Taabu, H. L; Ndyomugyenyi, E. K; Mutetikka, D. and Wasswa, D. (2015). Dynamics of cyanogens and in-vitro degradability of cassava peels as an indicator of its nutritional value as...

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Dynamics of cyanogens and *in-vitro* degradability of cassava peels as an indicator of its nutritional value as animal feed

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Abstract

The *in-vitro* degradability of cassava peels and its relationship with the cyanogenic potential of five cassava varieties grown in Uganda was investigated to study the potential of cassava peels as an energy source in animal feeds. The cassava varieties included NASE 3, NASE 4, NASE 10, TME 14 and Tongolo. The first four varieties were considered to be of low to medium cyanogenic potential (CNp), while the last one contained high CNp.

Cassava peels had 25.5 ± 6.6% dry matter (DM), 86.6 ± 0.9% organic matter (OM) and 56.8 ± 5.8% neutral detergent fibre (NDF). Degradability of DM, OM and NDF was 74.8± 4.2%, 74% ± 7.5% and 44.2 ± 7.5% respectively. Total cyanogenic potential and free hydrogen cyanide (HCN) in the fresh peels ranged from 923 to 2815 and 33 to 676 mg HCN equivalent kg^{-1} DM respectively. These levels dropped by 27- 88% and over 90% respectively, when the peels were dried. For all varieties, the residual cyanogenic potential after drying the peels fell within the safety limits recommended by FAO/WHO for food and feed. The study showed that using fresh peels in sufficient amounts for feeding animals, particularly monogastrics, is potentially harmful due to high cyanogenic potential. Drying the peels can, however, reduce the cyanide toxicity risks. The low NDF degradability reduces the nutritional value of cassava peels if they are used as a sole source of energy in ruminant diets.

Keywords: *cassava varieties, cyanogenic potential, cyanide toxicity, drying, energy source, livestock*

Introduction

Cassava and its by-products are high sources of energy and potentially able to substitute cereal sources for specific classes of livestock (Serres 1992). Fresh cassava foliage is used as a source of by-pass protein for cattle fed basal diets of rice straw (Sath et al 2010; Tham et al 2010). Peels from adopted varieties are often used to feed livestock in the cassava growing areas as well as

near urban centers where cassava forms a staple food for the low income bracket (Otim-Nape et al 1996). The roots contain about 35% DM, 1-2% CP, 1.5% CF, 0.3% fat, 1.4% ash and 3% NFE (Otim-Nape et al 1996). The leaves plus tender stems have a CP value of up to 23%, decreasing to about 10.6% in the peels and 4.6% in the edible parenchyma (Tie'moko 1994; Akinfala and Tewe 2001). Evaluation of utilization of cassava and its by-products for animal feeds has shown that peels are the most economical and practical cassava feed for smallholder livestock (Sonaiya 1995). However, utilization of cassava and its by-products as animal feeds is limited by the high cyanogenic potential in some varieties; high crude fiber; low CP and mineral content; low palatability and dustiness of the ground meal (Sonaiya 1995). Additionally, cassava contains cyanogenic glucosides, namely linamarin and lotaustralin present in all parts of the plant, which are hydrolyzed by the enzyme linamarase (β-glucosidase) producing corresponding cyanohydrins (Sonaiya 1995). While cyanogenic glucosides are found in the cytosol, linamarase is located in the cell wall (Poulton 1990). Damage of the tissue cellular structure during harvesting and processing of cassava into its products brings linamarin and linamarase in contact resulting in the hydrolysis of linamarin to cyanohydrins.

Although live plants contain only the cyanogenic glucosides, linamarin and lotaustralin, processed cassava products may in addition contain cyanohydrins and traces of HCN. The sum of concentrations of cyanogenic glucosides, cyanohydrins and HCN is referred to as cyanogenic potential (CNp). The safety level of cyanide is 300mg equivalent kg^{-1}DM for fresh tubers (10mg HCN equivalent/100g fresh weight) and 10mg HCN equivalent kg^{-1} DM for processed cassava products (FAO and WHO 1991). The main factors influencing HCN toxicity in animals consuming cassava and its by-products include the level of intake; CNp of the ingested feed, chemical composition of rumen contents (ruminants); proportion of readily fermentable carbohydrates and availability of sulfur for conversion to thiocyanate (Clarke and Myra 1978). Persistent intake of less toxic levels of HCN may not cause death but could lead to chronic cyanide intoxication. Despite the toxicity of some cassava varieties ('bitter' varieties) to livestock due to presence of high HCN concentration, there are positive attributes of these varieties. The 'bitter' cassava varieties are used for industrial starch production (Vongsamphanh et al 2014) and reduction of methane emissions, hence protecting the environment (Phuong et al 2012). However, fresh cassava leaves were reported to be better than dried leaves in reducing methane emissions when they were used as protein sources in *in vitro* incubations with sugar cane (Phommasack et al 2011) and cassava root meal (Sangkhom et al 2012).

A number of methods to overcome cyanide toxicity in cassava and its by-products have been investigated. Katongole (2001) reported three different but related methods to detoxify cassava peels, which included traditional sun drying, dry fermentation and wet fermentation. Heap fermentation followed by sun drying was reported to give the lowest residual cyanogenic content and the CNp dropped from 330mg to 58mg of HCN equivalent kg^{-1} DM (Iyayi and Losel 2000). The detoxification of cassava by-products by fungal solid-state fermentation reduced HCN, which was attributed to changes in the texture of plant tissue, increased β-glucosidase activity and utilization of cyanogenic glucosides by micro-organisms (Iyayi and Losel 2000). Incorporation of palm oil has been found effective in moderating the effect of cyanide in poultry feeds (Sonaiya1995). To design rational recommendations on the integration of cassava products into existing livestock feeding systems, there is need to assess the nutritive value and quantify potential utilisability of these feeds by livestock. Several studies indicate that feeding cassava

peels of certain varieties can lead to poor performance in animals due to HCN toxicity (Clarke and Myra 1978; Bokanga et al 1993; Tweyongyere et al 2000; Katongole 2001). *In-vitro* studies on degradability of feeds are one of the quickest ways of assessing their potential utilisability by livestock (Madsen and Hvelplund 1985). This study was conducted to evaluate the nutritional value for livestock of cassava peels from five cassava cultivars commonly grown in Uganda.

Materials and methods

Sampling and sample selection

Five cassava varieties were selected based on the level of adoption for human consumption with special consideration to provide for low, medium and high cyanogenic potential (CNp). The selected varieties were: NASE 3 (sweet variety and medium CNp); NASE 4 (sweet variety and low CNp); NASE 10 (sweet variety and low CNp); TME 14 (sweet variety and low CNp) and Tongolo (bitter variety and high CNp). All samples were collected from Namulonge Research Station in Wakiso district ($00^{o}24'N\ 32^{o}29'E$), central Uganda, about 27 km Northeast of Kampala ($00^{o}19'N\ 032^{o}\ 35'E$) and 1150m above sea level. The soils are predominantly deep tropical red clay loam soils, characteristic of the lower slopes of the Buganda catena (Yost and Eswaran 1990). The mean annual rainfall is 1170mm, which is bimodal (March-May and September-December). All the varieties were sampled at 11 months during the rains, from a RBD experimental field. A single tuber was harvested per plant over the entire experimental strip. A total of 12 tubers per variety were collected and quickly delivered to the laboratory. The tubers were washed in ice-cold water. For each sample, the thick top skin was carefully removed and the thicker inner coat peeled off under ice-cold conditions. For each cassava variety, two composite samples of fresh peels from the 12 tubers were obtained for subsequent laboratory analysis. One of the composite samples was used to determine CNp, DM, and HCN on as-is-basis, while the other samples for determination of CNp, HCN, OM and NDF on DM-basis.

Extraction of cassava peels

The peels were processed both as wet and dry samples to determine CNp and HCN on “as is” or DM basis. A composite sample of the fresh tubers was prepared under ice-cold condition by mincing into 1cm cubes and then homogenizing in a warring blender using cold orthophoric acid. The resulting homogenates were centrifuged at 1000 r.p.m for 10 minutes and the supernatant used as extract to determine both the CNp and HCN of fresh cassava peels. The second set of cassava composite samples was dried in air-forced oven at $60^{0}C$ for 48 hours, milled through 1mm screen and the resulting flour dissolved in cold orthorphoric acid. The tubes and their contents were centrifuged at 1000 r.p.m for 10 minutes and the resultant supernatant used to determine both CNp and HCN content for dry samples.

Determination of cyanogens

Total cyanide was determined using the enzymatic assay method (Cooke 1978). Free cyanide (CN^{-}) was determined using the improved assay method developed by O’Brien et al (1991). Linamarin was used as a standard for total cyanide calibration curve, as it is the major

component of total cyanide in the cassava peels, usually more than 60%. Calculation of free CN^- was done based on the assumption that the cyanogens have stronger preference for the extraction media compared with the residue (O'Brien et al 1991) thus:

$$CN = \frac{Q\{(V + S)*(M/100)\}}{Q\{(I - M)*(M/100)\}} * 0.026$$

Where:
S: Sample weight (g)
V: Volume of extracting media (ml)
D: Volume of extract assayed (ml)
M: Moisture content (%)
Q: Quantity of cyanogens in the tube calculated from the calibration curve using the formula: Q = (A605-a)/s; where A605 is the maximum absorbance at 605 wavelength; a and s are the intercept and slope of the calibration curve respectively.

Determination of NDF content

The NDF content was determined using the modified Van Soest (1970) method involving use of Ankom Technology fiber analyzer and sample bags to minimize errors involved in conventional filtration processes during NDF determination. The NDF content was then calculated using the formula: %NDF (DM basis) = $100\{W_3 - (W_1 \times C_1)\}/W_2$ where:

W_1 = Weight of filter bag
W_2 = Sample weight expressed on DM basis
W_3 = Final bag and sample weight
C_1 = Blank bag correction (oven-dried bag weight /original blank bag weight)

***In-vitro* incubation**

This was done using the two-stage method developed by Tilley Terry (1963). Residual NDF after incubation was calculated from the formula:

$$\%NDF = \frac{(W2 + C1) - (W3 + C2)}{W1 \times \%DM} \times 100$$

Where:
W_1 = Weight of air dry sample
W_2 = Weight of crucible + sample
W_3 = Weight of crucible + ash
C_1 = Correction for W_2 read from balance (due to hot weighing)
C_2 = Correction for W_3 read from balance (due to hot weighing)

NDF degradability was then calculated as a difference between the NDF content before and after incubation of samples.

Statistical analysis of data

Data generated were subjected to the GLM procedure of SAS (2001) and differences between means were separated by Duncan's Multiple Range test.

Results and discussion

Dry matter, organic matter and neutral detergent fiber composition of cassava peels

Cassava peels had DM values similar to those reported by Katongole (2001) (Table 1). Varieties NASE 3 and NASE 4 had the highest DM content while Tongolo had the lowest DM. Mean ash content of the peels was more than twice the average ash content of 1.4% reported for the parenchyma (Tie'moko1994; Akinfala and Tewe 2001). The difference in ash content could be attributed to the type of soil and use of organic fertilizers in the study area at Namulonge research station. The OM and NDF varied among the varieties. The highest NDF content was realized in NASE 4 and TME 14 varieties, while the lowest NDF was found in NASE 10.

Table 1: The DM, OM and NDF of peels of selected cassava varieties in Uganda (DM basis)

Variety	Fresh sample DM (%)	Dry sample DM (%)	Ash (%)	OM (%)	NDF (%)
NASE 3	31.0^{a}	89.0^{b}	3.00^{ab}	86.0^{c}	58.0^{a}
NASE 4	30.0^{a}	90.0^{b}	3.20^{ab}	87.0^{ab}	61.0^{a}
NASE 10	27.0^{b}	91.0^{a}	3.80^{a}	87.0^{ab}	47.0^{b}
NASE 14	24.0^{c}	91.0^{a}	3.50^{a}	88.0^{a}	61.0^{a}
Tongolo	15.0^{d}	89.0^{b}	3.80^{a}	85.0^{c}	57.0^{a}
p		<0.0001	0.620	0.0455	0.0016
SEM	0.600	0.200	0.400	0.300	1.20

^{abc} *Means in the same column followed by different subscripts differ (p<0.05)*

Cyanogenic content of cassava peels

The level of cyanogens in the cassava peels was expressed as free hydrogen cyanide (HCN) and cyanogenic potential (CNp), which is the sum of concentrations of cyanogenic glucosides, cyanohydrins and hydrogen cyanide (Table 2). Fresh peels had very high CNp ranging from 923 to 2815mg kg^{-1} DM. The highest was in Tongolo, a bitter variety, while the lowest CNp was in TME 14, grown as a sweet variety for food. The CNp levels were reduced by 88% in Tongolo to 27% in NASE 3 when the peels were dried. Drying also caused a drop in the free HCN levels by over 93% for all the varieties although the mechanism involved in the reduction of cyanogens when cassava products were dried could not be substantiated from literature.

Table 2: Cyanogenic content (mg HCN equiv. kg^{-1} DM) of peels from selected cassava varieties

	Fresh peels			Dry peels		
Variety	CNp (10^3)	HCN	CNp:HCN	CNp	HCN	CNp:HCN
NASE 3	1.55^{b}	252^{b}	6.20^{c}	185^{a}	6.00^{a}	29.2^{a}
NASE 4	1.09^{c}	197^{c}	5.50^{c}	85.0^{b}	3.00^{b}	28.0^{a}
NASE 10	1.06^{c}	141^{d}	7.60^{b}	37.0^{c}	1.20^{c}	29.2^{a}
TME 14	0.924^{c}	33.0^{c}	27.9^{a}	22.0^{d}	2.00^{c}	11.9^{b}
Tongolo	2.82^{a}	676^{a}	4.20^{d}	79.0^{b}	7.00^{a}	11.5^{b}
p	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0039

SEM	0.050	9.80	0.400	2.20	0.300	2.30

[abcd] Means in the same column followed by different subscripts differ (p < 0.005)

The results revealed that although Tongolo had the highest CNp in fresh peels, its residual potential after drying was lower than that of NASE 3 and NASE 4 with lower CNp in fresh peels. All varieties had a CNp lower than 10mg HCN equivalent kg^{-1} DM recommended by FAO and WHO (1991) for all cassava products to be used for food or feeds. The guide for cassava safety by Bolhuis (1954) gave a level of <50mg HCN equivalent as safe; 50-100mg HCN equivalent kg^{-1} as moderately toxic and >100mg equivalent kg^{-1} as highly toxic. Tongolo cassava, a 'bitter' variety would be best used to reduce methane emissions because of its high HCN concentration. 'Bitter' cassava varieties are known to reduce methane emissions, thereby conserving the environment (Phuong et al 2012).

In-vitro degradability of dry matter, organic matter, NDF and cyanogens in cassava

The *in-vitro* degradability of DM, OM, NDF and cyanogens in cassava are shown in Table 3. Degradability of DM and OM for all varieties was above 66%. Mean DM degradability was 74.8%, while that of OM was 74%.

Table 3: *In-vitro* degradability of dry matter, organic matter and NDF in cassava peels

	Degradability (%)		
Variety	**DM**	**OM**	**NDF**
NASE 3	79.3[a]	79.9[a]	48.7[a]
NASE 4	73.5[ab]	72.7[bc]	50.8[a]
NASE 10	75.5[a]	74.1[ab]	32.2[c]
TME 14	77.7[a]	76.0[ab]	43.5[b]
Tongolo	68.8[b]	66.9[c]	42.8[b]
p	0.041	0.0021	0.0013
SEM	1.70	1.70	1.40

[abcd] Means in the same column followed by different subscripts differ (P < 0.05)

Variety NASE 3 showed the highest DM and OM degradability and lowest in Tongolo. Although there was variation in the DM and OM degradability among the five varieties, Tongolo variety gave the lowest values. The ratio between the cyanogenic potential and free hydrogen cyanide did not give a linear relationship for DM and OM degradability. Degradability of NDF varied among the varieties though without any demonstrated pattern based on cyanogenic content. The present data could not readily explain why the lowest cyanogenic containing variety, NASE 10 had the lowest degradability values against the 'bitter' variety, Tongolo.

Conclusions

- High cyanogenic potential in most cassava varieties commonly cultivated in Uganda is a hindrance to usage for animal feeding unless appropriate measures are undertaken to detoxify the product.

- Drying cassava products significantly lowers the cyanogenic potential to sufficiently reduce the toxicity risk in cassava peels of the varieties investigated although the optimum temperatures and duration of drying under farm conditions need further investigation.

- Although cassava products are often used as an energy source, their low DM, CP and NDF content and NDF degradability pose limitations to usage in animal feeding.

- Cassava peels can be a valuable supplementary energy source, especially for the smallholder livestock farmers in developing countries.

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Received 31 March 2015; Accepted 7 May 2015; Published 3 June 2015

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