

In Vitro Evaluation of Grass and Legume/Browse Species Grazed by West African Dwarf Goats in South-Western Nigeria

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Abstract: Four grass species (*Panicum maximum*, *Imperata cylindrica*, *Axonopus fissifolius* and *Brachiaria decumbens*) and three legume/browse species (*Leucaena leucocephala*, *Centrosema pubescens*, and *Gliricidia sepium*) were evaluated for proximate composition and in vitro gas production characteristics. The completely randomized design with four grass species and three legume/browse species, each in triplicates was employed. The dry matter of the grass species ranged between 79.38 and 89.95%, while that of legume/browse species ranged from 85.00 to 93.38%. The crude protein contents of the grasses ranged from 5.68 to 8.72% and the legumes from 16.02 to 28.86%. Other ranges of values were for crude fibre, 18.34 to 24.32% in the grasses and 12.16 to 61.41% in the legume/browse species. Gas production (23.00 – 54.00 ml/200 mg DM) at 24 h incubation, potential gas production (a + b) also (23.00 – 54.00 ml/200 mg DM), metabolizable energy (ME) (6.01 – 7.07 MJ/kg DM), organic matter digestibility (OMD) (44.53 – 78.91%) and short chain fatty acids (SCFA) (0.49 – 1.23 μ mol) were observed. These values were higher in the legume/browse than in the grass species. In conclusion, the observed nutritive values of the forages understudied have shown that they could be beneficial for improved goat production in Nigeria.

Keywords: Proximate composition, in vitro gas production, forages, goat.

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I. Introduction

Ruminant production is one of the ways of reducing animal protein deficiency in most developing countries. In most of these countries, the recommended minimum daily animal protein intake of 35g per head was difficult to attain due to low levels of livestock production (FAO, 2008). The scarcity of forage for ruminants, particularly during the dry season result in weight loss, reduced meat and milk production and sometimes leads to high mortality of animals. The best solution to this problem could be to intensify research into the sourcing of cheaper and available feedstuffs that can meet the nutritional requirement of ruminants. The native pasture is known to be the most widely available low cost feed for ruminants in the tropics. However, majority of tropical livestock have access only to limited quantities of pastures with relatively low nutritive value. Also, both milk yield and growth of ruminants are largely limited by forage quality which is reflected in low feed intake and digestibility. Kamalak (2005) stated that tree and shrub leaves are important component of sheep and goats diets. However, there can only be improvement in the productivity of these ruminants when the nutritional qualities of these forages are assessed and improved upon.

Ajayi and Babayemi (2008) made a comparative *in vitro* evaluation of sole *Panicum maximum* with *Stylosanthes guianensis*, *Lablab purpureus*, *Centrosema pubescens* and *Aeschynomene histrix* using female West African Dwarf does. They observed a crude protein content of 7.63% for the sole *Panicum maximum* grass, and for the *Panicum maximum* with legume mixtures, the crude protein ranged from 18.05 to 23.29%. Ether extract was 2.76% in sole *Panicum maximum* grass but ranged between 8.42 and 12.66% in the *Panicum maximum* with legume mixtures. The ash content was 6.78% in sole *P. maximum* grass but increased to the range of 8.42 to 12.66% for the mixtures. The potential gas production, 'b' value from the insoluble fraction of the sole *P. maximum* was 16.90 ml/g DM while the 'b' values ranged from 14.55 to 22.10 ml/g DM in the *Panicum maximum* with legume mixtures. Thus Ajayi and Babayemi (2008) found out that there were increased proximate composition and *in vitro* gas production values in the grass/ legume mixtures than sole grass. Other earlier researchers, Evitayani *et al.* (2004) studied the nutritive values of tropical forages commonly used as ruminant feeds in North Sumatra, Indonesia. They observed that crude protein content ranged from 6.6 to 16.2% in grass and from 17.5 to 29.1% in legumes. The organic matter digestibility of grass ranged from 51.9 to 64.4% while in legumes the values ranged from 65.2 to 72.0%. The metabolizable energy content of grass varied from

6.4 to 9.3 MJ/kg and from 6.5 to 8.3 MJ/kg for legumes. Thus the crude protein content and organic matter digestibility values were higher for legumes than in the grass species studied. Odeyinka (2001) stated that since ruminants cannot meet their maintenance needs on grass alone, leguminous plants such as *Leucaena leucocephala*, *Centrosema pubescens*, and *Gliricidia sepium* can be fed in combination with grass especially during the dry season.

The aim of this study is to assess proximate composition and *in vitro* gas production characteristics of four indigenous grass and three legume/browse species grazed by West African Dwarf goats in South-Western Nigeria. This could serve as preliminary investigation for studying other grass and legume/browse mixtures.

II. Materials And Methods

2.1 Collection of forages

Samples of four grass species (*Panicum maximum*, *Imperata cylindrica*, *Axonopus fissifolius* and *Brachiaria decumbens*) and three legume/browse species (*Leucaena leucocephala*, *Centrosema pubescens*, and *Gliricidia sepium*) were collected during the dry season in January 2014. They were harvested in the environment of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The leaves were defoliated, oven dried at 105°C to a constant weight for 24 hours for dry matter determination. Another set of the leaf samples were oven dried at 65°C for 48 hours to a constant weight, milled to pass through 1.0 mm sieve and stored in air tight containers pending laboratory analysis.

2.2 Chemical analysis

Ash content of samples was determined by igniting the samples in a muffle furnace at 600°C for 8 hours. Nitrogen (N) content of the forages was determined by the standard Kjeldahl method, also crude fibre and ether extract were determined according to the methods of AOAC (2005). Crude protein was calculated ($N \times 6.25$) and NFE was also calculated ($100 - (\% CP + \% CF + \% EE + \% Ash + \% moisture)$). Samples were determined in triplicates.

2.3 *In vitro* gas production experiment

Rumen fluid was obtained with suction tube from three female WAD goats that were previously fed (two days prior) concentrate feed (40% maize, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewer grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). In addition, foliages of *Panicum maximum*, *Imperata cylindrica*, *Axonopus fissifolius* and *Brachiaria decumbens* grass species and three legume/browse species *Leucaena leucocephala*, *Centrosema pubescens*, and *Gliricidia sepium* were fed to the goats (the feed was about 5% of their body weights). Two hundred (200) mg of diet samples (containing the experimental grass and legume/ browse species) labelled (G1- G4) and (L1-L3) were placed in triplicates in 120 ml calibrated syringes. About 30 ml inoculums containing (cheese-cloth) strained rumen liquor and buffer solutions (1:4 v/v) under constant flushing with CO₂ were incubated. The liquor was collected into pre-warmed thermos flasks. Ingredients of the buffer solution were 9.8 NaHCO₃ + 2.77 Na₂HPO₄ + 0.57 KCl + 0.47 NaCl + 2.16 MgSO₄·7H₂O + 0.16 CaCl₂·2H₂O. Incubation was carried out at 3, 6, 9, 12, 15, 18, 21 and 24 h at 39°C. At post incubation period, the gas production was measured at 3, 6, 9, 12, 15, 18, 21 and 24 h. Average volumes of gas produced from the blanks were deducted from the volume of gas produced per sample. (The blanks contained only the inoculums and buffer). Rates and extent of gas production were determined for each substrate using the linear equation: $Y = a + b(1 - e^{-ct})$ (as described by Orskov and McDonald (1979). Where: Y = volume of gas produced at time 't'; a = intercept (gas produced from the soluble fraction); b = potential gas production (ml) from the insoluble fraction; c = gas production rate constant (h⁻¹) for the insoluble fraction (b); t = incubation time. Metabolizable energy (ME, MJ/kg DM) was calculated as $ME = 2.20 + 0.136 GV + 0.057 CP + 0.0029 CF$ (Menke and Steingass, 1988). Organic matter digestibility (OMD %) was estimated as $OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA$ (Menke and Steingass, 1988). Short chain fatty acids (SCFA) = $0.0239 GV - 0.0601$ (Getachew *et al.*, 1999). Where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively of the incubated samples.

2.4 Statistical analysis

Data from the experiment were subjected to one way analysis of variance (ANOVA) procedure of SAS (2000), using the completely randomized design. Significant means were ranked using the Duncan's multiple range test of the same package. Mean differences were considered significant at $P < 0.05$.

III. Results And Discussion

3.1 Proximate composition of grass and legume/browse species

3.1.1 Proximate composition of the grass species

Table 1 shows the proximate composition of the grass and legume/browse species. The dry matter contents of the four grass species ranged from 79.38% for *Panicum maximum* to 89.95% for *Imperata cylindrica*. The crude protein content of the grasses were 5.68% for *Brachiaria decumbens*, 7.30% for *Axonopus fissifolius*, 7.91% for *Panicum maximum* and 8.72% for *Imperata cylindrica*. These values are comparable to the values of 7.9% crude protein of *P. maximum* (Babayemi, 2007), 7.63% crude protein of *P. maximum* (Ajayi and Babayemi, 2008), 9.55% crude protein of *P. maximum* (Yusuf *et al.*, 2013), 9.40% crude protein of *P. maximum* (Asaolu *et al.*, 2014) and 10.47% crude protein of *P. maximum* (Tona, 2014). The crude protein levels of (5.68 – 8.72%) for the grass species in this study were close to the 8% crude protein required to provide the minimum ammonia levels required by micro-organisms for optimum rumen activity (Norton, 1995). It however did not meet the required range of 11.0 to 13.0% crude protein required to supply adequate protein for maintenance and moderate growth in goats (Poppi and McLennan, 1995). Since the crude protein contents of the grasses in the current study might not be adequate to meet the requirement for ruminants, proper supplementation with legume/browse species or concentrate diets could enhance ruminant performance as demonstrated in the research of Tona (2014). Tona (2014) investigated ruminant diet combinations of *P. maximum* with *Gliricidia sepium* and cassava peels.

3.1.2 Proximate composition of the legume/browse species

Dry matter contents of the legume/browse species ranged between 85.00 and 93.38%. The crude protein contents of the legume/browse species ranged from 16.02 in *C. pubescens* to 28.86% in *L. leucocephala*, and this range was close to the crude protein contents of 16.0% in *C. pubescens*, 25.7% in *G. sepium* and 27.6% in *L. leucocephala* reported by Babayemi (2007). It has been observed that the legume/browse species contained higher crude protein content than in the grass species. Similar findings were made in the works of Evitayani *et al.* (2004), Babayemi (2007), Ajayi and Babayemi (2008) and Asaolu *et al.* (2014). Also, the crude protein contents of the legume/browse species in the current study were well above the minimum requirement of 11.0 to 13.0% recommended for maintenance and moderate growth of goats (Poppi and McLennan, 1995). Other ranges of values observed in the present study were, 12.16 to 61.41% of crude fibre, 2.60 to 8.45% of ether extract and 4.10 to 10.52% of ash, for both the grass and legume/browse species. These are close to the ranges of 56.8 – 74.1%, crude fibre; 2.1 – 12.5%, ether extract; and 3.6 – 10.8% ash found in the study of Babayemi (2007), for grasses, legumes and browse plants. The high crude fibre content observed in *C. pubescens* (61.41%), in this study is favourable for the environmental conditions of the rumen which is normally in favour of the fibrolytic micro-organisms which aid the degradation of high fibre diets in contrast to the negative effects of concentrates high in carbohydrates (Nouala *et al.*, 2006).

Table 1 Proximate composition of grass and legume/browse species

Composition (%)	DM	CP	CF	EE	Ash	NFE
Grass species						
<i>Panicum maximum</i>	79.38	7.91	23.58	4.21	5.82	49.86
<i>Imperata cylindrica</i>	89.95	8.72	18.34	5.74	8.11	50.04
<i>Axonopus fissifolius</i>	80.65	7.30	24.32	3.85	6.37	47.51
<i>Brachiaria decumbens</i>	81.75	5.68	22.04	8.45	5.34	46.74
Legume/browse species						
<i>Leucaena leucocephala</i>	93.38	28.86	12.16	5.67	9.68	37.01
<i>Centrosema pubescens</i>	85.00	16.02	61.41	2.60	4.10	0.87
<i>Gliricidia sepium</i>	91.02	18.55	21.18	2.64	10.52	38.13

DM = Dry matter, CP = Crude protein, CF = Crude fibre, EE = Ether extract, NFE = Nitrogen free extract

3.2 In vitro gas production of grass and legume/browse species for a period of 24 hour

3.2.1 In vitro gas production of grass species

The gas production during the 24 hour period of incubation is presented in Table 2. All the values of the gas production in the grasses were significant ($P < 0.05$). At the 24 hours of incubation, the gas production in the grasses ranged between 24.00 ml/200mg (*I. cylindrica*) and 32.00 ml/200mg (*P. maximum*). In earlier studies, gas production values of 5 ml/200mg DM (Tona, 2014), 40 ml/200mg DM (Babayemi, 2007; Asaolu *et al.*, 2014) at 24 h of incubation period were reported for *P. maximum* grass.

3.2.2 In vitro gas production of legume/browse species

The *in vitro* gas production of the legume and browse plants ranged from 23.00 ml/200 mg DM (*G. sepium*) to 54.00 ml/200mg DM in (*C. pubescens*). The values observed in this research are comparable with the net gas volume of *C. pubescens* (49.7 ml/200 mg DM), *G. sepium* (59.7 ml/200 mg DM) and *L. leucocephala* (41.0 ml/200 mg DM) observed by Babayemi (2007). Asaolu *et al.* (2014) reported gas production volumes of

25.67 ml/200 mg DM in *G. sepium* and 18.00 ml/200 mg DM in *L. leucocephala*. Ajayi and Babyemi (2008) observed lower gas production volume of 17.50 ml/200 mg DM in *C. pubescens*. Fieves *et al.* (2005) reported that *in vitro* gas production is comparable with feed digestibility, so that the higher the gas production, the higher the digestibility. The differences in the values of gas production volumes could be due to differences in the presence of secondary metabolites (anti-nutrients) as were explained by Babayemi (2007) and Ajayi and Babayemi (2008). In this study, the highest ($P < 0.05$) gas production observed for *Centrosema pubescens* (54.00 ml/24 h) and *Leucaena leucocephala* (50.33 ml/24 h) could be due to their high CP contents. This could have enhanced microbial multiplication in the rumen fluid, which in turn could have determined the extent of their fermentation. This is supported in the research of Sommart *et al.* (2000), which suggested that gas volume is a good parameter from which to predict *in vitro* digestibility, fermentation end product and microbial protein synthesis of a substrate by rumen microbes in the *in vitro* system. In this study, the lower range of gas production from the grass species (24.00 – 32.00 ml) could signify lower fermentation potential than in the legume/browse species (23.00 – 54.00 ml). Babayemi and Bamikole (2006) explained that gas production from protein fermentation was relatively higher compared to carbohydrate fermentation. This confirms that the feeding of small ruminants with grass needs to be supplemented with legume/browse forage for efficient productivity.

Table 2 *In vitro* gas production volumes (ml/200 mg sample) of grass and legume/browse species for a period of 24 hour

Incubation period (h)	3	6	9	12	15	18	21	24
Grass								
<i>P. maximum</i>	2.33 ^c	6.00 ^b	10.00 ^b	16.00 ^a	22.67 ^a	28.33 ^a	32.00 ^a	32.00 ^a
<i>I. cylindrical</i>	2.67 ^b	5.33 ^c	10.00 ^b	14.67 ^b	19.67 ^b	23.00 ^b	23.67 ^c	24.00 ^c
<i>A. fissifolius</i>	3.00 ^a	7.00 ^a	12.00 ^a	16.33 ^a	19.00 ^b	21.33 ^c	22.67 ^c	25.00 ^c
<i>B. decumbens</i>	2.33 ^c	5.33 ^c	8.67 ^c	14.67 ^b	20.67 ^b	23.00 ^b	27.00 ^b	29.00 ^b
± SEM	0.16	0.39	0.69	0.44	0.80	1.52	2.10	1.85
Legume/browse								
<i>L. leucocephala</i>	8.67 ^a	16.00 ^a	24.00 ^a	31.67 ^a	36.00 ^a	38.67 ^a	44.00 ^a	50.33 ^a
<i>C. pubescens</i>	6.33 ^b	15.00 ^b	23.33 ^a	29.67 ^b	35.00 ^a	38.67 ^a	45.67 ^a	54.00 ^a
<i>G. sepium</i>	3.33 ^c	6.00 ^c	9.33 ^b	14.67 ^c	17.33 ^b	19.67 ^b	23.00 ^b	23.00 ^b
± SEM	1.55	3.18	4.78	5.36	6.06	6.33	7.29	9.78

^{a,b,c} Means on the same column with different subscripts are significantly different ($P < 0.05$).
± SEM = standard error of the mean

3.3 *In vitro* gas production characteristics of grass and legume/browse species

3.3.1 *In vitro* gas production characteristics of grasses

The *in vitro* gas production characteristics are shown in Table 3. *In vitro* gas production characteristics of grasses were found to be significantly different ($P < 0.05$) in all the four grass species. The volume of gas produced at time ‘t’, that is the (Y) values ranged from 13.67 to 23.33 ml/200 mg DM, while the ‘a+b’ values ranged from 24.00 to 32.00 ml/200 mg DM. The Y value for *P. maximum* was 23.33 and ‘a+b’ was 32.00 ml/200 mg DM in this study. Other researchers observed lower Y and ‘a+b’ values for *P. maximum*. Ajayi and Babayemi (2008) recorded the volume of gas production of Y = 6.52 and ‘a+b’ = 18.30 ml/200 mg DM for *P. maximum*. Tona (2014) observed Y = 10.00 and ‘a+b’ = 6.00 ml/200 mg DM for *P. maximum*. Asaolu *et al.* (2014) reported Y = 9.00 and ‘a+b’ = 17.67 ml/200 mg DM for sole *P. maximum*. The differences in the ‘Y’ and ‘a+b’ values were probably due to the differences in the crude fibre percentages of their proximate compositions. Babayemi *et al.* (2004) stated that there are many factors that may determine the amount of gas to be produced during fermentation, depending on the nature and level of fibre, the presence of secondary metabolites and potency of the rumen liquor used for incubation. They also explained that it was only possible to attain potential gas production of a feedstuff if the donor animal from which rumen liquor used for incubation was collected got their nutrients requirement met.

3.3.2 *In vitro* gas production characteristics of legume/browse species

The *in vitro* gas production characteristics were also observed to vary significantly ($P < 0.05$) in the three legume/browse plants. The values of the volumes of gas produced at time ‘t’ (Y) and ‘a+b’ values ranged from 14.67 and 23.00 ml/200 mg DM (*G. sepium*), 24.67 and 50.33 ml/200 mg DM (*L. leucocephala*) and 19.33 and 54.00 ml/200 mg DM (*C. pubescens*). Ajayi and Babayemi (2008) found ‘Y’ and ‘a+b’ values of 16.48 and 25.34 ml/200 mg DM (*C. pubescens*). In the research of Asaolu *et al.* (2014), 8.67 and 25.00 ml/200 mg DM were observed for *G. sepium*. Also for *L. leucocephala*, ‘Y’ and ‘a+b’ values were 10.00 and 18.00 ml/200 mg DM. Differences could be attributed to the reasons outlined above for grasses.

Table 3 *In vitro* gas production characteristics of grass and legume/browse species

Treatments	Y	b	a+b	c
Grass				
<i>P. maximum</i>	23.33 ^a	29.67 ^a	32.00 ^a	0.10 ^a
<i>I. cylindrical</i>	15.33 ^b	21.33 ^c	24.00 ^c	0.08 ^b
<i>A. fissifolius</i>	13.67 ^b	22.00 ^c	25.00 ^c	0.07 ^b
<i>B. decumbens</i>	14.67 ^b	26.67 ^b	29.00 ^b	0.05 ^c
± SEM	2.22	1.98	1.85	0.01
Legume/browse				
<i>L. leucocephala</i>	24.67 ^a	41.67 ^b	50.33 ^b	0.05 ^b
<i>C. pubescens</i>	19.33 ^b	47.67 ^a	54.00 ^a	0.04 ^c
<i>G. sepium</i>	14.67 ^c	19.67 ^c	23.00 ^c	0.08 ^a
± SEM	2.89	8.51	9.78	0.01

^{a,b,c} Means on the same column with different subscripts are significantly different (P<0.05). Y = volume of gas produced (ml/200 mg DM) at time 't', a = gas production (ml) from the soluble fraction, b = gas production (ml) from insoluble fraction, c = gas production rate (h⁻¹) constant from insoluble fraction 'b', a + b = potential gas production (ml), t = incubation time; ± SEM = standard error of mean

3.4 *In vitro* metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) levels of grass and legume/browse species

3.4.1 Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of grass species

Table 4 shows the *in vitro* metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of grass and legume/browse species. The values observed were all significantly (P<0.05) different. The values of ME, OMD and SCFA in the grasses ranged as follows: 6.01 to 7.07 MJ/kg DM of ME, 44.53 to 50.67 % of OMD and 0.51 to 0.70 µmol of SCFA. The highest values found were 7.07 MJ/kg DM of ME, 50.67 % of OMD and 0.70 µmol of SCFA in *P. maximum*. Lower values were reported in earlier research works; Ajayi and Babayemi (2008) observed 2.94 MJ/kg DM of ME, 29.15 % OMD and 0.06 µmol of SCFA for sole *P. maximum*. Tona (2014) reported 3.87 MJ/kg DM of ME, 31.23 % OMD and 0.08 µmol of SCFA for sole *P. maximum*. Asaolu *et al.* (2014) obtained predicted values of 5.24 MJ/kg DM of ME, 41.58 % OMD and 0.36 µmol of SCFA for sole *P. maximum*.

3.4.2 Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) of legume/browse species

Among the legume/browse species, the highest (P<0.05) values were observed for *C. pubescens* legume forage with 10.73 MJ/kg DM of ME, 78.91 % OMD and 1.23 µmol of SCFA. Next was for *G. sepium* browse plant with 10.64 MJ/kg DM of ME, 72.76 % OMD and 1.15 µmol of SCFA. The lowest values of 6.45 MJ/kg DM of ME, 50.13 % OMD and 0.49 µmol of SCFA were recorded for *L. leucocephala*. Lower values were reported in the study of Asaolu *et al.* (2014) as follows: 6.86 MJ/kg DM of ME, 52.35 % OMD and 0.54 µmol of SCFA for *Gliricidia sepium*; and for *Leucaena leucocephala* they observed; 6.28 MJ/kg DM of ME, 44.91 % OMD and 0.37 µmol of SCFA. Thus the grass and legume/browse species investigated in the current study have potential to make energy available to ruminants as was also reported in other studies (Ajayi and Babayemi (2008); Yusuf *et al.*, 2013; Tona *et al.*, 2014).

Table 4 *In vitro* metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) levels of grass and legume/browse species

Treatments	ME	OMD	SCFA
Grass			
<i>P. maximum</i>	7.07 ^a	50.67 ^a	0.70 ^a
<i>I. cylindrical</i>	6.53 ^b	46.69 ^b	0.63 ^a
<i>A. fissifolius</i>	6.09 ^c	44.53 ^b	0.54 ^b
<i>B. decumbens</i>	6.01 ^c	45.41 ^b	0.51 ^b
± SEM	0.24	1.36	0.04
Legume/browse			
<i>L. leucocephala</i>	6.45 ^b	50.13 ^b	0.49 ^b
<i>C. pubescens</i>	10.73 ^a	78.91 ^a	1.23 ^a
<i>G. sepium</i>	10.64 ^a	72.76 ^a	1.15 ^a
± SEM	1.41	8.75	0.23

^{a,b,c} Means on the same column with different subscripts are significantly different (p<0.05). ± SEM = standard error of mean

IV. Conclusion

The observed nutritive values of the forages understudied have shown that they could be beneficial for improved goat production in Nigeria. The legume/browse species contained higher amounts of crude protein, ash, enhanced gas production and also, higher predicted metabolizable energy, organic matter digestibility and short chain fatty acids than the grass species. Among the species of grass and legume/browse, the highest

metabolizable energy, organic matter digestibility and short chain fatty acids values were recorded for *Panicum maximum* and *Centrosema pubescens* respectively.

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