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ORIGINAL ARTICLE

Chemical composition and nutritive value of four varieties of cassava leaves grown in South-Western Nigeria

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Summary

The nutritive value of leaves of four varieties of cassava – MS 6, TMS 30555, *Idileruwa* and TMS 30572 was evaluated based on their chemical composition and *in vitro* fermentation. Crude protein (CP) contents of cassava leaves ranged from 177 to 240 g/kg dry matter (DM), with TMS 30555 showing the highest CP contents. Neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) contents of cassava leaves ranged from 596 to 662 and 418 to 546 g/kg DM respectively. Condensed tannin (CT) and hydrocyanic acid contents ranged from 1.0 to 3.8 g/kg and 58.5 to 86.7 mg/kg DM respectively. The range of volatile fatty acids (VFA) in the supernatant after *in vitro* incubation of the cassava varieties was: acetate (14.7–31.5 mmol/l); propionate (4.5–6.3 mmol/l); butyrate (3.1–3.9 mmol/l); valerate (0.4–0.6 mmol/l); iso-butyrate (0.6–1.3 mmol/l); iso-valerate (1.1–1.9 mmol/l). The acetate:propionate ratio resulting from fermentation of TMS 30555 was higher($p < 0.05$) than that of the other leaves. The highest *in vitro* gas production of 50.5 ml/200 mg DM was recorded for MS6 being higher ($p < 0.05$) than for TMS 30572, but similar to TMS 30555 and *Idileruwa*. The DM, CP, ADF and HCN contents of cassava leaves were positively correlated with gas production, while CT content was negatively correlated with gas production. The study showed that leaves of the varieties MS 6 and TMS 30555 are superior to the others in terms of CP and gas production indicating a higher digestibility and energy content and thus nutritive potential. They may therefore serve as supplements for ruminants fed on poor roughages.

Introduction

Cassava (*Manihot esculenta Crantz*) is one of the most important staple food crops grown in tropical Africa, Asia and Latin America. Nigeria alone as at 2005 produced over 14 M tonnes, representing about 25% of sub-Saharan Africa's output (Ayodeji, 2005). Although it is the third most important food source in the tropical world after rice and maize, and provides energy for over 160 M people in Africa (Polsen and Spencer, 1991), its food value is greatly compromised by the presence of endogenous cyanogenic glucosides. Unlike the roots that largely consist of carbohydrates, cassava leaves, a by-product of

cassava root harvest, are (depending on the variety) rich in protein [14–40% of dry matter (DM)], minerals, vitamins B1, B2 and C, and carotenes (Adewusi and Bradbury, 1993). The leaves compare favourably with other green browses generally regarded as good protein sources. Available literature clearly suggests, that apart from lower methionine, lysine and perhaps isoleucine contents, the amino acid profile of cassava leaf protein compares favourably with those of milk, cheese, soybean, fish and egg (Devendra, 1977). However, while the macro- and micro-nutrient content of the leaves may be high, the processing techniques used can lead to huge losses of nutrients (Hahn, 1988). Recently, the Nigerian Government launched a campaign promoting the large-scale production of cassava for export purposes, for the production of glucose syrup and the inclusion in wheat flour for bread baking. This has led to the production and distribution of seedlings of some improved cultivars, especially TMS 30572, TMS 30555, MS 6 and the local cultivar of *Idileruwa* to farmers by government agencies and research institutes. This study evaluates and characterizes chemical composition and *in vitro* fermentation of the leaves of these four varieties of cassava grown in South-Western Nigeria.

Materials and methods

Leaves samples

Leaves (leaf with petiole) of four different varieties of cassava were collected by hand at the time of root harvest from three replicate plots established in the experimental field of the Research and Development Centre, University of Agriculture, Abeokuta, Nigeria. The area was located in the derived savannah vegetation zone of south-western Nigeria. The climate in this area is tropical, with a wet season from March to October and a dry season from November to February. Annual rainfall averages approximately 1100 mm and the peak rainfall occurs in the period of June to September. Leaves were shade dried and representative dry samples from each plot were taken to a laboratory and milled as described below before chemical analysis and use in *in vitro* fermentation studies.

Chemical analyses of cassava leaves

The foliage samples of the cassava leaves were subsampled and weighed fresh at the time of collection, and then oven dried at 65 °C to constant weight to determine DM content. The dried foliage samples were ground with hammer mill through a 1-mm sieve and stored for subsequent chemical and *in vitro* analyses. Parameters analysed according to the standard methods of AOAC (1995) included ash (ID; 942.05), crude protein (CP; ID 984.13), ether extract (EE; ID 963.15), crude fibre (Foss fibertec 3428), neutral detergent fibre (NDF), acid detergent fibre (ADF; including ash; ID 973.18) and acid detergent lignin (ADL). NDF and ADL (by solubilization of cellulose with sulphuric acid) were determined by the methods of Van Soest et al. (1991). NDF was analysed using sodium sulphite and amylase and expressed with residual ash. Hydrocyanic acid (HCN) content was determined using the method of Bradbury et al. (1999). Condensed tannin (polyphenols) was estimated by the HCl–butanol method as outlined by Porter et al. (1986).

Volatile fatty acids (VFA) in the supernatant after the *in vitro* fermentation were determined using high performance liquid chromatography (La Chrom, Merck Hitachi, Tokyo, Japan) according to the method of Ehrlich et al. (1981), subsequent to preparation of the samples as described by Doane et al. (1998). The column used (HPX-87H, Bio-Rad Laboratories, Hercules, CA, USA) was 7.8 mm × 300 mm in size. Separation was at an oven temperature of 30 °C, and isocratic elution with 5 mM H_2SO_4 as the mobile phase. The pH of the *in vitro* supernatant was determined using JENWAY pH meter, model 3150. For the determination of ammonia N, the *in vitro* supernatants were acidified with 1 ml sulphuric acid (200 ml H_2SO_4/l, 10 M, final concentration 2%) per 5 g sample to stop fermentation and stored at −20 °C. Samples were later thawed and after settling, an amount of 5 ml of upper clear layer was combined with 10 ml of 1 M NaOH (400 ml/l) and steam distilled (Kjedahl) and titrated to determine ammonia N (Lanyasunya et al. 2007).

In vitro fermentation study

The *in vitro* fermentation experiment was carried out at the Key Laboratory of Agricultural Ecological Engineering, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, Hunan Province, China following the procedure of Menke and Steingass (1988). Rumen fluid was collected before the morning feed from three ruminally cannulated Chinese Liuyang black goats (body weight 14.2 ± 0.5 kg) previously fed with 40% concentrate feed (40% maize, 45% wheat offal, 1% sodium chloride and 4% oyster shell) and 60% rice straw. The rumen fluid was mixed using equal volumes of

fluid from the three goats. The rumen fluid mixture was filtered through three layers of cheese cloth into a pre-warmed, insulated bottle and taken to the laboratory immediately after collection. The fluid was then mixed (1:2, v/v) with a buffer and mineral solution containing, per litre, g: 8.75 $NaHCO_3$, 1.00 NH_4CO_3, 1.43 Na_2HPO_4, 1.55 KH_2PO_4, 0.15 $MgSO_4{\cdot}7H_2O$, 0.52 Na_2S, 0.017 $CaCL_2{\cdot}2H_2O$, 0.015 $MnCl_2{\cdot}4H_2O$, 0.002 $CoCl_3{\cdot}6H_2O$, 0.012 $FeCl_3{\cdot}6H_2O$ and 0.125 resazurin. The laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. About 200 ± 10 mg of DM of each sample was accurately weighed into a nylon bag (12 × 12 mm, pore size: 45 μm). Each sample was measured in triplicate. Each nylon bag was put into a 100 ml glass syringe fitted with a plunger. The syringes were filled with 30 ml of incubation medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution and were placed in a shaking water bath (DS H2-300; Taicang, Jiangsu, China) with 50 movements per minute at 39 °C. The volume of gas released was read directly on the graduated syringe. A blank syringe containing 30 ml of the buffered inoculum only was also included as control. Gas volume was recorded at 2, 4, 6, 8, 12, 24, 36, 48 and 72 h of incubation. The average of the volume of gas produced from the blanks was deducted from the volume of gas produced by any other sample at that time. The data obtained were fitted to the non-linear equation (Larbi et al., 1996):

$$V(\text{ml}/200\text{mg DM}) = \text{GV}(1 - \text{e}^{-ct})$$

where V = potential gas production at time t, GV= the volume of gas that will evolve with time, and c = the fractional rate of gas production. Initial gas production rate (Abs_g) was calculated as the product of GV and c (Larbi et al., 1996).

Calculations and statistical analyses

The post-incubation parameters such as metabolizable energy (ME) and *in vitro* organic matter digestibility (IVOMD) were estimated at 24 h post-gas collection (Menke and Steingass, 1988) as follows:

$$\text{ME}(\text{MJ}/\text{kg DM}) = 2.20 + 0.136\text{GV} + 0.057\text{CP} + 0.0029\text{CF}$$

(Menke and Steingass, 1988).

$$\text{IVOMD}(\%) = 14.88 + 0.889\text{GV} + 0.45\text{CP} + 0.651\text{XA}.$$

where GV (ml/200 mg DM) = total gas volume, CF (%) = crude fibre and XA (%) = ash.

Data collected were subjected to analysis of variance using SPSS (1999). Duncan's multiple range test was used to identify significant differences among means (Duncan, 1955). The relationships between variables were analysed using correlation analysis.

Results

Table 1 shows the chemical composition of the leaves of cassava varieties. The CP content ranged from 177 ± 0.30 g/kg DM in *Idileruwa* to 240 ± 0.60 g/kg in TMS 30555 and was higher ($p < 0.05$) in TMS 30555 than other varieties. The mean NDF content was 621 g/kg DM ranging from 596 ± 3.00 g/kg DM in MS6 to 662 ± 4.00 g/kg DM in *Idileruwa* ($p < 0.05$). The contents of EE and ash ranged from 60.0 ± 3.50 g/kg in TMS 30555 to 73.0 ± 2.90 g/kg DM in MS6 and 64.9 ± 4.70 g/kg

Table 1 Chemical composition (g/kg DM) of four varieties of cassava leaves (means ± SE)

	Varieties			
Parameters*	MS 6	TMS 30555	*Idileruwa*	TMS 30572
Dry matter	900 ± 1.1^{a}	884 ± 0.9^{b}	891 ± 1.3^{b}	901 ± 4.9^{a}
Crude protein	235 ± 0.4^{b}	240 ± 0.6^{a}	177 ± 0.3^{d}	208 ± 1.2^{c}
Ether extract	73.0 ± 2.90^{a}	60.0 ± 3.50^{b}	66.0 ± 3.50^{ab}	69.7 ± 4.60^{ab}
Ash	161 ± 1.0^{a}	160 ± 0.8^{ab}	153 ± 0.4^{b}	64.9 ± 4.70
Neutral detergent fibre	596 ± 3.0^{c}	613 ± 1.4^{b}	662 ± 4.0^{a}	613 ± 3.5^{b}
Acid detergent fibre	546 ± 5.9^{a}	529 ± 8.0^{a}	418 ± 7.7^{c}	480 ± 8.7^{b}
Acid detergent lignin	293 ± 5.1^{a}	279 ± 2.6^{a}	245 ± 6.5^{b}	254 ± 6.7^{b}
Condensed tannin	1.0 ± 0.00^{d}	1.4 ± 0.02^{c}	3.8 ± 0.11^{a}	2.2 ± 0.02^{b}
Hydrocyanic acid (mg/kg DM)	83.7 ± 2.92^{a}	58.5 ± 3.35^{b}	86.7 ± 2.19^{a}	78.6 ± 2.95^{a}
Metabolizable energy (MJ/kg DM)†	3.5 ± 0.04^{b}	4.3 ± 0.06^{a}	3.8 ± 0.04^{ab}	4.0 ± 0.05^{ab}

Means within the same row with different superscripts are significantly different ($p < 0.05$).
*Mean values (n = 3).
†Estimated according to MAFF (1984) equation (ME = DOM% × 0.15).

in TMS 30572 to 161 ± 1.00 g/kg in MS6 respectively. The cyanogenic potential ranged from 58.5 ± 3.35 mg HCN/kg in TMS 30555 to 86.7 ± 2.19 mg HCN/kg in *Idileruwa* ($p < 0.05$). The total polyphenols (as tannic acid equivalent) varied ($p < 0.05$) from 1.0 g/kg in MS6 to 3.8 g/kg in *Idileruwa*.

The profile of VFA in the supernatant after *in vitro* incubation of the cassava varieties is shown in Table 2. There were differences ($p < 0.05$) in the acetate concentration between the cassava varieties ranging from 31.5 ± 7.01, 23.7 ± 4.00, 20.6 ± 3.81 to 14.7 ± 1.44 mmol/l in TMS 30555, TMS 30572, MS 6 and *Idileruwa* respectively. Also, the isovalerate values were different ($p < 0.05$) among the varieties. The acetate to propionate ratios were different ($p < 0.05$) ranging from 3.3 ± 0.06 mmol/l in *Idileruwa* to 5.0 ± 0.43 mmol/l in TMS 30555.

There were steady increases in the volume of gas production as incubation period progressed from 2 to 72 h (Fig. 1). The highest volume of 50.5 ml/200 mg DM was recorded for MS6 at the end of the 72 h of incubation. This gas production was higher ($p < 0.05$) than that from TMS 30572 at 36 to 72 h, but similar with TMS 30555 and *Idileruwa*. The total fractional rate of gas production of the four cassava varieties studied showed that TMS 30572 was highest with 0.14 ml/h; *Idileruwa* had 0.10 ml/hr; TMS 30555 had production rate of 0.06 ml/h while MS 6 had the lowest rate with 0.05 ml/h (Table 3).

The post-incubation parameters described by ME and IVOMD are presented in Table 4. The values for ME and IVOMD ranged from 10.1 ± 0.55 (MJ/kg) in TMS 30572 to 11.8 ± 0.68 (MJ/kg DM) in TMS 30555, and 66.4 ± 2.16 in TMS 30572 to 80.5 ± 2.61(%) in TMS 30555.

Table 5 shows the results of correlation coefficients between chemical composition and gas production

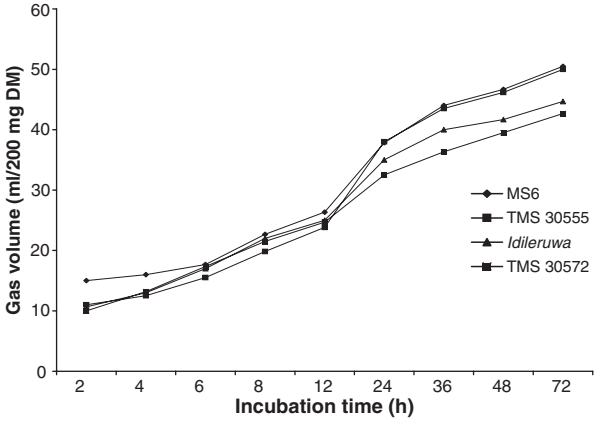

Fig. 1 Evolution of *in vitro* gas production of four varieties of cassava leaves.

of the four varieties of cassava. There were both positive and negative correlations especially with contents of DM, CP, ADF, HCN and tannin. DM was positively correlated at 8 and 12 h of incubation while CP was positively correlated at 48 and 72 h of incubation. NDF was negatively correlated with gas production throughout incubation.

Discussion

The chemical composition of four cassava varieties presented in this study showed that their CP contents compared favourably with, and in certain cases surpassed, those reported for leguminous browse plants grown in West Africa (Aletor and Aladejimi, 1989). Wanapat (2001) reported an even higher CP content of 200 to 300 g/kg DM for dried cassava leaves. The variation in the CP content may at least partly be attributed to varietal leaf differences.

The NDF, ADF and ADL values reported for the four varieties exceeded values reported in literature.

Table 2 Volatile fatty acid (mmol/l), pH and ammonia concentrations in *in vitro* incubations after incubating dried cassava leaves of four varieties for 72 h

	Varieties			
Parameters	MS 6	TMS 30555	*Idileruwa*	TMS 30572
Acetate	20.6 ± 3.81^{ab}	31.5 ± 7.01^{a}	14.7 ± 1.44^{b}	23.7 ± 4.00^{ab}
Propionate	5.2 ± 0.49	6.2 ± 0.97	4.5 ± 0.48	6.3 ± 0.56
Butyrate	3.3 ± 0.27	3.5 ± 0.26	3.1 ± 0.13	3.9 ± 0.17
Isobutyrate	1.1 ± 0.31	1.3 ± 0.41	0.6 ± 0.65	1.1 ± 0.35
Isovalerate	1.5 ± 0.20^{ab}	1.4 ± 0.12^{b}	1.1 ± 0.13^{b}	1.9 ± 0.07^{a}
Valerate	0.5 ± 0.03	0.4 ± 0.10	0.4 ± 0.13	0.6 ± 0.09
Acetate:Propionate ratio	3.9 ± 0.34^{b}	5.0 ± 0.43^{a}	3.3 ± 0.06^{b}	3.7 ± 0.55^{b}
pH	6.8 ± 0.02	6.8 ± 0.01	6.8 ± 0.03	6.8 ± 0.01
Ammonia (g/kg DM)	2.4 ± 0.08	2.2 ± 0.07	2.4 ± 0.05	2.4 ± 0.04

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

Table 3 Gas production characteristics of four varieties of cassava leaves

	Varieties				
Parameters	MS 6	TMS 30555	*Idileruwa*	TMS 30572	SEM
GV(ml/200 mgDM)	33.3^{a}	32.8^{a}	25.4^{b}	23.0^{b}	2.60
c (ml/h)	0.05^{b}	0.06^{b}	0.10^{a}	0.14^{a}	0.03
abs_g (ml)	1.71^{c}	1.97^{c}	2.46^{b}	3.24^{a}	0.24
Lag time (h)	3.42^{b}	1.67^{ab}	1.51^{ab}	0.79^{b}	1.83

Means within the same row with different superscripts are significantly different ($p < 0.05$).
GV = volume of gas produced per unit of time (*t*); *c* = fractional rate of gas production; abs_g = absolute initial gas production during the first hour.

Table 4 Estimated metabolizable energy (ME; MJ/kg DM) and organic matter digestibility (OMD; %) of four varieties of cassava leaves

	Varieties			
Parameters	MS 6	TMS 30555	*Idileruwa*	TMS 30572
ME	11.8 ± 0.56	11.8 ± 0.68	10.5 ± 0.08	10.1 ± 0.55
OMD	80.3 ± 2.18^{a}	80.5 ± 2.61^{a}	72.6 ± 0.27^{b}	66.4 ± 2.16^{b}

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

Table 5 Correlation coefficients (*r*) of relationship of chemical composition with *in vitro* gas production

Incubation time (h)	Chemical constituents (g/kg DM)					
	DM	CP	NDF	ADF	HCN	Tannin
2	−0.12	0.48	−0.49	0.60**	0.25	−0.56*
4	0.21	0.21	−0.28	0.29	0.30	−0.33
6	0.41	−0.22	−0.03	−0.11	0.52*	0.07
8	0.53*	−0.20	0.01	−0.07	0.39	0.03
12	0.52*	−0.01	−0.10	0.02	0.25	−0.08
24	0.27	0.38	−0.18	0.29	−0.14	−0.32
36	0.17	0.48	−0.24	0.42	−0.13	−0.41
48	0.16	0.56*	−0.31	0.47	−0.17	−0.51*
72	0.06	0.59*	−0.35	0.51*	−0.17	−0.53*

*$p < 0.05$; **$p < 0.01$.
DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; HCN, hydrocyanic acid.

For example, Khang (2004) reported a range in NDF of 260–510 g/kg DM and ADF contents from 190 to 370 g/kg DM. Wanapat et al. (2000) obtained NDF, ADF and ADL values of 296, 241 and 47 g/kg DM, respectively, for dried cassava leaves. The fact that the varieties of this study contained >500 g NDF/kg DM showed that they have only fair proportions of soluble carbohydrate which is helpful to maintain a proper rumen function (Oni et al., 2008). This study shows that apart from cyanide, the leaves contained some CT (1.00–3.83 g/kg). According to Wanapat (2001), levels of CT are generally higher in mature cassava leaves than in cassava hay (leaves + top soft stem) harvested at a younger growth stage. Reed (1995) reported that if CT in the feed exceeds 60 g/kg DM, it would reduce feed intake and digestibility. In contrast, if the level of CT was between 20 and 40 g/kg DM, the positive effect, namely its help to protect protein from rumen digestion and thus increase bypass protein, would be overriding. According to Ayodeji (2005), CT brings about their nutritional influences (especially in non-ruminants) largely, by binding dietary proteins into complexes that are not readily degradable at the pH conditions in the rumen. The concentration of VFA in incubation liquid, especially that of acetate, propionate and butyrate, were lower in the variety that contained most CT. This result was contrary to the report by Ngamsaeng et al. (2006) that propionate production was slightly higher in local tropical plants using *in vitro* fermentation, but agrees with the findings that acetate-to-propionate ratio was lower in the group with higher CT. The lower production of VFAs especially in *Idileruwa* could therefore, be due to the elevated content of CT consistent with the finding by Getachew and Makkar (2002). *In vitro* gas production during the fermentation periods did not significantly differ indicating that contents of degradable carbohydrates were also quite similar. MS6 and TMS 30555, however, may have had more slowly fermentable carbohydrate contents than the other two varieties. Gas production parameters suggested differences in nutritional values that were generally closely related to chemical composition (Cerrillo and Juarez, 2004). The high *in vitro* gas production at 72 h of incubation in MS6 and TMS 30555 indicates a higher extent of fermentation throughout the incubation periods versus the other varieties, especially TMS 30572. Differences in gas production among the leaves could be due to proportion, and nature of their fibre (Rubanza et al., 2003). Additionally the higher levels of secondary compounds (Salem et al., 2006) especially tannin in *Idileruwa* and TMS 30572, could have been responsible for their reduced gas production compared to the other varieties by exerting adverse effects on ruminal microbes (McSweeney et al., 2005). This seems to be an effect of a reduction in microbial attachment to feed particles (McAllister et al., 1994) and inhibition of microbial growth and enzyme activity (McSweeney et al., 2005). However, differences in gas production among browses could also be due to

the extent of lignification of NDF (Fonesca et al., 1998). The low gas production values of *Idileruwa* and TMS 30572 could also be due to its NDF being bound by polyphenols (Ndlovu and Nherera, 1997). Finally, some variations among leaves could be due to genotypic characteristics including the type of secondary compound activity on digestibility (Salem et al., 2006).

The values for ME ranging from 10.1 to 11.8 MJ/kg DM obtained in this study fell within the range reported for several other dry season forages by Babayemi (2007) and foliage and fruits of *Enterolobium cyclocarpum* by Babayemi (2006). The IVOMD values of 66.4 to 80.5% were also within the range obtained by the same author for some dry season forages and foliage and fruits of *E. cyclocarpum* respectively. The range of gas volume recorded for these cassava varieties indicated that they are capable of producing approximately 50.4 mg/g of microbial mass (Blummel et al., 1997).

The presence of correlations between nutrient composition and *in vitro* gas production is consistent with the findings of Nsahlai et al. (1994), Getachew et al. (2003) and Cerrillo and Juarez (2004). This may be a result of feed constituents such as NDF and ADF, which are less degradable than soluble carbohydrates and therefore reduce gas production. In case they are better degradable, this might be different. The positive and relatively high correlation between CP and gas production especially between 36 and 72 h of incubation time is consistent with the reports of Larbi et al. (1998) and Nherera et al. (1999). It can be estimated that 30% of the variation in the gas production can be explained by variation in the CP concentration of the leaves of the four cassava varieties. Extra CP may enhance ruminal fermentation in case rumen-degradable protein is lacking. However, it is important to note that there might be a confounding by the fact that NDF contents (not ADF contents, though) as well as CT were higher with lower CP. At most of the incubation times, except 6–8 h, gas production was negatively correlated with CT content. This result is not in agreement with findings of Abdulrazak et al. (2000) but in agreement with Tolera et al. (1997) and Yavuz (2007).

Conclusion

The results from these studies showed that leaves of MS 6 and TMS 30555 with high CP, ADF and ADL contents, a moderate NDF and low CT contents had a higher nutritive value in terms of energy content and *in vitro* organic matter digestibility. Thus, leaves of MS 6 and TMS 30555 have the potential to contribute substantially as feed supplements to ruminants in animal production systems in Nigeria and other sub-Saharan African countries despite the presence of some cyanide and other anti-nutritional factors found in cassava leaves.

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