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IN VITRO GAS PRODUCTION AND DRY MATTER DEGRADATION OF FOUR BROWSE LEAVES USING CATTLE, SHEEP AND GOAT INOCULA

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ABSTRACT

The effects of rumen inoculum from Bunaji cattle, West African Dwarf (WAD) sheep and WAD goat on the in vitro gas production and dry matter (DM) degradation of *Moringa oleifera, Millettia griffoniana, Enterolobium cyclocarpum* and *Gmelina arborea* leaves were compared in an *in vitro* study using incubation periods ranging from 0 – 48 h. Oven-dried samples of the leaves were incubated in three replicates with each inoculum source and incubations run at two consecutive times to make six replicates per treatment for estimation of the kinetics of gas production using non-linear equation. Leave samples were analyzed for crude protein (CP), lignin (ADL), acid (ADF) and neutral (NDF) detergent fibres. Concentrations of CP (165 – 247 $g.kg^{-1}$ DM), NDF (413 – 538 $g.kg^{-1}$ DM) and ADF (300 – 346 $g.kg^{-1}$ DM) differed among species. Inoculum sources varied ($P < 0.05$) in volume of gas production at 12 and 24 h along the incubation but not at later incubation times of 36 and 48 h. Gas production between cattle, sheep and goat were correlated ($r = 0.98$; $P < 0.001$). Kinetics of gas production differed ($P < 0.05$) among inoculum sources with cattle inoculum showing a shorter ($P < 0.05$) lag time and higher ($P < 0.05$) rate of fermentation. Gas production also varied ($P < 0.05$) among browse species with *M. oleifera* recording the highest volume of production. *M. oleifera* and *E. cyclocarpum* were higher ($P < 0.05$) in dry matter degradation than *M. griffoniana* and *G. arborea* irrespective of inoculum source. Results indicated that *in vitro* gas production and dry matter degradation of the forages varied due to browse species and not inoculum source. Rumen fluid from cattle, sheep and goats could therefore, serve as inoculum source for the screening of these forages for ruminants.

Key words: browse species; *in vitro* degradation; cattle; sheep; goat

INTRODUCTION

In vitro techniques in feed evaluation for ruminants has gained wider acceptance due to its ease of adoption, repeatability, minimized use of animals and the decrease in funding for *in vivo* evaluation of feeds (Getachew *et al.*, 2005). Although these techniques are more rapid and precise, requiring less substrate than *in situ* procedures, they still require an inoculum to create the fermentative environment (Mould *et al.*, 2005). Different ruminant species including cattle, sheep, goats, deer, chamois and buffalo have been used as inoculum donors. Small ruminants especially sheep and goat are commonly used as donor animals for *in vitro* trials in most African countries, more particularly in Nigeria (Abegunde *et al*., 2009; Anele *et al*., 2009; Ajayi and Babayemi, 2008; Babayemi and Bamikole, 2009), probably due to ease of animal handling, reduced cost of maintenance and lower feed requirements relative to larger ruminants such as cattle (Bueno *et al*., 2005).

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Several studies have reported the comparative ability of different ruminant species to digest fibrous feeds, fodder trees and shrubs (Larbi *et al*., 1997; Bueno *et al.*, 1999; Cone *et al*., 2000; Calabro *et al.*, 2004; Bueno *et al.*, 2005; Ammar *et al.*, 2008). Most studies made comparisons only between two ruminant species and quantitative data is limited to *in vitro* digestibility of shrub fodder among West African indigenous breeds of cattle, sheep and goats. This study compared *in vitro* gas production and dry matter (DM) degradation of foliage from four tropical browse species using inoculum from Bunaji cattle (BUC), West African Dwarf (WAD) sheep and WAD goats. This is an attempt to ascertain if rumen fluid from any of the three donors could accurately serve as inoculum for *in vitro* testing to predict the digestibility of these feeds for ruminants.

MATERIALS AND METHODS

Experimental design

A 3 (inoculum source) × 4 (browse species) factorial arrangement in a randomized complete block design was used in this study.

Collection of browse species

Fresh leaves plus edible twigs of *Moringa oleifera*, *Millettia griffoniana*, *Enterolobium cyclocarpum* and *Gmelina arborea* were harvested in triplicates from mature trees at the experimental plot of the College of Animal Science and Livestock Production, University of Agriculture, Abeokuta, southwestern Nigeria in May 2010. The region has a humid climate with a mean annual rainfall of 1037 mm; and mean annual temperature and humidity are 34.7 °C and 82 %, respectively. Freshly harvested foliage from each species was sub-sampled and initially weighed fresh on the field and then oven-dried at 65 °C to constant weight. Dried samples were weighed and hammer-milled to pass through a 1mm sieve and then stored for subsequent analysis.

Chemical analysis of browse leaves

Total nitrogen (N) was determined by the Kjeldahl method (AOAC, 1990; ID 973.18). Crude protein (CP) was then calculated as N x 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to Van Soest *et al.* (1991). Neutral detergent fibre was determined without amylase and sodium sulphite. Both NDF and ADF were both exclusive of residual ash. Lignin was determined by solubilisation of cellulose with sulphuric acid on the ADF residue (Van Soest *et al*. (1991).

Animal donors and collection of rumen fluid

Two 350 kg BUC, three WAD sheep (45 kg average weight) and three WAD goats (35 kg average weight) were used as inoculum donors. The animals were previously fed with 600 $g.kg^{-1}$ DM of *Pennisetum purpureum* and 400 $g.kg^{-1}$ DM of concentrate diet. The concentrate consisted of (as fed basis, $g.kg^{-1}$) 400 corn, 100 wheat offal, 100 palm kernel cake, 200 groundnut cake, 50 meal, 100 dried brewers grain, 10 common salt, 37.5 oyster shell and 2.5 fish meal. Rumen fluid was collected in equal proportions from the donor animals, under the same feeding regime within 15 min before the morning meal into thermo flasks and strained through four-layered cheesecloth and kept at 39 °C soon after collection. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. The rumen liquor and a buffer solution were mixed in the ratio 1:2 (v/v), respectively as described by Menke and Steingass (1988).

***In vitro* procedure**

Incubation was carried out at 39 °C and the volume of gas production was measured at 3 h interval from 3 to 48 h using procedures described by Menke and Steingass (1988). Three blanks containing 30 ml of medium only were included in the run. Average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. Gas volumes obtained at varying incubation hours were fitted to the non-linear equation model of France *et al*. (2002):

$$A = b\,(1-e^{-c(t-L)})$$

where: **A** is the volume of gas produced at time *t*, *b* is the asymptotic/potential gas production (ml/g DM) from the fermentable fraction of forage, *c* is the fractional rate of gas production (/h) from the slowly fermentable feed fraction *b*, and *L* is the discrete lag time prior to gas production. Two runs of incubations were performed consecutively resulting in six replicates per treatment.

***In vitro* dry matter degradation**

The *in vitro* dry matter degradation was determined at 48 h by centrifuging the incubation residues at 20, 000 x g for 30 min following placement in iced cubed (-4 °C) to end fermentation. Residues obtained were filtered and oven-dried to determine their dry weight. The blanks were also centrifuged and residues weighed and used to correct for residues from the ruminal inoculum. *In vitro* dry matter degradation was then calculated as:

$$\frac{\text{Substrate dry matter incubated} - (\text{residue dry matter} - \text{blank dry matter})}{\text{Substrate dry matter incubated}}$$

Gas production ratios

The ratio of cumulative gas production at 24 h and 48 h (GP24/GP48) were compared in an attempt to ascertain how much of the fermentation was completed in the first 24 h (Bueno *et al.*, 2005). Similarly, the ratio of 48 h cumulative gas production and asymptotic gas production, *b* (GP48/b) were compared in order to determine how close 48 h gas production is from *b*. The closer 48 h gas production is to *b* (i.e. higher ratio), the better the feed quality and/or the incubation time was long enough to express the fermentation potential of the feed.

Statistical analyses

Data were analyzed as a 3 x 4 factorial arrangement in a randomized complete block design using the general linear models (GLM) procedure of SAS (1999) with the model:

$$Y_{ijk} = \mu + B_i + R_j + \beta n + (BR)_{ij} + \varepsilon_{ijk}$$

where: Y_{ijk} is the observation, μ is the population mean, B_i is the browse species effect ($i = 1 – 4$), R_j is the inoculum source (cattle, sheep, goat) effect ($j = 1 – 3$), βn is the block effect (repeated incubation; n = 1 – 2), $(BR)_{ij}$ is the interaction between browse species and inoculum source and ε_{ijk} is the residual error. Regression analysis was used to

Table 1: Chemical composition of leaves of browse species

Browse species	Composition				
	[a]DM	[b]CP	[c]NDF	[d]ADF	[e]ADL
Moringa oleifera	245	247	413	300	79
Millettia griffoniana	345	165	537	346	84
Enterolobium cyclocarpum	344	182	514	319	86
Gmelina arborea	372	179	538	349	88
Mean	326	193	501	329	84
SEM	14.98	9.59	15.76	7.06	1.11

[a]DM: dry matter (g.kg^{-1} as fed basis), [b]CP: crude protein (g.kg DM^{-1}), [c]NDF: neutral detergent fibre (g.kg DM^{-1}), [d]ADF: acid detergent fibre (g.kg DM^{-1}), [e]ADL: acid detergent lignin (g.kg DM^{-1}).

Table 2: Cumulative gas production using rumen fluid from Bunaji cattle, and West African Dwarf sheep and goats as inoculum

Browse species	GP-12 h[a]			GP-24 h[b]			GP-36 h[c]			GP-48 h[d]		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa oleifera	143	133	133	168	153	158	188	183	187	205	198	197
Millettia griffoniana	93	85	90	115	113	107	143	140	145	160	160	167
Enterolobium cyclocarpum	127	120	120	153	137	138	170	167	172	190	183	188
Gmelina arborea	97	83	82	125	110	113	145	143	140	170	168	167
SEM												
Browse (B)		2.10			3.46			2.25			1.94	
Inoculum source (R)		6.54			6.52			5.90			4.79	
B*R		3.74			3.78			3.32			2.71	
P > F												
B		< 0.001			< 0.001			< 0.001			< 0.001	
R		0.0002			0.0138			ns			ns	
B*R		ns			ns			ns			ns	

[a]GP-12 h: cumulative gas production after 12 h of incubation (ml.g DM^{-1}), [b]GP-24 h: cumulative gas production after 24 h of incubation (ml.g DM^{-1}), [c]GP-36 h: cumulative gas production after 36 h of incubation (ml.g DM^{-1}), [d]GP-48 h: cumulative gas production after 48 h of incubation (ml.g DM^{-1}), [e]ns: not significant.

establish relationships between gas production using cattle, sheep and goat rumen fluid. Means were separated with Fisher's Least Significant Difference (SAS, 1999) when $P \leq 0.05$.

RESULTS

Chemical composition of browse leaves

Crude protein concentration was highest in the leaves of *M. oleifera* and lowest in *M. griffoniana* (Table 1). The cell wall concentration was highest in *G. arborea* and lowest in *M. oleifera*.

Cumulative gas production

Cumulative gas volumes varied ($P < 0.05$) among inoculum sources at 12 and 24 h of incubation when gas volumes measured were higher with feeds incubated with cattle rumen fluid than those measured with sheep and goat rumen fluid (Table 2). Cumulative gas volumes with cattle rumen fluid were relatively higher than using the rumen fluid of sheep and goats (Fig. 1). Browse species affected ($P < 0.05$) cumulative gas production (Table 2). Gas production from *M. oleifera* was higher than those of the other browse species at all incubation periods in cattle, sheep and goats (Fig. 2 a, b, c).

Kinetics of gas production

Potential gas production was significantly affected by browse species, with potential gas production of *M. oleifera* and *E.cyclocarpum* being higher than *M. griffoniana* and *G. arborea* (Table 3). The rate of fermentation varied ($P < 0.05$) among browse species and source of inoculum (Table 3). *M. oleifera* had relatively higher rate of fermentation than *M. griffoniana*, *E.cyclocarpum* and *G. arborea*; while rate of fermentation using cattle rumen fluid was higher than sheep and goats. The browse species*source of inoculum interaction significantly affected the lag time (Table 3). *Gmelina* had the highest lag time with cattle and goat rumen fluid, while *Millettia* had the highest lag time with sheep rumen fluid. Cattle rumen fluid had shorter ($P < 0.05$) lag times than sheep and goat rumen fluid.

Ratio of GP24 to GP48 and GP48 to *b*

The ratio of cumulative gas production at 24 h to that of 48 h (GP24/GP48) was influenced ($P < 0.05$) by the browse species and source of inoculum (Table 4). The GP24/GP48 using cattle rumen fluid was higher than sheep and goats; while *M. oleifera* and *E. cyclocarpum* had higher ($P < 0.05$) ratio than *M. griffoniana* and *G. arborea*.

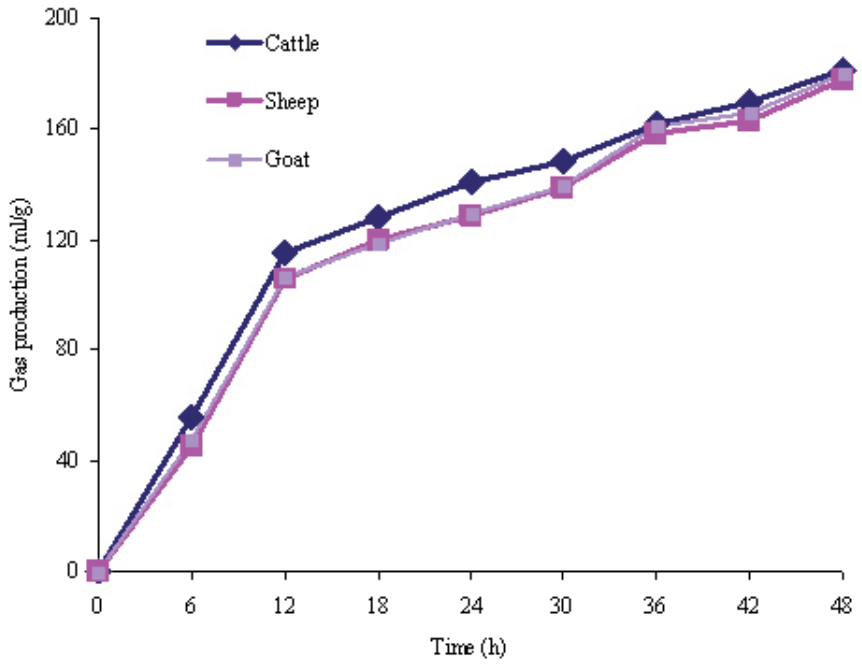

Fig. 1: Gas production from fermentation of leaves of four browse species at varying incubation times using cattle, sheep and goat rumen fluid as inoculum

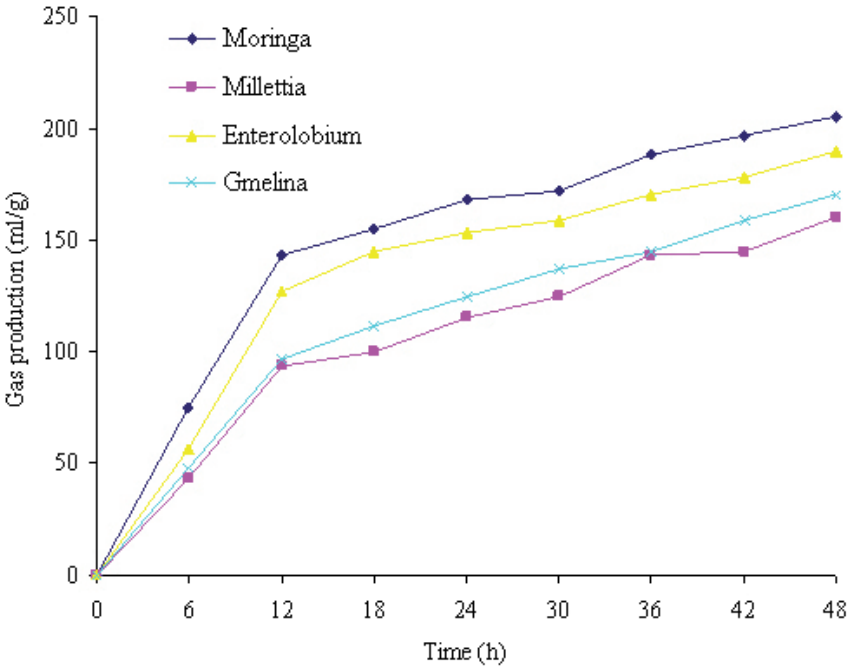

Fig. 2a: ***In vitro*** **gas production profile of four browse species at varying incubation times using rumen fluid from Bunaji cattle**

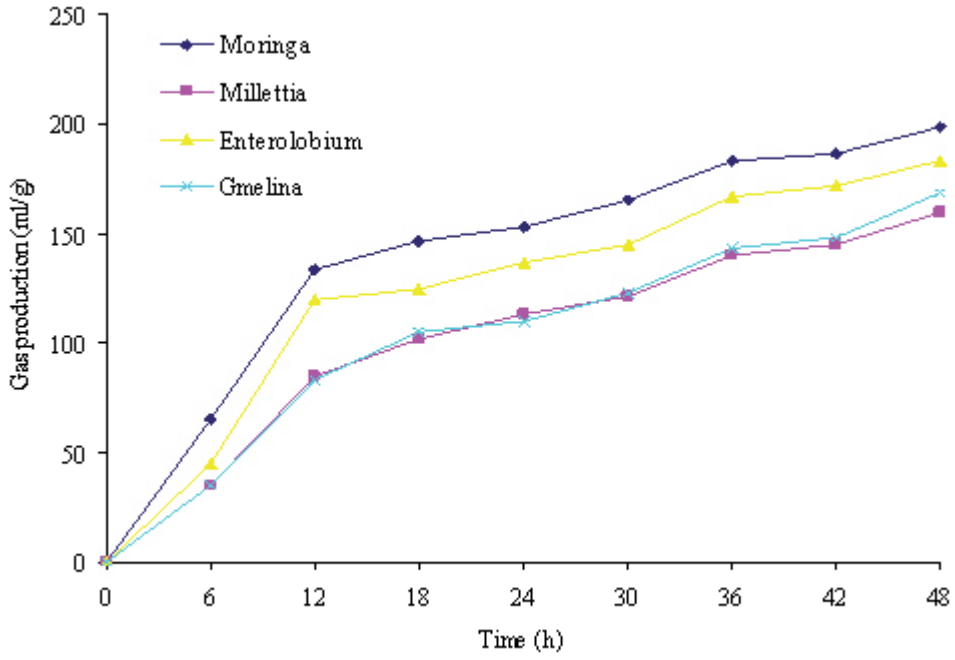

Fig. 2b: ***In vitro*** **gas production of four browse species at varying incubation times using rumen fluid from West African dwarf sheep**

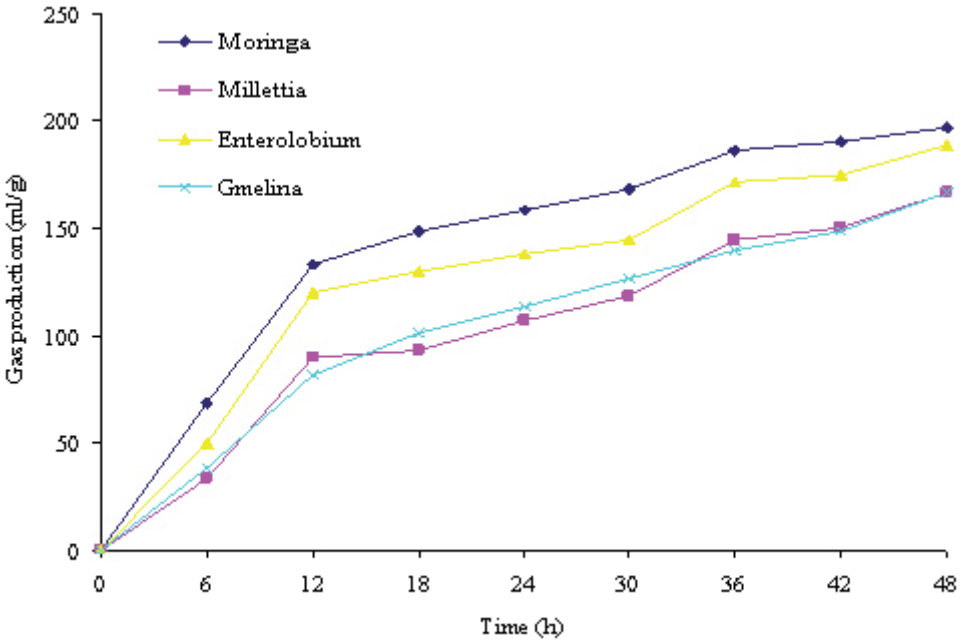

Fig. 2c: ***In vitro*** **gas production of four browse species at varying incubation times using rumen fluid from West African dwarf Goat**

Table 3: Gas production kinetics of browse leaves using rumen fluid from cattle, sheep, and goats

Browse species	*b*[a]			*c*[b]			lag time[c]		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa. oleifera	210	201	201	0.073	0.062	0.067	1.10	1.16	1.14
Millettia griffoniana	165	163	172	0.060	0.050	0.051	1.00	1.24	1.08
Enterolobium cyclocarpum	193	189	191	0.058	0.051	0.054	1.06	1.20	1.20
Gmelina. arborea	176	172	170	0.058	0.047	0.056	1.18	1.22	1.22
SEM									
Browse (B)		2.09			0.0026			0.0250	
Inoculum source (R)		4.73			0.0025			0.0199	
B*R		2.68			0.0016			0.0140	
P > F									
B		< 0.0001			0.0014			0.0028	
R		ns			0.0147			< 0.0001	
B*R		ns			ns			0.0194	

[a]*b*: asymptotic gas production from insoluble fraction (ml.g DM^{-1}; France *et al*., 2002); [b]*c*: rate of gas production (/h);
[c]lag time (h); ns: not significant

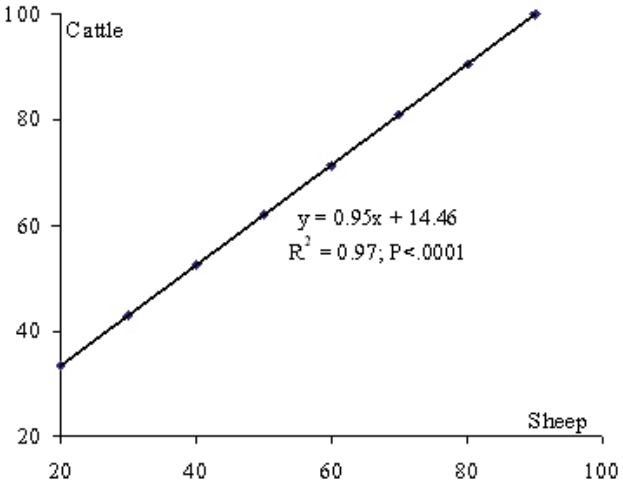

Fig. 3a: Relationships between *in vitro* cumulative gas production using cattle and sheep inoculum

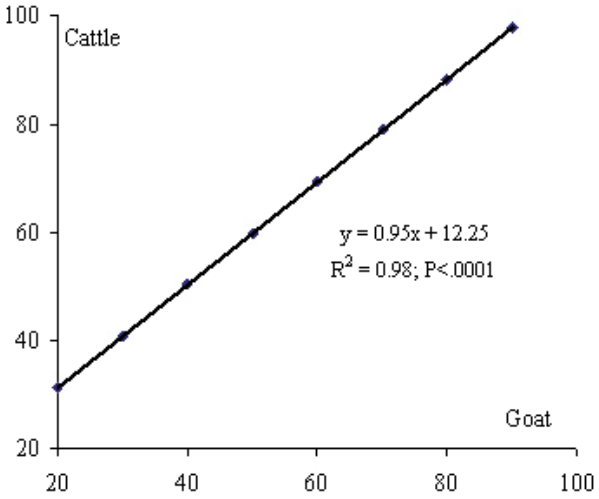

Fig. 3b: Relationships between *in vitro* cumulative gas production using cattle and goat inoculum

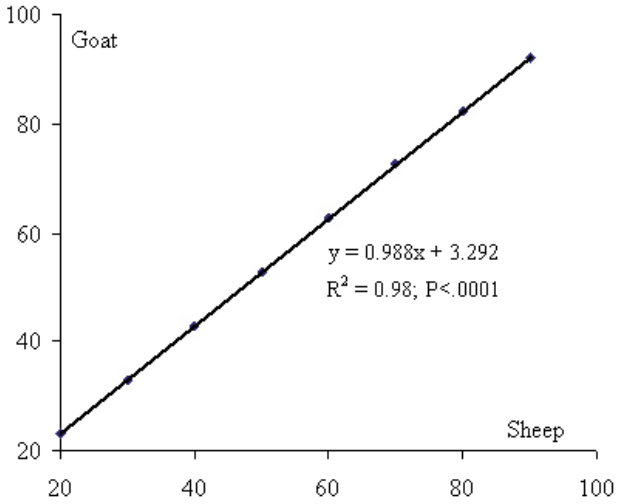

Fig. 3c: Relationships between *in vitro* cumulative gas production using goat and sheep inoculum

Relationship between gas production using cattle, sheep and goat inocula

As shown in Fig. 3a, 3b, 3c, cumulative gas production at 48 h from cattle and sheep inocula, cattle and goat inocula, as well as goat and sheep inocula were significant and highly correlated ($r = 0.98$; $P < 0.001$) .

Dry matter degradability

Browse species significantly affected *in vitro* DM degradation (IVDMD), with degradation of *M. oleifera* and *E. cyclocarpum* being higher than *M. griffoniana* and *G. arborea* (Table 5).

DISCUSSION

Chemical composition

Chemical composition varied among browse species in support of earlier studies with shrubs and trees (Ammar *et al.*, 2004b; Camacho *et al.*, 2010; Larbi *et al.*, 1997; Larbi *et al.*, 2011; Mbugua *et al.*, 2008; Salem *et al.*, 2007). Variations in CP and cell wall content could possibly be due to differences in ratio of leaves and twigs in forages as similarly reported by Larbi *et al.* (2011). The mean CP (193 g.kg DM^{-1}) and NDF (501 g.kg DM^{-1}) values for browse species in this study are consistent with earlier reports for browse species in the tropics (Anele *et al.*, 2009; Larbi *et al.*, 1998). The CP of browses was above 80 g.kg DM^{-1}, the reported minimum required in diet for adequate digestive activities of rumen microbes (Orskov, 1982). The high CP content in these species is an advantage to rumen microbes that depend on dietary source of nitrogen to build up their body proteins.

Table 4: Ratio between cumulative gas production at 24 h and 48 h incubation (GP24/GP48) and ratio between gas production at 48 h and *b* (GP48/b) of browse leaves using rumen fluid from cattle, sheep and goat

Browse species	GP24/GP48			GP48/*b*		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Moringa oleifera	0.821	0.773	0.807	0.977	0.989	0.979
Millettia griffoniana	0.720	0.709	0.642	0.971	0.980	0.968
Enterolobium cyclocarpum	0.807	0.746	0.734	0.985	0.968	0.986
Gmelina arborea	0.734	0.651	0.679	0.965	0.979	0.980
Means	0.770	0.720	0.715	0.974	0.979	0.978
SEM						
Browse species (B)		0.018			0.005	
Inoculum source (R)		0.020			0.004	
B*R		0.012			0.003	
P > F						
B		0.0002			ns	
R		0.0302			ns	
B*R		ns			ns	

ns: not significant

Table 5: *In vitro* dry matter degradation (g.kg DM^{-1}) of browse leaves using rumen fluid from cattle, sheep, and goat[$]

Browse species	IVDMD[a]		
	Cattle	Sheep	Goat
Moringa. oleifera	617	603	610
Millettia griffoniana	511	503	505
Enterolobium cyclocarpum	558	536	559
Gmelina arborea	521	507	512
SEM			
Browse species (B)		10.54	
Inoculum source (R)		15.30	
B*R		8.69	
P > F			
B		< 0.0001	
R		ns	
B*R		ns	

[a]IVDMD: *in vitro* dry matter degradation; ns: not significant

Cumulative gas production

The variation in cumulative gas production among inoculum sources at 12 h and 24 h incubation period could possibly be due to variation in microbial activity such as a shorter lag time and higher rate of fermentation with cattle rumen fluid than with either sheep or goat rumen fluid (Table 3). Comparable with our results, variation in gas production at intermediate times along the incubation periods were reported when feeds were incubated with sheep and buffalo rumen fluids (Calabro *et al.*, 2005), as well as at earlier incubation times with cattle and sheep rumen fluid (Bueno *et al*., 2005). Our results suggest that variation may occur along the incubation period but differences among inoculums would be insignificant

at end incubation hours, in agreement with earlier studies (Bueno *et al*., 2005; Calabro *et al*., 2005).

Cumulative gas production volumes varied among browse species. Differences in chemical composition (i.e. CP and NDF) (Table 1), differences in morphological composition (i.e. leaf, stem), as well as reported concentrations of anti-nutritional components such as tannin (Larbi *et al.*, 2011; Ndijja and Nasiru, 2010; Rittner and Reed, 1992) could be responsible. A higher gas volume among browse species corresponded to a higher CP and lower cell wall content. Positive correlation between crude protein and gas production in browse species have been reported (Gasmi-Boubaker *et al*., 2005; Ndlovu and Nherera, 1997).

The browse species followed similar trend in extent of gas production with cattle, sheep and goat rumen fluid (Fig. 2a, b, c). This observation implies that the browse species were ranked similarly with cattle, sheep and goat inocula suggesting that any of cattle, sheep or goat rumen fluid could be used in *in vitro* fermentation studies to examine differences between browse species. Coppock *et al*. (1988) and Calabro *et al*. (2005) reported similar trends when feeds were incubated with rumen fluid from some species of ruminants in a comparative study.

Kinetics of gas production

Potential gas production b did not vary among cattle, sheep or goat inoculums in agreement with earlier work by Cone *et al.* (2000) and Bueno *et al*. (2005) who reported similarity in the estimates of total gas production between inoculums collected from cattle and sheep. Variation in *b* among browse species confirm earlier works (Ammar *et al*. 2004a; Ammar and Gonzalez, 2005; Bueno *et al*., 1999), which could be due to variation in chemical composition of the browse species (Table1).

Rate of fermentation was higher and lag time shorter with cattle rumen fluid. Shorter lag time observed in our study with cattle rumen fluid is consistent with Bueno *et al*. (1999) who reported a longer lag period with sheep rumen fluid than rumen fluid from cattle. Lag time is indicative of the time taken for microbes to adhere themselves to the substrates, and microbial attachment to insoluble substrate has been reported to be a pre-condition for digestion to proceed (Kudo *et al*., 1995). The shorter lag time could be responsible for the faster rate of fermentation with cattle rumen fluid (Table 4), implying that fermentation of browse species incubated with cattle rumen fluid proceeded faster than those with sheep and goat. This could also explain the variation observed among cattle, sheep and goat inocula in cumulative gas production along the incubation period. Variation in dynamics of gas production has been reported with cattle and sheep rumen fluid (Bueno *et al*., 2005; Cone *et al*., 2000) and is indicative of the fact that one species could not be used to predict the gas production profile of the other (Bueno *et al*., 2005).

The higher extent of gas production and rate of degradation of *M. oleifera* suggests that rumen microbes were able to utilize the feed better probably due to a higher content of fermentable nutrients. A higher potential gas production can contribute significantly to energy supply via short chain fatty acid production (Remesy *et al*., 1995). The longer lag times observed for cattle and goat inocula with *G. arborea* and that observed for sheep with *M. griffoniana* could not be clearly explained but these could be responsible for the lower potential gas production of the two browse species relative to *M. oleifera* and *E. cyclocarpum.*

Ratio between gas production parameters

The ratio between GP-24 h and GP-48 h was higher with cattle rumen fluid than with sheep and goat. This is comparable with results obtained by Bueno *et al.* (2005) who reported a higher ratio with cattle rumen fluid than with sheep rumen fluid. Our findings imply that a greater extent of fermentation had taken place half time the incubation period with cattle rumen fluid, suggesting cattle rumen fluid as having a higher fermentation efficiency half time the incubation period. Sheep and goat rumen fluid could be considered as requiring extended periods of incubation for efficient substrate fermentation *in vitro*.

***In vitro* dry matter degradability**

Inoculum source did not affect IVDMD in agreement with earlier studies (Ammar *et al.,* 2004b; Dalmau *et al*., 2006; Mabjeesh *et al.*, 2000) suggesting possible similarity in microbial species and activity. Microbial population is dependent on the type of diet fed and since the three donors were maintained on the same diet, microbial species were not expected to vary. Differences between ruminant species in terms of digestive capability become noticeable only when each species are fed a different diet, and are considerably reduced when all animals receive the same diet (Ammar *et al.*, 2008; Dalmau *et al*., 2006; Mould *et al*., 2005). Several authors (Aerts *et al*., 1985; Bueno *et al*., 1999; Larbi *et al*., 1993; Tolkamp and Brouwer, 1993) suggested good agreement in the digestive capacity of cattle, sheep and goat. The often stated superiority of goats over sheep and cattle in terms of forage digestibility (Domingue *et al*., 1991) was not confirmed in this study.

The IVDMD varied with browse species possibly due to differences in cell wall content. The higher IVDMD observed in *M. oleifera* is attributed to its higher potential gas production (Table 3). Digestibility has been reported to be synonymous to *in vitro* gas production, with a high positive correlation obtained between gas production and dry matter digestibility (Datt and Singh, 1995; Fieves *et al.*, 2005).

Relationship between gas production using cattle, sheep and goat rumen fluid

Average values of gas production with cattle, sheep and goat inocula were highly correlated suggesting that rumen fluid from Bunaji cattle, WAD sheep or WAD goat could be reliable in predicting the gas production by any of the browse species. Similar to our results, significant correlations between sheep and cattle, sheep and goat or sheep and buffalo have been reported (Ammar *et al*., 2008; Bueno *et al.*, 2005; Calabro *et al*., 2005; Cone *et al*., 2000).

CONCLUSION

In conclusion, our results indicate that inoculum from cattle, sheep or goats were well correlated and can be used to determine the potential gas production and IVDMD of any of the four browse species. Browse species with higher crude protein and lower cell wall content showed better potential for gas production and in vitro dry matter digestibility. Variation in gas production and IVDMD would therefore vary due to species differences rather than inoculum source in an *in vitro* medium.

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