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# Comparative evaluation of chemical composition, in vitro fermentation and methane production of selected tree forages

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**Abstract** The objective of this study was to investigate the nutritive value of leaves of six tree forage species [*Acacia albida* (Del.), *Acacia nilotica* (L.) Del., *Balanites aegyptiaca* (L.) Del., *Leucaena leucocephala* (Lam.) de Wit, *Moringa stenopetala* (Baker f.) Cufodontis and *Morus alba* (L.)] sampled from south-western part of Ethiopian rift valley. The leaf samples were analyzed for chemical composition using official methods, and in vitro gas test was conducted to estimate their metabolizable energy content, organic matter digestibility (OMD), short-chain fatty acid (SCFA), ammonia nitrogen ($NH_3$-N) and gas production characteristics. Crude protein was highest in *L. leucocephala* (213.09 g $kg^{-1}$ DM) and *M. stenopetala* (209.80 g $kg^{-1}$ DM) and the lowest was in *M. alba* (101.63 g $kg^{-1}$ DM). The fiber (NDF, ADF and ADL) fractions were highest in *B. aegyptiaca* and lowest in *M. stenopetala*. Condensed tannin concentration ranged from 10.76 g $kg^{-1}$ DM in *B. aegyptiaca* to 81.89 g $kg^{-1}$ DM in *A. nilotica*. The OMD, cumulative gas volume, SCFA and $NH_3$-N production were highest ($p < 0.05$) in *M. stenopetala* and *M. alba* followed by the values measured for *L. leucocephala*, *B. aegyptiaca* and *A. albida* and lowest was for *A. nilotica*. Highest methane ($CH_4$) production per gram of dry matter was noted for *M. stenopetala* and the lowest for *A. nilotica* though opposite situation was observed when $CH_4$ production was expressed as a ratio to total gas produced. Overall, most of the studied browse plants are desirable candidate species for mitigation of enteric methane emission while supplying optimum level of nitrogen if used as a supplement to low-quality forages.

**Keywords** Chemical composition · In vitro gas · Methane

**Abbreviations**

| | |
|---|---|
| ADF | Acid detergent fiber |
| ADL | Acid detergent lignin |
| CT | Condensed tannin |
| CP | Crude protein |
| DM | Dry matter |
| DMI | Dry matter intake |
| ME | Metabolizable energy |
| aNDF | Neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash |
| $NH_3$-N | Ammonia nitrogen |
| OM | Organic matter |
| OMD | Organic matter digestibility |
| SCFA | Short-chain fatty acid |

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## Introduction

Feed shortage is among the main constraints to Ethiopian livestock production (Bediye et al. 2001; Mengistu 2006). Forage availability in grasslands depends on rain fall pattern. Grazing lands are steadily shrinking by being converted to arable lands and are restricted to areas that have little value for farming potential (Mengistu 2006; Tolera 2007). This resulted in animals increasingly being fed on crop residues. At national level, cereal and pulse crop residues contribute about 50% of the total feed supply followed by grazing (44%), whereas the balance is supplied by other agricultural and agro-industrial by-products (Tolera 2007). However, crop residues are not capable of enhancing productivity of animals when fed solely because of their deficiency in essential nutrients, low digestibility and low voluntary intake by animals (Migwi et al. 2013). As these feeds have high content of lingo-cellulosic components, their digestibility in the rumen is very low resulting in low nutrient release and also high enteric methane production. High methane production is given by high fiber content in diet, which produces more acetate and butyrate and less propionate. The amount and proportion of these volatile fatty acids determine methane production. Fibrous feeds are characterized in general by less propionate shifted fermentation with more methane production as compared with concentrates (McDonald et al. 2011). Due to this, the intake of high-fiber/low-protein-containing forages in ruminants is often associated with a significant loss of feed energy as heat increment and $CH_4$ gas production with the later contributing significantly to global warming (Migwi et al. 2013).

Increasing the proportion of concentrate in the diet of ruminant animals has been suggested (Johnson and Johnson 1995; Lascano and Cárdenas 2010) as potential strategy to reduce enteric methane emission. However, in grassland and crop residue-based production systems which are dominant in Ethiopia, ruminants usually receive relatively small amounts of concentrates during their production cycle. Feeding ruminants with leaves of nitrogenous trees and shrubs have been reported by several authors (Beauchemin et al. 2009; Hook et al. 2010; Bhatta et al. 2012; Goel and Makkar 2012) as an alternative low cost strategy to enhance the efficiency of rumen fermentation and subsequent animal performance as well as reduce rumen methane emission.

Various tree and shrub species were identified to be browsed by ruminant livestock in Ethiopia. Their diversity, nutrient composition and digestibility have been studied in different parts of the country (Shenkute et al. 2012; Yisehak and Janssens 2013; Weldemariam and Gebremichael 2015). However, information about their potential to reduce methane production is limited. Screening feed stuffs for their methane production potential would be advantageous in formulating low-methane-producing diets for ruminant animals. Therefore, this study was undertaken to comparatively evaluate leaves of six tree forage species viz. *Acacia albida* (Del.), *Acacia nilotica* (L.) Del., *Balanites aegyptiaca* (L.) Del., *Leucaena leucocephala* (Lam.) de Wit, *Moringa stenopetala* (Baker f.) Cufodontis and *Morus alba* (L.) in terms of their chemical composition, organic matter digestibility, short-chain fatty acid, ammonia nitrogen, gas production characteristics and methane production.

## Materials and methods

### Sample collection and preparation

Leaf samples of the studied browse species were collected from Alage Agricultural Technical and Vocational Education and Training (ATVET) College's compound during dry season (December 2015). The area is located at 38°28′E, 07°42′N at elevation ranging from 1580 to 1600 m above sea level in agro-ecologically semi-arid south western part of the Ethiopian rift valley. The area has three distinct seasons: short rainy (March to May), main rainy (June to September) and dry (October to February) seasons with average annual rain fall of 810 mm and minimum and maximum temperatures of 14.9 and 29.2 °C, respectively (Alage weather station—unpublished data). The browse species were purposely selected based on their multiple uses and drought tolerance. Mixtures of young and mature leaves with tender stems (twigs) were collected from three randomly selected trees of each species from five sampling sites and oven dried at 60 °C for 48 h. The dried samples were ground by using a Willey Mill to pass through a 1-mm sieve and kept in airtight containers until they were used for chemical analysis, in vitro digestibility and gas production determinations.

## Chemical analysis

Standard methods described in AOAC (1995) were used to determine dry matter (DM, method no 930.15), ash (method no. 924.05) and crude protein (CP, method no. 984.13) contents. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were analyzed according to Van Soest and Robertson (1985) using ANKOM F-57 filter bags in Ankom200 fiber analyzer (Ankom Technology, Macedon, NY, USA). For NDF analysis, the samples were extracted by neutral detergent solution (NDS) containing heat stable α-amylase and sodium sulfite and the residues were not corrected for ash. Hemi-cellulose and cellulose contents were calculated as NDF–ADF and ADF–ADL, respectively. The condensed tannin (CT) concentration was analyzed according to the method of Makkar (2003) by using butanol-HCl and ferric reagents and expressed as leucocyanidin equivalent.

## In vitro fermentation and gas production studies

The in vitro fermentation and gas production measurements were completed as described by Menke and Steingass (1988). A 200 mg milled leaf samples of each browse species was incubated in triplicate with buffered rumen fluid in calibrated glass syringes. The rumen fluid was collected from three rams to pre-heated (39 °C) thermos flask before morning feeding (grass hay *ad libitum* and 400 g concentrate/day) using stomach tube and mixed with buffered mineral solution in the ratio of 1:2 under continuous stirring and flushing with carbon dioxide. Then 30 ml of incubation medium (mixture of rumen fluid and buffered mineral solution) was dispensed into pre-heated sample containing and blank syringes and incubated in a water bath maintained at 39 °C. The reading of gas volume was recorded after 3, 6, 12, 24, 48 and 72 h of incubation, and the data were fitted to the model $y = a + b(1 - e^{-ct})$ to analyze gas production kinetics (Ørskov and McDonald 1979), where $y$ = total gas production at time $t$; $a$ = gas production from the immediately fermentable organic matter (OM), $b$ = gas production from slowly but potentially fermentable OM; $a + b$ = the potential gas production (the asymptote of the gas production curve); $c$ = the rate constant for the gas production $b$, $t$ = incubation time and $e$ = base of natural logarithm.

The amount of methane gas component was measured at the end of fermentation period by injecting 4.0 ml of NaOH (10 M) into each syringe containing the incubated samples following the technique described by Fievez et al. (2005). The measured methane gas volume was related to its respective total gas volume in order to estimate the methanogenic potential of the digestible OM (Moss et al. 2000).

Volume of gas produced after 24 h of incubation was used as an index of energy content and organic matter digestibility (OMD) as described by Menke and Steingass (1988) and short-chain fatty acid (SCFA) production according to Girma et al. (2002).

$$\text{ME (MJ/kg DM)} = 2.20 + (0.136 * \text{GP}_{24}) + (0.057 * \text{CP})$$

$$\text{OMD (\%)} = 14.88 + (0.889 * \text{GP}_{24}) + (0.45 * \text{CP}) + (0.651 * \text{XA})$$

$$\text{SCFA (mmol)} = (0.0222 * \text{GP}_{24}) - 0.00425$$

where GP, CP and XA are corrected 24 h gas volume (ml/200 mg), crude protein (%DM) and ash (%DM) of the incubated samples, respectively.

## Ammonia nitrogen ($NH_3$-N)

Ammonia nitrogen ($NH_3$-N) concentration in fermentation liquid of each syringe was determined according to Preston and Leng (1987) using Kjeldahl method.

## Statistical analysis

One-way analysis of variance (ANOVA) was carried out to compare the browse species in terms of their chemical composition, OMD, SCFA, $NH_3$-N, total gas and $CH_4$ production using general linear model (GLM) procedures of statistical analysis system (SAS) (2008). Significant differences between individual means were tested by using Duncan's Multiple Range Test, and differences among means at 5% level of significance were accepted as significant.

## Results

### Chemical composition

Considerable variations ($p < 0.05$) were observed among the browse species studied in terms of their chemical composition (Table 1). The ash content was highest ($p < 0.05$) in *M. alba* (206.8 g kg$^{-1}$ DM) followed by *B. aegyptiaca* (103.95 g kg$^{-1}$ DM), while the lowest was in *A. nilotica* (43.36 g kg$^{-1}$ DM). The OM contents varied from 793.16 g kg$^{-1}$ DM in *M. alba* to 956.63 g kg$^{-1}$ DM in *A. nilotica*. The highest ($p < 0.05$) CP content was noted in *L. leucocephala* (213.09 g kg$^{-1}$ DM) and *M. stenopetala* (209.80 g kg$^{-1}$ DM) and the lowest in *M. alba* (101.63 g kg$^{-1}$ DM). The CP contents of the other species were not significantly ($p > 0.05$) different from one another. The aNDF, ADF, ADL and cellulose contents were highest ($p < 0.05$) in *B. aegyptiaca* followed by *L. leucocephala,* whereas *M. stenopetala* had the lowest content of the fiber fractions except for cellulose. The cellulose content was lowest in *A. nilotica*.

The highest concentration ($p < 0.05$) of CT was measured in *A. nilotica* (81.89 g kg$^{-1}$ DM), whereas the lowest concentration was measured in *B. aegyptiaca* (10.76 g kg$^{-1}$ DM) and *M. stenopetala* (13.48 g kg$^{-1}$ DM) which were similar to each other. The CT concentration of *A. albida* (57.88 g kg$^{-1}$ DM) was significantly ($p < 0.05$) higher than that of *L. leucocephala* (23.20 g kg$^{-1}$ DM) and *M. alba* (16.32 g kg$^{-1}$ DM).

### Gas production

#### *Cumulative gas*

The volume gas released from fermented leaf samples increased with increasing incubation time over a period of 72 h (Fig. 1). Lowest gas volume was recorded for *A. nilotica* at each fermentation time, while *M. stenopetala* and *M. alba* produced higher ($p < 0.05$) gas volume than the other species over the whole fermentation period. At 12 h of incubation*, B. aegyptiaca* produced higher ($p < 0.05$) gas volume than *A. albida* though they were similar ($p > 0.05$) at 3 and 6 h of incubation. The gas volumes from *L. leucocephala* were similar ($p > 0.05$) with that of *B. aegyptiaca* and *A. albida* at 3 and 12 h of incubation, respectively, but lower than ($p < 0.05$) that of *A. albida* at 3, *B. aegyptiaca* at 12 and both at 6 h of incubation. From 24 h onwards, there were no differences among *A. albida*, *B. aegyptiaca* and *L. leucocephala* in their measured gas volume.

The gas volume from slowly but potentially fermentable OM "*b*" and potential gas production ($a + b$) were lowest in *A. nilotica* and highest in *M. stenopetala* and *M. alba*, whereas the remaining

**Table 1** Mean values of dry matter and its nutrient composition of leaf samples of the studied browse species

| | *Browse species* | | | | | | SEM |
|---|---|---|---|---|---|---|---|
| | *Acacia albida* | *Acacia nilotica* | *Balanites aegyptiaca* | *Leucaena leucocephala* | *Moringa stenopetala* | *Morus alba* | |
| DM (g/kg) | 431.26$^{b}$ | 365.97$^{c}$ | 363.81$^{d}$ | 333.90$^{f}$ | 438.52$^{a}$ | 335.73$^{e}$ | 0.39 |
| 105 °C DM (g/kg) | | | | | | | |
| Ash | 80.06$^{d}$ | 43.36$^{e}$ | 103.95$^{b}$ | 89.33$^{c}$ | 92.89$^{c}$ | 206.83$^{a}$ | 1.21 |
| OM | 919.93$^{b}$ | 956.63$^{a}$ | 896.04$^{d}$ | 910.66$^{c}$ | 907.10$^{c}$ | 793.16$^{e}$ | 1.21 |
| CP | 170.43$^{b}$ | 161.06$^{b}$ | 149.40$^{b}$ | 213.09$^{a}$ | 209.80$^{a}$ | 101.63$^{c}$ | 8.70 |
| aNDF | 198.69$^{c}$ | 190.83$^{c}$ | 310.07$^{a}$ | 230.29$^{b}$ | 148.63$^{d}$ | 200.02$^{c}$ | 4.83 |
| ADF | 130.32$^{c}$ | 112.89$^{d}$ | 228.17$^{a}$ | 139.97$^{b}$ | 93.33$^{e}$ | 125.38$^{c}$ | 3.06 |
| ADL | 46.21$^{bc}$ | 48.07$^{bc}$ | 98.71a | 53.84$^{b}$ | 19.74$^{d}$ | 44.32$^{c}$ | 2.86 |
| Hemi-cellulose | 68.36$^{c}$ | 77.93$^{b}$ | 81.89$^{b}$ | 90.31$^{a}$ | 55.29$^{d}$ | 74.64$^{bc}$ | 2.67 |
| Cellulose | 84.11$^{b}$ | 64.81$^{d}$ | 129.46$^{a}$ | 86.12$^{b}$ | 73.59$^{c}$ | 81.06$^{b}$ | 2.33 |
| CT | 57.88$^{b}$ | 81.89$^{a}$ | 10.76$^{d}$ | 23.20$^{c}$ | 13.48$^{d}$ | 16.32 $^{cd}$ | 2.62 |

Means within the same row with different superscripts are significantly different ($p < 0.05$)

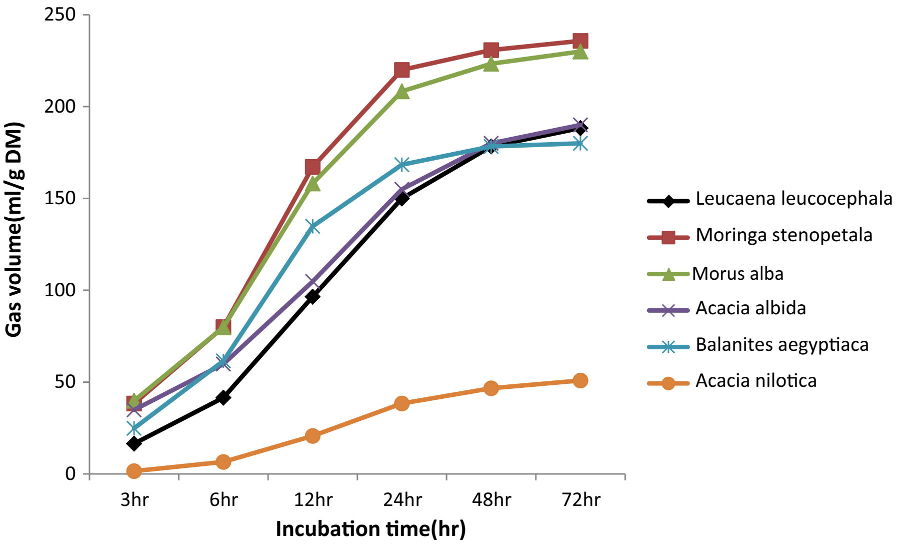

**Fig. 1** In vitro gas production patterns of the studied browse species

**Table 2** Mean values of gas production parameters (gas volume from immediately fermentable organic matter "*a,*" gas volume from slowly but potentially fermentable organic matter "*b,*" potential gas production "$a + b$" and gas production rate "*c*") of fermented leaf samples of the studied browse species

| Gas production Parameters | Browse species | | | | | | SEM |
|---|---|---|---|---|---|---|---|
| | *Acacia albida* | *Acacia nilotica* | *Balanites aegyptiaca* | *Leucaena. leucocephala* | *Moringa stenopetala* | *Morus alba* | |
| $a$ (ml) | $-2.38^{a}$ | $-9.98^{a}$ | $-51.91^{c}$ | $-27.17^{b}$ | $-48.68^{c}$ | $-35.39^{b}$ | 2.89 |
| $b$ (ml) | $192.45^{d}$ | $61.37^{e}$ | $232.14^{bc}$ | $215.70^{cd}$ | $284.46^{a}$ | $263.85^{ab}$ | 12.08 |
| $a + b$ (ml) | $190.07^{b}$ | $51.39^{c}$ | $180.22^{b}$ | $188.53^{b}$ | $235.77^{a}$ | $228.45^{a}$ | 10.71 |
| $c$ (ml $h^{-1}$) | $0.07^{c}$ | $0.06^{c}$ | $0.12^{a}$ | $0.07^{c}$ | $0.11^{b}$ | $0.10^{b}$ | 0.003 |

Means within the same row with different superscripts are significantly different ($p < 0.05$)

species had intermediate values which were comparable to one another (Table 2). The rate of gas production "*c*" was highest ($p < 0.05$) in *B. aegyptiaca* (0.12 ml $h^{-1}$) followed by *M. stenopetala* and *M. alba*, whereas *A. nilotica*, *A. albida* and *L. leucocephala* had lowest gas production rate.

### *Methane gas*

Methane gas production varied among fermented leaf samples. The highest ($p < 0.05$) $CH_4$ gas volume (51.66 ml $g^{-1}$ DM) was measured in *M. stenopetala*, whereas the lowest volume (18.33 ml $g^{-1}$ DM) was recorded for *A. nilotica*. There were no significant differences ($p > 0.05$) among *M. alba*, *L. leucocephala* and *B. aegyptiaca* in the volume of $CH_4$ released per gram of substrate fermented. The ratio of $CH_4$ to total gas volume was found to be highest ($p < 0.05$) in *A. nilotica* (0.36 ml) than in other species, which ranged from 0.15 to 0.21 ml.

### Energy content and organic matter digestibility

The ME content was highest ($p < 0.05$) in *M. stenopetala* (9.38 MJ $kg^{-1}$ DM) followed by *M. alba* (8.45 MJ $kg^{-1}$ DM), whereas the lowest ME value (4.16 MJ $kg^{-1}$ DM) was recorded for *A. nilotica*. Similarly, OMD values were lowest in *A. nilotica* and highest in *M. stenopetala* and *M. alba* with intermediate values in the other species.

### Short-chain fatty acid and ammonia nitrogen production

Significant variations ($p < 0.005$) were observed among the browse species in their SCFA and $NH_3$-N profiles

(Table 3). *M. stenopetala* was highest in both SCFA and $NH_3$-N production followed by *B. aegyptiaca*, *A. albida* and *L. leucocephala.* SCFA production was also highest in *M. alba,* while *A. nilotica* was observed to be lowest in both SCFA and $NH_3$-N production.

## Discussion

### Chemical composition

The chemical compositions of the browse species were comparable with values reported for similar species studied in different part of Ethiopia (Girma et al. 2002; Melesse 2011; Shenkute et al. 2012; Yisehak and Janssens 2013). All the species had above the minimum critical levels of CP content (80 g $kg^{-1}$ DM) required for normal function of rumen microorganisms (NRC 2000), and except for *B. aegyptiaca* and *M. alba*, the values observed for the other species were above the optimal range of 110–160 g $kg^{-1}$ DM recommended by NRC (2001) for maintenance requirements of small ruminants. This suggests the possibility of using these browse species as a dry season fodder and/or feed supplement to low-quality pastures and crop residues. Furthermore, their low to moderate fiber content is their positive attribute since the voluntary DM intake and digestibility are dependent on the cell wall content especially the NDF and lignin contents (Bakshi and Wadhw 2004).

Except *A. nilotica* and *A. albida*, the CT values of the other browse species were less than 50 g $kg^{-1}$ DM which is within a range with beneficial effect (Frutos et al. 2004; Mueller-Harvey 2006) if included in the diet of ruminants. Low levels of tannins have nutritional benefits for ruminants by protecting dietary proteins from excessive microbial hydrolysis and deamination in the rumen, thereby increasing the availability of feed proteins for intestinal digestion and enabling more amino acids to be absorbed postruminally (Girma et al. 2000; Bunglavan and Dutta 2013). This reduces the excretion of urea through urine by promoting greater nitrogen retention and by increasing urea recycling to the rumen (Bunglavan and Dutta 2013). However, the actual CT values in the browse species could be higher than the values reported, since a considerable amount of tannins is bound to either fiber and/or proteins and remains unextracted (Makkar 2003).

### Gas production characteristics

The volume of gas produced from fermented leaf samples of the studied browse species increased with increasing time of incubation. This shows that the DM in their leaves can still be degraded beyond 72 h of incubation which further reflects the sampling season (December) when most of the forages in the study area are fibrous and therefore took longer time for the DM to be degraded. The amount of gas produced in the rumen is a reflection of the extent to which the feed is

**Table 3** Mean values of organic matter digestibility (OMD, $g^{-1}kg$), metabolizable energy (ME, MJ $kg^{-1}$ DM), short-chain fatty acids (SCFAs, mmol), ammonia nitrogen ($NH_3$-N, mg $L^{-1}$), methane gas volume ($CH_4$, ml $g^{-1}$ DM) and its ratio (v/v) to total gas volume of fermented leaf samples of the studied browse species

| | Browse species | | | | | | SEM |
|---|---|---|---|---|---|---|---|
| | *Acacia albida* | *Acacia nilotica* | *Balanites aegyptiaca* | *Leucaena leucocephala* | *Moringa stenopetala* | *Morus alba* | |
| OMD | $615.55^b$ | $340.88^c$ | $604.14^b$ | $638.06^b$ | $722.88^a$ | $735.37^a$ | 1.97 |
| ME | $7.39^c$ | $4.16^d$ | $7.63^{bc}$ | $7.49^{bc}$ | $9.38^a$ | $8.45^b$ | 0.3 |
| SCFA | $0.68^b$ | $0.17^c$ | $0.74^b$ | $0.66^b$ | $0.97^a$ | $0.92^a$ | 0.05 |
| $NH_3$-N | $17.01^c$ | $9.07^d$ | $19.85^b$ | $20.98^b$ | $30.05^a$ | $20.41^b$ | 0.73 |
| $CH_4$ | $29.16^c$ | $18.33^d$ | $37.50^{bc}$ | $34.16^{bc}$ | $51.66^a$ | $40.83^b$ | 2.8 |
| $CH_4$: total gas | $0.15^b$ | $0.36^a$ | $0.21^b$ | $0.18^b$ | $0.20^b$ | $0.18^b$ | 0.02 |

Means within the same row with different superscripts are significantly different, $p < 0.05$

degraded and fermented (Girma et al. 2004) and can vary among different feed stuffs due to inherent characteristics of their chemical composition (Sejian et al. 2011). The maximum gas production volume (ml $g^{-1}$DM) and higher values of calculated parameters ($a + b$, $b$ and $c$) observed for *M. stenopetala* and *M. alba* indicated that they are the most fermentable and digestible browses as evidenced from their higher OMD. This could be due to their relatively lower CT and fiber contents. Tannins can make feed constituents less digestible by binding to them (Mueller-Harvey 2006). The fiber contents also reduce feed digestibility through their interwoven matrix of polymers which creates barriers against the microbial invasion and limits their access to digestible cell wall components (McDonald et al. 2011). Contrary to this concept, *B. aegyptiaca* with its highest fiber (NDF, ADF, Cellulose and ADL) contents showed higher OMD and produced relatively high volume of gas next to *M. stenopetala* and *M. alba* with highest production rate (0.12 ml $h^{-1}$). This could be due to better digestibility of its fiber fraction though it was not tested in this study. The highest gas production rate ($c$) of *B. aegyptiaca* also suggests its highest intake due to its fast ruminal passage rate (Khazaal et al. 1995). Despite its higher CT content, *A. albida* had higher gas volume records at 3, 6, 24 and 72 h of incubation similar to *B. aegyptiaca* which had lowest CT content. This shows that the CT content of *A. albida* had less depressing effect on rumen microbial activity. The tannins of different plant species have different physical and chemical properties, and therefore they have very diverse biological properties (Frutos et al. 2004). On the other hand, the lowest gas volume (ml $g^{-1}$ DM) of *A. nilotica* over the whole incubation period with slow production rate ($c$) was possibly due to its highest CT content that might reduced its OMD through formation of tannin–carbohydrate and tannin–protein complexes that are less degradable or toxicity to rumen microbes (Bhatta et al. 2009). The observed negative values of gas volume from immediately fermentable OM “$a$” for the examined browse species do not conform to the concept of gas production from the soluble and immediately fermentable fraction. This could be due to delayed onset of fermentation caused by delayed microbial colonization (Blummel and Becker 1997) or insufficiency of the instantly fermentable OM to produce significant amount of gas.

Enteric methane production primarily depends on the quantity and quality of the diet as they affect rate of fermentation and passage (Kumar et al. 2009). In most cases, feedstuffs that show high capacity for gas production are also observed to show high methane production (Njidda and Nasiru 2010; Seresinhe et al. 2012). This possibly explained why *M. stenopetala* and *M. alba* in the present study had relatively higher $CH_4$ production. Their higher $CH_4$ gas volume was due to their higher fermentation potential that could be associated with their low CT contents. The lower CT content of these browse species may have minimum depressing effect on rumen microbes that are responsible for production of $CH_4$. On the other hand, the lowest $CH_4$ volume of *A. nilotica* (18.33 ml $g^{-1}$ DM) could be related to its highest content of CT that might depress fermentation of the substrate through its bacteriocidal and bacteriostatic effects on rumen microbes and inactivation of their enzymes. The inhibitory effect of CT in *A. albida* on rumen microbes seems more pronounced on methanogens than its effect on substrate degrading microbes, as evidenced by its higher cumulative gas volume record (190.07 ml $g^{-1}$ DM) and lower methane (29.16 ml $g^{-1}$ DM). Despite its lowest CT (10.76 g $kg^{-1}$ DM) content, *B. aegyptiaca* produced less methane (37.50 ml $g^{-1}$ DM) as compared to that of *M. stenopetala* (51.66 ml $g^{-1}$ DM) which had also lowest CT (13.48 g $kg^{-1}$ DM) content. This could be because of its lower OMD due to its higher cell wall fraction content compared to *M. stenopetala*.

However, when ranking forages according to their $CH_4$-emission potential, the ratio of methane-to-total gas is more relevant than absolute methane formation: a low value for this proportion indicates a low methanogenic potential of the digestible part of the feed, i.e., fewer methane production per unit net gas volume production (Moss et al. 2000; Bezabih et al. 2013). In this light, range of $CH_4$ to total gas ratio values (0.15–0.36 ml) observed in the present study shows the potential opportunity to screen the available browse species with the goal of providing low $CH_4$-emission forage diets to ruminants. *Acaciqa nilotica* had the highest methane-to-total gas ratio value (0.36 ml), which is opposite to its lowest absolute methane volume record. This indicates that the $CH_4$ concentration in the total gas produced from its digested OM was higher as compared to that of the other species. Therefore, *A. nilotica* with its highest

$CH_4$ to total gas volume ratio (0.36 ml) may contribute more to the green house effect than the others if fed to ruminant animals.

### Short-chain fatty acids and ammonia nitrogen production

The observed difference among the browse species studied in their SCFA and $NH_3$-N production was possibly due to variation of their fermentable carbohydrate and protein available to microbes and chemical factors inhibiting feed digestibility. In the present study, CT and cell wall fractions showed depressing effect on the digestibility and fermentability of the substrates (Bakshi and Wadhw 2004; Bhatta et al. 2009). The lowest SCFA and $NH_3$-N production values of *A. nilotica* could be linked to its highest CT content which might reduced its OMD. On the other hand, the highest SCFA of *M. stenopetala* and *M. alba* may be because of their higher proportion of fermentable carbohydrate (Girma et al. 1999) and low CT and fiber contents. The higher CP content of *M. stenopetala* could also be the cause for its highest SCFA and $NH_3$-N since dietary crude protein can influence the amount of SCFA and $NH_3$-N (Njidda and Nasiru 2010) that are produced during organic matter fermentation. *Acacia albida* produced higher SCFA next to *M. stenopetala* and *M. alba* and lower $NH_3$-N regardless of its relatively higher CT content. This shows that the binding effect of its CT is more pronounced on protein than carbohydrates. The higher fiber and CT contents of *L. luecocephala* seem to be the cause for its lower $NH_3$-N value though it had highest CP content similar to *M. stenopetala*.

## Conclusion

There were variations among the examined browse species in their chemical composition, *in vitro* fermentation and gas production parameters, presenting an opportunity to select forage species with high nutritional quality and lower $CH_4$-emission potential. With its highest CP and ME contents as well as highest OMD, SCFA and $NH_3$-N production, coupled with its lower $CH_4$ to total gas ratio, *M. stenopetala* was found to be nutritionally and environmentally best followed by *B. aegyptiaca* and *L. leucocephala*. Even though *M. alba* had the lowest CP content, its highest OMD and substantial $NH_3$-N generation with low methanogenic potential make it potentially useful browse of low $CH_4$ producing fodder and /or supplement. In contrast, *A. nilotica* had lowest ME and showed lowest OMD, SCFA and $NH_3$-N production with highest methanogenic potential indicating its lower nutritional quality and higher potential to contribute to the green house effect than the others. However, these browse species need to be characterized further in vivo and/or in vitro so that optimal level of inclusion in the diet and feeding conditions are properly defined.

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