.9</td>
<td>17.8</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.02</td>
<td>0.155</td>
<td>6.23</td>
<td>6.39</td>
<td>4.62</td>
<td>1.49</td>
<td>0.175</td>
</tr>
</tbody>
</table>

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

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 feed (Geta-chew 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 Rhexum 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 (Holthausen 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 methanogen 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 VFA produced at 24 h of incubation of Algerian browse species.

<table>
<thead>
<tr>
<th>Plant family</th>
<th>Plant species</th>
<th>Gas production (ml/g DM)</th>
<th>Methane (ml/g DM)</th>
<th>ml methane/100 ml gas</th>
<th>CH4:VFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicotyledons</td>
<td>Arthrocneum macrostachyum</td>
<td>57.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.5&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>20.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.325&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Amplex canescens</td>
<td>58.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.313&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asteraceae – Leguminosae</td>
<td>Astragalus gulosus</td>
<td>107.4&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>22.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.269&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Callobota saharae</td>
<td>102.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>20.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.310&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Hedysarum coronarium</td>
<td>92.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>21.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.364&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Medicago sativa</td>
<td>119.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.335&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Ononis natrix</td>
<td>67.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.9&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>20.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.276&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monocotyledons</td>
<td>Hordeum vulgare (straw)</td>
<td>110.9&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>21.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.318&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Stipa tenacissima</td>
<td>40.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td>11.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>SEM</td>
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<td>0.83</td>
<td>1.26</td>
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<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.005</td>
</tr>
</tbody>
</table>

Notes: CH4:VFA = mmol of methane per mmol total VFA produced. 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).
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**


