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Original Research Article

Investigation of proximate composition and *in vitro* fermentation characteristics of *Panicum maximum*, *Gliricidia sepium* with cassava peels as feed for ruminants in Nigeria

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A B S T R A C T

The proximate composition and *in vitro* gas production of diet combinations, T1 (60 % *Panicum maximum* + 40 % *Gliricidia sepium*); T2 (100 % *Panicum maximum*); T3 (60 % *Panicum maximum* + 30 % *Gliricidia sepium* + 10 % cassava peels) and T4 (100 % cassava peels) were evaluated in WAD goats. Samples of the four dietary treatments were analyzed for the proximate composition, and also incubated in triplicates for *in vitro* gas production for 24 hours. Methane (CH_4) gas produced was measured. Metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were estimated from the *in vitro* gas production parameters. The proximate composition values ranges were as follows: crude protein (CP), 7.02 to 17.58 %; crude fibre (CF), 16.59 to 23.62 %; ether extract (EE), 2.75 to 7.52 %; and ash, 5.24 to 8.54 %. Potential gas production (a+b) varied from 6.00 to 25.00 mL/200 mg DM. The highest ($P<0.05$) potential gas (a+b) value of 25 mL/200 mg DM was obtained for diet T4, next were T3 > T1 >T2. Least methane was obtained for diet treatment T2 (100 % *P. maxiumum*), which was indicative of a good energy source. The highest values of ME (6.53 MJ/kg DM), OMD (49.09 %) and SCFA (0.54 μmol) were obtained. This study revealed that efficient rumen fermentation could be achieved with the diet combination of 60 % *Panicum maximum* + 30 % *Gliricidia sepium* + 10 % cassava peels (T3), for optimal utilization by ruminants.

Keywords

Gas production, grass, browse plant, cassava peels, combinations, WAD goats

Introduction

In Nigeria, ruminant livestock producers are faced with the challenge of provision of good quality forage and other feed supplements during the dry season. Ajayi and Babayemi (2008) stated that *P. maximum* is one of the most common grasses in the derived Savanna region of Nigeria. Under adequate conditions, its nutritional value is high, having up to 12.5% crude protein, total digestible nutrients (TDN) of 10.20% and also has some minerals such as calcium, phosphorus and magnesium. Other researchers (Aderinola et al, 2014) affirmed that *P.maximum* has been classified among the best forage grass due to its high nutritive value. Also, *P. maximum* produces high yield of palatable fodder and is suited for grazing, but rapidly declines in nutritive value with age and could also die off if continually grazed close to the ground. Odeyinka (2001) mentioned that ruminants cannot meet their maintenance needs on grass alone. Bamikole and Babayemi (2004) stated that although ruminants relish *P. maximum* (guinea grass), this grass become scarce during the dry season, and this cause normads to travel long distances with their livestock in search of greener pastures. These findings led to the search for leguminous forages which are more palatable and well accepted by ruminants all year round (Odeyinka, 2001). One of such shrubs in Nigeria is *Gliricidia sepium* which is a medium sized, semi-deciduous tree that grows up to between 10 and 15 m high. *Gliricidia sepium* is described as a suitable feed for ruminants (Bawala et al, 2006) and Asaolu et al (2012) used *Gliricidia sepium* as a diet supplement to cassava peels for growing WAD goats. Oni et al. (2010) stated that despite the presence of cyanide anti-nutrient, cassava is an important food crop. In recent times, the interest of livestock farmers have been focused on the use of cassava peels as an animal feed. The goat is reported as the best ruminant species in the utilization of course materials for the production of meat, preferring feedstuffs relatively rich in crude fibre (Ososanya et al, 2013).

The *in vitro* gas production method is a laboratory estimation of degraded feeds which are important in livestock nutrition (Ajayi and Babayemi, 2008). The method is less expensive, fast and allows a large number of samples to be handled at a time, such that research results could be obtained within short time period. Also, metabolizable energy, organic matter digestibility and short chain fatty acids values can be estimated from gas production parameters (Babayemi, 2007; Tona et al, 2013; Yusuf et al, 2013). There is dearth of information on the use of *in vitro* gas fermentation method to determine the performance of ruminants fed different combinations of *P. maximum*, *G. sepium* forages with cassava peels.

The aim of this study is thus to determine the proximate composition and *in vitro* gas production characteristics of different combinations of *P. maximum*, *G. sepium* forages with cassava peels as diets for ruminants.

Materials and Methods

Location of experiment and sample collection

The proximate analysis was carried out at the Animal Production and Health laboratory, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. The *in vitro* gas production experiment was done at the Animal Science Laboratory, University of Benin, Benin, Nigeria. *Panicum maximum* (guinea grass) and *Glyricidia sepium* (forage legume) were harvested from the existing stands within the LAUTECH Teaching and Research Farm, while the cassava peels was purchased from a reputable feeds store in Ogbomoso, Nigeria.

Preparation of samples

The dietary treatments were prepared as follows:

T1 = 60% *P. maximum* + 40% *G. sepium*
T2 = 100% *P. maximum*
T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels
T4 = 100% cassava peels

Three hundred (300) g samples of each of the treatment diets (T1, T2, T3 and T4) were oven dried at 65^oC for 48 hours to a constant weight to determine the dry matter. Samples were also milled to pass through 2.0 mm sieve and stored in air tight containers pending laboratory analysis.

Chemical analysis

Milled samples of the *P. maximum*, *G. sepium* and cassava peels were weighed out and mixed in proportions to make up for the treatment diets T1, T2, T3 and T4. The samples were then analysed for dry matter, crude protein, crude fibre, ether extract and ash by the procedures of AOAC (2005). Nitrogen free extract (NFE) was calculated as follows: NFE = 100 - (% CP +% CF + % EE + % ash + % moisture).

***In vitro* gas production study**

This procedure has been documented in previous research works (Babayemi, 2007; Yusuf et al, 2013). Rumen fluid was obtained with suction tube from three female WAD goats that were previously fed (two days prior) with *Panicum maximum*, *Gliricidia sepium* and cassava peels at 5% of their body weights. The rumen liquor was collected into a themo flask that had been pre-warmed to a temperature of 39^oC, from the goats before they were offered their morning feed. Incubation procedure was as reported by Menke and Steingass (1988) using 120 ml calibrated transparent plastic syringes with fitted silicon tube.

The dietary treatments (T1, T2, T3 and T4) samples, in triplicates were carefully dropped into the syringes and then 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (g/liter) of 9.8 $NaHCO_3$ + 2.77 Na_2HPO_4 + 0.57 KCl + 0.47 NaCl + 2.16 $MgSO_4.7H_2O$ + 0.16 $CaCl_2.2H_2O$) (1:4 v/v) under continuous flushing with CO_2 was dispensed using another 50 ml plastic calibrated syringe. The syringe was tapped and pushed upward by the piston in order to completely eliminate air in the inoculums. The silicon tube in the syringe was then tightened by a metal clip so as to prevent escape of gas. Incubation was carried out at 6, 12, 18, and 24 h. At post incubation period, 4 ml of NaOH (10 M) was introduced to estimated methane production as reported by Fievez et al (2005). The average of the volume of methane gas produced from the blanks was deducted from the volume of gas produced from the samples. (The blanks contained only the inoculums and buffer).

Calculations: Rates and extent of gas production were determined for each substrate from 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/ g DM) 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 assessed as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988). Short chain fatty acids (SCFA) as 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.

Statistical analysis

All data were subjected to one way analysis of variance (ANOVA) procedure of SAS (2002). Significant means were ranked using the Duncan's multiple range test of same software package.

Results and Discussion

Proximate composition

The proximate composition of *P. maximum*, *G. sepium* with cassava peels in different combinations is presented in Table 1. The dry matter ranged between 89.65 and 93.79 %, which is similar to the range of 85.70 to 95.86 % reported by Asaolu et al (2012) for fodder, cassava peel and concentrate mixture. It is also comparable to the range of 86.76- 92.47 % obtained by Tona et al (2013) for cassava peels based diets. The least (7.02 %) and the highest (17.58 %) CP is recorded for dietary treatments T4 and T1 respectively. The CP content of 7.02 % for T4 is slightly below the minimum level for maintenance of 7.70 % for goats (NRC, 1981). In diets T1 to T3, the CP content range of 10.47 % in T2 (100 % *P. maximum*) to 17.58 % in T1 (60 % *P. maximum* + 40 % *G. sepium*) is above this minimum level and also exceeded the 8 % CP observed by Norton (1994) to provide the ammonia levels required by rumen microbes for optimum activity. The low CP content of 7.02 % observed for T4 (100 % cassava peels) is similar to the low value of 4.90 % CP in dried cassava peels recorded in earlier research work (Ukanwoko et al, 2009). However, Smith (1988) reported from his research findings that cassava peels should not be fed alone, as the protein and mineral contents cannot support optimum rumen function and productivity. That the optimal utilization of cassava peels require supplementation with readily fermentable protein and by-pass protein, as well as micronutrients including sulphur, phosphorus and vitamin B. He also mentioned that if fed in a balanced diet, cassava peels are valuable feed for ruminants. The ranges of CF, EE and Ash levels of the dietary treatments T1 to T4 are as follows: 16.59 – 23.62 % CF; 2.75 – 7.52 % EE; and 5.24 – 8.54 % ash. The values obtained in a similar research are also as follows: 7.51 to 17.18 % CF: 1.72 to 8.32 % EE; and 7.46 to 19.19 % ash (Asaolu et al, 2012). Nouala et al (2006) outlined that the environmental conditions of the rumen are 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. This reiterates the fact that treatment diet T4 (100 % cassava peels) might need to be supplemented with readily fermentable protein or by-pass protein sources as well as micro-nutrients for optimal utilization by ruminants.

***In vitro* gas production characteristics**

Table 2 and Figure 1 show the *in vitro* gas production of dietary treatments T1 to T4 during the 24 hours of incubation. Gas production increases as incubation progresses and there are significant differences ($P<0.05$) in the gas production characteristics of T1 to T4. The highest ($P<0.05$) potential gas production (a + b) value of 25.00 mL/200 mg DM is recorded for dietary treatment T4 (100 % cassava peels). This might be attributed to its high content of degradable carbohydrates as is reported in similar research by Tona et al (2013), next are T3 (60 % *P. maximum* + 30 % *G. sepium* + 10 % cassava peels) > T1 (60 % *P. maximum* + 40 % *G. sepium*) > T2 (100 % *P. maximum*). These probably reflect the proportions of the degradable carbohydrate sources in these diets (Blummel and Becker, 1997). The potential gas production (a + b) value for T2 (100 %

P. maximum) was lowest (6.00 ml) and this probably is due to its crude fibre (highest) content of 23.62 %. Babayemi and Bankole (2006) explained that sole guinea grass (100 % *P. maximum*) is high in crude fibre and this may reduce its digestibility. Also, digestibility has been described to be synonymous to *in vitro* gas production (Fievez et al, 2005). The low gas production in *P. maximum* in this study does not however reduce its importance in ruminant feeding. This is because it is one of the commonest grasses around and is relished by ruminants in the tropics (Bamikole and Babayemi, 2004). The potential gas production in T1 (60 % *P. maximum* + 40 % *G. sepium*) with 17.58 % CP and T3 (60 % *P. maximum* + 30 % *G. sepium* + 10 % cassava peels) with 13.21 % CP are higher than in T4 (100 % cassava peels) with 7.02 % CP. Babayemi and Bamikole (2006) explained that there is relatively lower gas production from diets having higher protein content.

Methane gas production in diets T1 to T4 is shown in Figure 2. The range of 4.00 to 10.00 ml/200 mg DM recorded in this study is lower than 29.75 – 53.25 ml/200mg DM observed in a similar study by Yusuf et al (2013) for selected forages. In the current research, methane gas production is lowest in T2 (100 % *P. maximum*), followed by T1 (60 % *P. maximum* + 40 % *G. sepium*), then T3 (60 % *P. maximum* + 30 % *G. sepium* + 10 % cassava peels), which contain some of the *G. sepium* browse plant, while methane level is highest in T4 (100 % cassava peels). Yusuf et al (2013) reported that high methane production has a negative effect on ruminants as it implies an energy loss and when it accumulates in the rumen, this could result in bloat. The high methane production observed in diets high in legume (T1 and T3) and carbohydrate (T4, 100% cassava peels) might indicate that browse legumes and sole carbohydrate diets would need to be supplemented with grasses, which have higher crude fibre content for adequate utilization by ruminants.

Metabolizable energy, organic matter digestibility and short chain fatty acids levels

Metabolizable energy (MJ/kg DM), organic matter digestibility (%) and short chain fatty acids (µmol) levels are shown in Table 3. The values of ME, OMD and SCFA were all significant ($P<0.05$) among the treatments T1 to T4 and ranged between 3.87 and 6.53 MJ/kg DM; 31.23 and 49.09 % and 0.08 and 0.54 µmol, respectively. The values observed in the current study are higher than the ranges of 2.99 to 4.75 MJ/kg DM (ME) and 21.46 to 33.80 % (OMD) cited by Babayemi and Bamikole (2006) for *P. maximum* (grass) and *Tephrosia candida* (browse plant) mixtures. The values obtained in this study are however lower than the ranges of 7.25 to 10.10 MJ/kg DM (ME), 51.87 to 80.19 % (OMD) and 0.74 to 1.22 µmol (SCFA) observed in another study on browse plants (Yusuf et al, 2013). Short chain fatty acids are volatile fatty acids (VFA) and their presence denote energy is available in a feedstuff. These observations imply that the dietary treatments T1 – T4 have potential to make energy available to ruminants. In another research (Ajayi and Babayemi, 2008), it was further explained that SCFA is one of the end products of rumen fermentation, and that a high volume of gas is produced when substrate is fermented to acetate and butyrate, however, relatively lower gas production is associated with propionic production. This is in agreement with the observation of Ngamsaeng et al (2006) that *in vitro* fatty acid production has low correlation with volatile fatty acid production particularly that of propionate.

Table.1 Proximate composition of *P. maximum*, *G. sepium* with cassava peels in different combinations

Parameters (%)	T1	T2	T3	T4
Dry matter	89.65	90.37	92.72	93.79
Crude protein	17.58	10.47	13.21	7.02
Crude fibre	16.59	23.62	18.82	21.09
Ether extract	7.52	3.46	2.75	3.52
Ash	5.24	8.54	7.68	7.74
Nitrogen free extract	42.72	44.28	49.26	54.42

T1 = 60% *P. maximum* + 40% *G. sepium*, T2 = 100% *P. maximum*, T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels and T4 = 100% cassava peels

Table. 2 *In vitro* gas production characteristics of *P. maximum*, *G. sepium* with cassava peels in different combinations

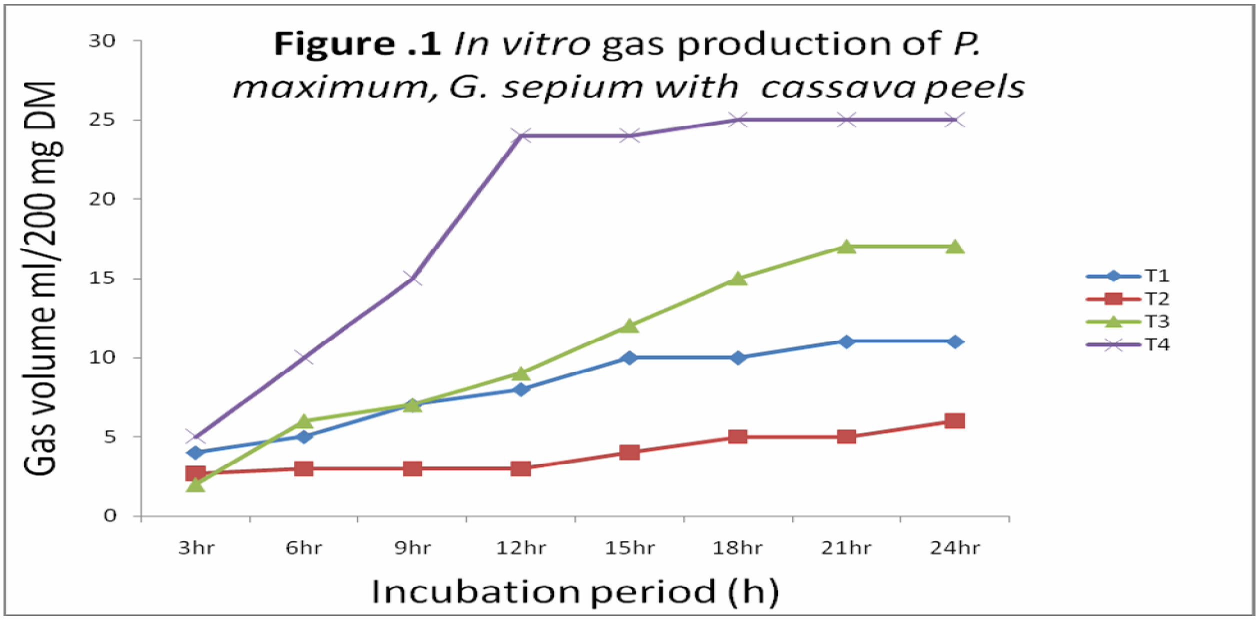
Treatment	a	b	a + b	c	T	Y
T1	4.00^{ab}	7.00^{c}	11.00^{c}	0.08^{b}	8.00^{b}	7.00^{bc}
T2	3.00^{bc}	3.00^{c}	6.00^{d}	0.05^{b}	16.00^{a}	10.00^{b}
T3	2.00^{c}	15.00^{b}	17.00^{b}	0.08^{b}	11.00^{ab}	10.00^{b}
T4	5.00^{a}	20.00^{a}	25.00^{a}	0.18^{a}	11.00^{ab}	21.33^{a}
SEM	0.35	1.17	1.12	0.02	1.37	1.22

[a,b,c,d] 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 an insoluble fraction, c = gas production rate (h^{-1}) constant from insoluble fraction 'b', a + b = potential gas production (ml), t = incubation time, T1 = 60% *P. maximum* + 40% *G. sepium*, T2 = 100% *P. maximum*, T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels and T4 = 100% cassava peels

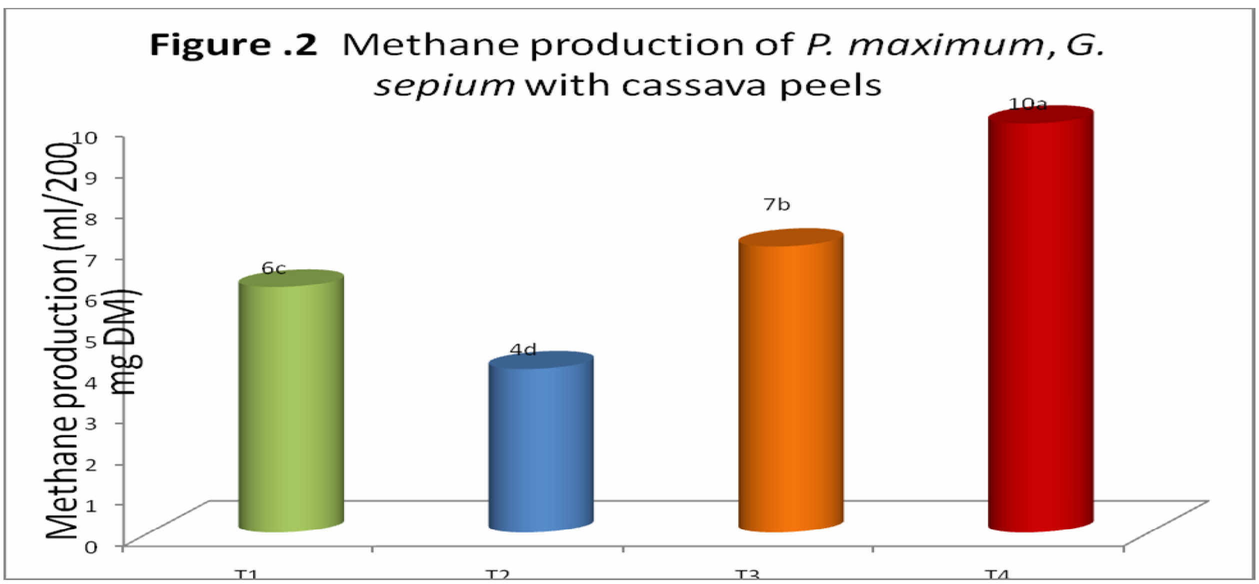
Table.3 Metabolizable energy (MJ/kg DM), organic matter digestibility (%) and short chain fatty acids (µmol) of *P. maximum*, *G. sepium* with cassava peels in different combinations

Treatment	ME	OMD	SCFA
T1	4.36^{c}	34.96^{c}	0.20^{c}
T2	3.87^{c}	31.23^{c}	0.08^{d}
T3	5.52^{b}	42.40^{b}	0.35^{b}
T4	6.53^{a}	49.09^{a}	0.54^{a}
SEM	0.15	0.10	0.03

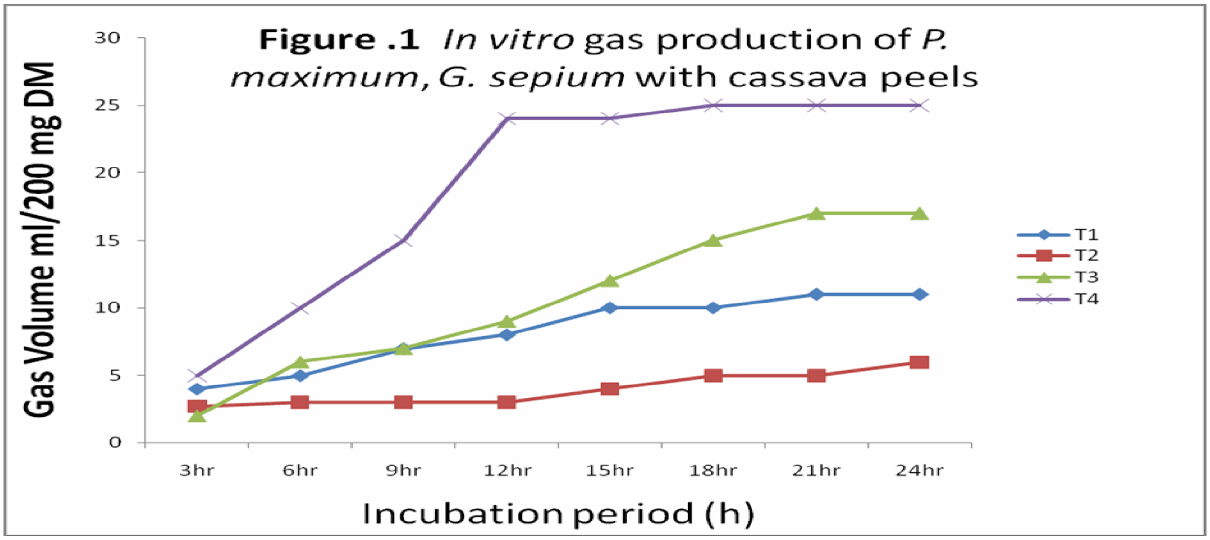
[a,b,c] Means on the same column with different subscripts are significantly different ($p<0.05$);
ME = metabolizable energy, OMD = organic matter digestibility, SCFA = short chain fatty acids,
T1 = 60% *P. maximum* + 40% *G. sepium*, T2 = 100% *P. maximum*, T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels and T4 = 100% cassava peels



T1 = 60% *P. maximum* + 40% *G. sepium*, T2 = 100% *P. maximum*, T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels and T4 = 100% cassava peels



T1 = 60% *P. maximum* + 40% *G. sepium*, T2 = 100% *P. maximum*, T3 = 60% *P. maximum* + 30% *G. sepium* + 10% cassava peels and T4 = 100% cassava peels



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