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Chemical characterization and in vitro methane production of selected agroforestry plants as dry season feeding of ruminants livestock

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Abstract The vagaries in climatic changes disrupt the prevailing weather conditions leading to temperature extremes and protracted rainfall pattern which subsequently affect the quality of forages. Ruminant animals had been implicated as a major source of enteric methane production to the greenhouse effect. Grazing on this low-quality forages extends the time of fattening thereby increasing the amount of methane produced. In this case, effort has been shifted to the feeding of agroforestry plants (browse and tree species) which are available all year round with high nutritive profile. Leaves of selected agroforestry plant species *Thevetia peruviana* (Pers) K. Schum, *Piliostigma thonningii* (Schumach.) Milne-Redhead*,* *Spondia mombin* L. and *Newbouldia laevis* (P. Beauv.) Seem were harvested, dried, milled and analysed for their chemical constituents, mineral composition, in vitro and methane gas production. Data collected were analysed using one-way analysis of variance. Significant ($p < 0.05$) differences were observed in the nutritive value of the selected plants except for dry matter and ether extract contents. NDF values ranged from 606.7 g/kg in *T. peruviana* to 666.7 g/kg in *P. thonngii. P. thonngii* recorded the highest value (533.3 g/kg) for acid detergent fibre with the lowest value (500.0 g/kg) observed in *N. laevis.* There were significantly ($p < 0.05$) differences among the recorded mineral components of selected plants except for Mn. *N. laevis* had the highest volume of gas, while *P. thonngii* had the least. However, *N. laevis* had the least ($p < 0.05$) insoluble fibre fraction (*b*), methane gas production with considerably highest metabolizable energy. With the appreciable level of nutrients, especially in terms of crude protein content and reduced methane production, research into various agroforestry plants will be of high interest to be adopted in ruminant diets.

Keywords Feed · Ruminants · Nutrient composition · Methane production

Introduction

The impact of climatic variability has been a major focal point of livestock scientist (Sanz-Sáez et al. 2012) with ruminants' livestock been implicated as the major source of enteric methane production. The rainfall distribution pattern has been disrupted, consequently, affecting the seasonal weather conditions as well as forage production. In this case, the available forages are lignified with adverse effects on voluntary

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intake, digestibility, productive and reproductive performance. Thus, ruminant animals in the affected regions survive on repeated shortage of feed resources of low nutritive value for most part of the year (Robles et al. 2009), thereby leading to extended production time and subsequently higher methane production. Abdelgawad et al. (2014) stated that temperature extremes have been experienced in some regions leading to physiological stress and reduced performance of these animals.

Consequently, interest has been shifted to rational utilization of potential livestock feed resources such as browse species, shrubs and tree species that are adapted to these environments and are evergreen all year round (Salem et al. 2004). In Nigeria, there abound many agroforestry trees and browse species which are underexploited.

Researches (Thornton and Herrero 2010; Hristov et al. 2013) indicated that modification of feeding practices like adopting the use of agroforestry plants, conservation of feed for different agro-ecological zones, alteration of feeding frequency/time among others can reduce the menace caused by climate change, improve feed intake and reduce shortage of feed during the long dry season.

Indigenous tree and browse species are useful agroforestry feed resources for livestock animals. These agroforestry trees are evergreen that provide vegetation with quality nutrients than other annual grass and herbaceous species. They prevent desertification, mitigate droughts, allow soil fixation and enhance the restoration of the vegetation and the recuperation of rangelands (Aregawi et al. 2008). They have been considered as useful sources of cheap feed for ruminant animals in developing countries, especially during dry period when herbaceous pasture grasses and legumes are senescence. Since they are able to retain their green leaves, they bridge the gap often created by decline in the nutritive potentials of natural pastures. Their ability to retain their greenish colouration helps maintain their nutritive content, thus, making them essential protein and energy sources (Olafadehan 2013; Yusuf et al. 2018).

In addition, they also contain useful phenols and other compounds to reduce the contribution of ruminant animals to overall methane production. Therefore, research into chemical characterization and methane production of selected agroforestry tree and browse species was evaluated in this study.

Materials and methods

Sample collection and preparation

Leaves and twigs of selected species *Thevetia peruviana* (Pers) K. Schum., *Piliostigma thonningii (*Schumach.) Milne-Redhead*,* *Spondia mombin* L. and *Newbouldia laevis* (P. Beauv.) Seem were harvested from the pasture plot of Oyo State College of Agriculture and Technology, Igboora, Oyo State. The harvested parts were air-died for a month after which they were ground and stored (for 3 weeks) in an airtight container for further analysis.

Chemical analysis

The dry matter was determined by oven drying the samples at 60 °C until constant weight was reached. Crude protein, ether extract and ash contents of the milled (ground to pass through the 2-mm screen) plant samples were determined as described by AOAC (2000). Non-fibre carbohydrate was calculated as NFC = 100 − (CP + Ash + EE + NDF).

Fibre fraction analysis

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) of the milled plants samples were determined with the procedure of Van soest et al. (1991) using ANKOM 2000 fibre analyser. Cellulose content was measured as the difference between ADF and ADL, while hemicellulose content was also calculated as the difference between NDF and ADF.

Mineral determination

Sub-samples of the dried plants were analysed for some macro-minerals (Ca, P, K and Mg) and micro-minerals (Cu and Fe). The concentration of potassium (K) was estimated after wet digestion in nitric acid and per chloric acid using Nexion ICP-MS machine. Concentrations of calcium, phosphorus, magnesium, copper and iron were determined with atomic absorption spectrophotometry as stated by Fritz and Schenk (1979).

In vitro gas production

This was determined following the procedure of Menke and Steingass (1988). Approximately, 200 mg of the samples (in triplicates) was measured and then placed into 100 ml graduated glass syringes. The rumen fluid (inoculum) collected from culled N'dama heifers was placed inside a pre-warmed flask (39 °C) early in the morning (6.00am). The inoculum was strained through two layers of cheese cloth, with sodium and ammonium bicarbonate buffer (35 g $NaHCO_3$ plus 4 g NH_4HCO_3 per litre) at a ratio of 1:2 (v/v) to prevent lowered pH of the rumen fluid which could result in decreased microbial activities. Thereafter, 30 ml of the buffered inoculum was drawn into each syringe containing the milled samples and the gas released was read from the graduated syringe. Empty syringes containing 30 ml of the buffered inoculums (in triplicate) only were included as the control. The syringes were agitated for 30 min afterwards and subsequently every four hours until the end of the incubation period. The gas produced was recorded at 0, 3, 6, 12, 24, 36 and 48 h of incubation.

The data obtained from in vitro gas production were fitted to the nonlinear equation (Larbi et al. 1996):

$$V(\mathrm{ml}/0.2\,\mathrm{g\,DM}) = \mathrm{GV}(I - \mathrm{e}^{-\mathrm{ct}}) \quad (1)$$

where V = the potential gas production, GV = the volume of gas and, ct = the fractional rate of gas production.

Organic matter digestibility (OMD) was estimated as (Menke and Steingass 1988)

$$\mathrm{OMD} = 14.88 + 0.889\,\mathrm{GV} + 0.45\,\mathrm{CP} + 0.651\,\mathrm{ash} \quad (2)$$

Short-chain fatty acids (SCFA) were estimated as (Getachew et al. 2000)

$$\mathrm{SCFA} = 0.0239\mathrm{GV} - 0.0601 \quad (3)$$

Metabolizable energy (ME) was calculated as (Menke and Steingass 1988)

$$\mathrm{ME} = 2.20 + 0.136\,\mathrm{GV} + 0.057\mathrm{CP} + 0.029\mathrm{CP}^2 \quad (4)$$

Total gas volume (GV) was expressed as ml/0.2 g DM, CP and ash as %, ME as MJ/kg DM and SCFA as μmol/g DM

Methane determination

The methane gas by each browse sample was determined by administration of 4 ml 10 N NaOH into each incubated samples at the end of 48 h incubation period. The NaOH was added to remove the carbon dioxide produced during fermentation process; the remaining volume of gas was recorded as methane gas (Demeyer et al. 1988; Fievez et al. 2005).

Statistical analysis

Data generated for proximate composition, fibre fractions and minerals composition were subjected to one-way analysis of variance (ANOVA), while the treatment means were separated using Duncan's Multiple Range Test as contained in the SAS (1999) package.

Results

Proximate composition (%) of selected plants

In Table 1, significant differences were observed ($p < 0.05$) in the nutritive value of the selected plants except for dry matter and ether extract contents. The crude protein (CP) content of *N. laevis* recorded the highest value (160.9 g/kg) with the lowest value (107.4 g/kg) observed in *P. thonngii,* ash contents of the plants ranged from 50.0 g/kg in *N. laevis* and *T. peruviana* to 83.3 g/kg in *P. thonngii. P. thonngii* had the least value (72.6 g/kg) for non-fibre carbohydrate (NFC) with the highest value (130.0 g/kg) recorded in *T. peruviana*.

Fibre composition (%) of selected plants

The fibre components of most of the selected plants were significantly ($p < 0.05$) different (Table 2). NDF values ranged from 606.7 g/kg in *T. peruviana* to 666.7 g/kg in *P. thonngii. P. thonngii* recorded the highest value (533.3 g/kg) for acid detergent fibre (ADF) with the lowest value (500.0 g/kg) observed in *N. laevis,* while acid detergent lignin (ADL) values ranged from 86.7 g/kg in *N. laevis* and *T. peruviana* to 113.3 g/kg in *P. thonngii*.

Table 1 Proximate composition (g/kg) of selected browse plants

Sample	DM	CP	EE	ASH	NFC
Piliostigma thonningii	956.7	107.4^{c}	70.0	83.3^{a}	72.6^{b}
Spondia mombin	950.0	145.1^{b}	60.0	63.3^{ab}	105.0^{ab}
Newbouldia laevis	943.3	160.9^{a}	60.0	50.0^{b}	115.8^{a}
Thevetia peruviana	953.3	156.8^{a}	56.7	50.0^{b}	130.0^{a}
SEM	2.3	6.5	2.7	5.2	7.8

SEM standard error of mean, *DM* dry matter, *EE* ether extract, *CP* crude protein, *NFC* non-fibre carbohydrate

^{a,b,c}Means in same column with different superscripts are significantly ($p < 0.05$) different

Table 2 Fibre composition (g/kg) of selected browse plants

Sample	NDF	ADF	ADL	HEM	CELL
Piliostigma thonningii	666.7^{a}	533.3^{a}	113.3^{a}	133.3	420.0
Spondia mombin	626.7^{b}	513.3^{ab}	93.3^{ab}	113.3	420.0
Newbouldia laevis	613.3^{b}	500.0^{b}	86.7^{b}	113.3	413.3
Thevetia peruviana	606.7^{b}	506.7^{ab}	86.7^{b}	100.0	420.0
SEM	7.6	5.1	4.4	0.56	4.6

SEM standard error of mean, *NDF* neutral detergent fibre, *ADF* acid detergent fibre, *ADL* acid detergent lignin, *HEM* hemicellulose, *CELL* cellulose

^{a,b}Means in same column with different superscripts are significantly ($p < 0.05$) different

Mineral contents (mg/kg) of selected plants

There were significant ($p < 0.05$) differences among the recorded mineral composition of selected plants except for Mn (Table 3). Calcium values ranged from 21.23 mg/kg in *P thonngi* to 32.71 mg/kg in *S mobin*. *T peruviana* recorded the highest values (35.27 and 157.58 mg/kg) for Mg and K, respectively, with lowest value of 27.47 and 66.00 mg/kg observed in *S. mobin* and *N. laevis*, respectively. *P. thonngii* recorded the highest value of 0.35 mg/kg for P. The value for Na ranged from 9.83 mg/kg in *N. thonngi* to 22.39 mg/kg in *S. mobin. T. peruviana* recorded the highest value of 1.78 mg/kg and 0.45 mg/kg for Fe and Cu, respectively, while Zn values ranged from 0.47 mg/kg *in N. laevis* to 0.65 mg/kg in *S. mobin*.

Table 3 Mineral contents (mg/kg) of selected browse plants

Sample	Ca	Mg	K	P	Na	Fe	Cu	Mn	Zn
Piliostigma thonningii	21.23^{d}	31.71^{c}	72.92^{c}	0.35^{a}	17.83^{b}	163^{b}	0.07^{b}	0.31^{a}	0.53^{b}
Spondia mombin	32.71^{a}	27.47^{d}	140.15^{b}	0.32^{a}	22.39^{a}	144^{c}	0.18^{ab}	0.31^{a}	0.65^{a}
Newbouldia laevis	26.43^{c}	34.05^{b}	66.00^{d}	0.33^{a}	9.83^{c}	131^{d}	0.11^{ab}	0.31^{a}	0.47^{c}
Thevetia peruviana	28.01^{b}	35.27^{a}	157.58^{a}	0.17^{b}	16.87^{b}	178^{a}	0.45^{a}	0.31^{a}	0.57^{b}
SEM	1.24	0.91	12.14	0.02	1.36	5	0.06	0.002	0.02

SEM standard error of mean

^{a,b,c,d}Means in same column with different superscripts are significantly ($p < 0.05$) different

In vitro gas production (ml/200 mg DM) of selected plants

The volume of gas produced increased from 2 h of incubation to 48 h on incubation. *N. laevis* had the highest slope followed by *S. mobin* and *T. peruviana,* while *P. thonngii* had the least (Fig. 1). The volume of gas produced from *P thonngii* from 3 to 18 h of incubation remains at 2 ml/200 mg DM. Thereafter, it increased to the end of incubation period. On the other hand, gas production from *N. laevis* started increasing from 9 h of incubation and before the other plants.

Post-incubation parameters and gas production kinetics of selected browse plants

The post-incubation parameters and gas production kinetics differed significantly ($p < 0.05$) among the selected plants. The values for SCFA ranged from 0.02 µmol in *N. laevis* and *T. peruviana* to 0.13 µmol in *P. thonngii*. The OMD values ranged from 28.10% in *N. laevis* to 32.49% in *P. thonngii,* whereas *N. laevis* recorded the highest values (5.67 MJ kg^{-1} and 11.55 ml/200 mgDM for ME and volume of gas produced in time (*b*), while the lowest value (4.00 ml/200 mg DM) for CH_4 was recorded for *N. laevis* and *T. peruviana* with the highest value (7.00 ml/200 mg DM) recorded in *P. thonngii* (Table 4).

Discussion

Agroforestry resources (tree species and other browse plants) would continue to be a an important feed resource owing to their appreciable level of nutrients in terms of crude protein content, mineral composition and digestible nutrients (Devendra 1990; Topps 1992). The observed variations in the nutrients composition among the selected species support the report of Dicko and Sikena (1992) who reported a significant variation in crude protein contents and nutrient digestibilities between species tree and shrubs. These distinctions can be linked to varying age of the plants, season of harvesting, location and between species variability (Solomon 2001). In addition, there could be morphological differences within the same species of plant (Beyene 2009). In comparison with natural pastures, most especially grasses, Brewbaker, (1986) was of the opinion that trees and browse species usually contain higher crude fat and ash. Generally, the crude protein (CP) contents in browse and tree species were indicated to be above the minimum level required (7%) for microbial activities in the rumen (Norton 1998; NRC 2001). This confirmed the range of 10.74–16.09% CP obtained in this study. The higher values of CP recorded in all the browse plants indicated that these plants could serve as potential protein supplements to enhance the intake and utilization of low-quality grass and fibrous crop

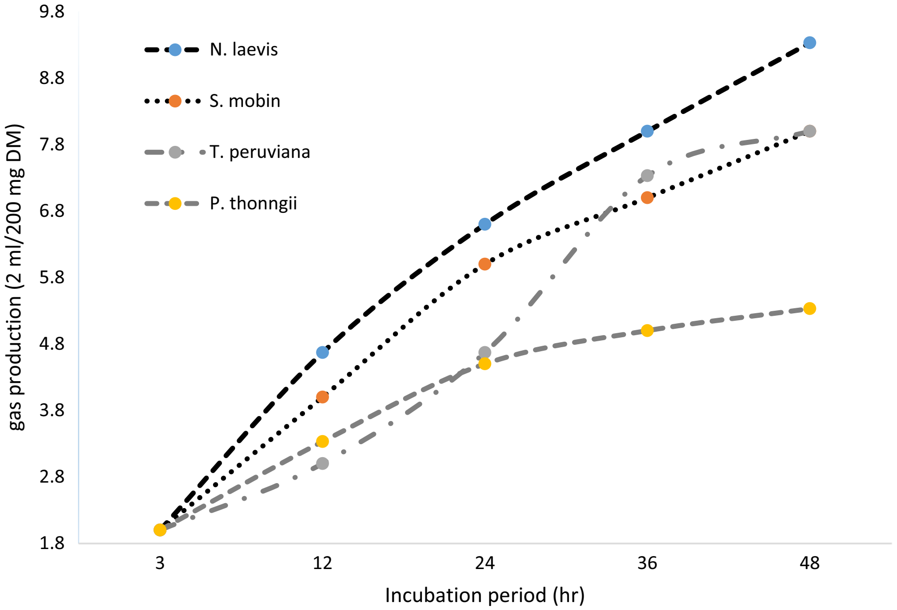

Fig. 1 In vitro gas production (ml/200 mgDM) of selected browse plants

Table 4 Post-incubation parameters and gas production kinetics of selected browse plants

Sample	SCFA (µmol)	OMD (%)	ME (MJ kg^{-1})	b (ml/200 mgDM)	c (ml/h)	Lag time (h)	CH_4 (ml/200 mgDM)
P. thonngii	0.02^{b}	28.10^{b}	4.71^{ab}	5.48^{b}	0.04	4.00	7.00^{a}
S. mobin	0.04^{b}	29.09^{b}	4.61^{ab}	8.37^{ab}	0.04	6.25	6.00^{b}
N. laevis	0.13^{a}	32.49^{a}	5.67^{a}	11.55^{a}	0.04	4.2	4.00^{c}
T. peruviana	0.02^{b}	28.15^{b}	4.50^{b}	6.55^{b}	0.04	3.50	4.00^{c}
SEM	0.02	0.59	0.12	0.82	0.003	0.48	0.41

SEM standard error of mean, *SCFA* short-chain fatty acid, *ME* metabolizable energy, *OMD* organic matter digestibility, *b* volume of gas produced in time (*t*), *c* fractional rate of gas production

[a,b,c]Means in same column with different superscripts are significantly ($p < 0.05$) different

residues by ruminants. The result is in line with the report of Getachew et al. (2004) who reported higher CP in browse forages than tropical grasses and roughages. Moreover, the CP contents of the agroforestry trees and shrubs used in this study were above the recommended value (8% CP) required for maintenance requirement of ruminant animals (Norton 1998) and above the minimum level necessary to provide sufficient nitrogen required by rumen microorganisms to support optimum activity (McDonald et al. 2002) and for adequate intake of forages. In addition to a high CP content, browse species also provide vitamins and mineral elements, which are often lacking in natural grassland, especially during the dry season (Skerman et al. 1988). The difference in the obtained values may also be linked to seasonal or climatic factors and ambient temperature (Agriculture 2011).

The observed NDF value in this present study is higher than those reported by Rittner and Reed (1992), and Gasmi-Boubaker et al. (2005). However, it was similar to that reported by Salem et al., (2006) for *E. camaldulensis* leaves. Gasmi-Boubaker et al. (2005) documented a range of 360-551 g/kg for Mediterranean browse species while Rittner and Reed (1992) in their work on West African browse plants reported a mean value of 342 g/kg for different *Acacia albida.* The higher NDF range (606.7–666.7 g/kg) obtained in this study revealed the contribution of twigs to the NDF content. Generally, higher level of fibre content of shrub and tree species could be partly influenced by the environmental conditions such as high temperatures and low precipitations which tend to increase the cell wall components and to decrease the soluble contents of the plants (Pascual et al. 2000).

Summarily, NDF is used to predict feed intake by the fibre content (Robinson et al. 1998), while ADF gives an expected digestibility and energy intake of the ruminants (Wright and Lackey 2008). The results obtained also showed higher value of ADF content in all the browse plants which might be due to a lower leaf to stem ratio in the sample analysed and an increase in cell wall lignification with advanced stages of growth as reported (Adane 2003; Yihalem 2004). Higher fibre levels of the browse species may hinder the activity of rumen microorganisms in colonization of ingesta, which in turn might induce higher fermentation rates. Hence, digestibility, intake and animal performance might be impaired. The phosphorus contents of the selected plants are optimum to supply the recommended requirements (0.12–0.34%) for growing to finishing beef cattle (McDowell 1992, 1997). Higher level of calcium in these browse plants could be due to the fact that the plant accumulated calcium to deal with all injuries which could happen as a result of water stress. Ejaz et al. (2011) supported that the possible mechanism to minimize negative effect of drought in crop plants was increasing absorption of certain minerals like calcium. The copper content of pastures and forages varied with the species, strain and maturity of the plant, with certain soil conditions and the fertilizers applied. The Cu content of the browse plants in this study was in line with the findings of MacPherson (2000). Iron (Fe) naturally well supplied by forage plants and deficiency of the element in grazing livestock is unlikely to occur but may result from blood loss due to heavy parasitic infestation or some other cause of haemorrhage. MacPherson (2000) reported > 30 mg/kg Fe as desirable for ruminants; hence, Fe contents of the selected browse plants were adequate to meet the requirement of all classes of ruminants.

Higher gas production observed for *N. laevis* suggested a higher nutrient digestibility of these browse plant compared to others. This result nonetheless is a reflection of higher proportion of soluble carbohydrate available for fermentation (Getachew et al. 1999). Utilization of forages by ruminants depends on microbial degradation and the extent of this degradation; the increased gas volume obtained suggested that *N. laevis* had more degradable and fermentable carbohydrates compared to other species. Higher gas production during in vitro fermentation of feed materials indicates higher rate of digestibility of such feed materials (Mebrahtu and Tenaye 1997). At 48 h of incubation, the variation in the cumulative gas production could be attributed to differences in their CP and fibre components. This is a reflection of the amount of substrate organic matter fermentation and production of volatile fatty acids. The increased gas volume observed for most of the plants could be attributed to the higher level of CP content more than required as reports had it that increased CP content of a plant material influence the amount of gas produced (Getachew et al. 2004). Hillman et al. (1993) stated that gas production is positively related to microbial protein synthesis. The amount of gas produced is also affected by the nature of feed and the presence of secondary metabolites (Babayemi et al. 2004). However, secondary metabolites of the selected plants were not determined in this study. Generally, gas production is a function and a mirror of degradable carbohydrate and the amount depends on the nature of the carbohydrate (Blummel and Becker 1997). These differences among browse species in digestibility and SCFA may be partly attributed to the variations in chemical composition (mainly cell wall content and composition). The higher the cell wall components (NDF, ADF and ADL), the lower the digestibility of such plant materials. Lower ADF and NDF (mg/kg) content in most browse species usually enhance high digestibility (Norton 1994) of the plant; however, high NDF, ADF and ADL can limit voluntary feed intake, digestibility and nutrient utilization of ruminant animals. This further buttressed the decreased ADF and NDF in plants with higher CP content and gas production.

The ME, OMD and SCFA observed among browse plants were lower than those reported by Babayemi et al. (2009) on some forage seeds. The ME, OM and SCFA could be translated to DM intake in ruminants. Methane production represents an energy loss to ruminant and also has environmental implication on the greenhouse gas contributing to global warming (Johnson and Johnson 1995), and higher methane production in *P. thonngi* might be as a result of the higher fibre component of the plant.

Conclusion

Generally, the selected species display higher nutritive status compared to natural pastures. However, in relation to the CP, fibre components, in vitro dry matter digestibility and methane production can be adopted in ruminant feeding. Adoption of these plants can improve ruminant productivity, reduce time spent on pasture and reduce methane contribution to the greenhouse gases; therefore, further research (in vivo studies) should be carried out to confirm the possible adoption of these agroforestry plants in ruminant feeding.

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