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THE POTENTIALS OF CASSAVA AS ANIMAL FEED AND IT'S TOXICITY IMPLICATIONS

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ABSTRACT

The potentials of cassava as animal feed and its toxicity implications were investigated in rabbits, using spectrophotometeric and enzymatic methods. Two groups of rabbits were maintained on cassava diets containing 110.93mg CN $100g^{-1}$(for high cyanide group) and 44.03mg (for low cyanide group) with protein supplement in the form of vitamin-free caesin for 28days. Analysis of the biochemical indicators of cyanide toxicity along the stomach, small intestine, as well as in the urine and serum of animals was carried out. The levels of total cyanide in their urine ranged between 2.08± 0.34-5.0 ± 0.72 µg/ml. The amount of thiocyanate measured in the serum and urine of these animals were 2.70± 74- 3.47± 0.21 and 5.01 ± 0.72- 10.20± 0.91 µg/ml respectively. The serum total protein was found to be within normal value, while the albumin levels were very low 2.63 ± 1.3- 2.82 ± 1.7 g/dl. Assay of some clinical important enzymes indicated increases in the activities of aspartate aminotransferase (ALT) by 41.7% and alanine aminotransferase (ALT) by 33.3% above normal (for high cyanide group) but within normal range for the other group and control. Alkaline phosphatase activity and bilirubin levels in all the groups were within normal range. Significant elevation in blood glucose of the two groups of rabbits was also observed. These results indicate exposure of the animals to cyanide poisoning resulting from ingestion of cassava diets containing substantial amount of cyanide. The toxicological implications of these findings are discussed.

Keywords: Cassava potential, animal diet, toxicity.

INTRODUCTION

The low nutritive value of tropical grasses and the lack of feed in dry season are very common features, which influence small ruminant production. This is evidenced by the fact the number of cattle, sheep, goats, pigs and chickens found in developing countries (corresponding mainly to tropical areas) in 1991 far outnumbered those in the developed countries, but the quantity of meat and milk produced in developed countries was far higher than that produced in developing countries (FAO, 1992). Therefore, there is need to find new feed resources to be used as low-cost supplements to improve animal productivity especially in dry season.

In this context, novel feeds resources such as sorghum spent grains and wheat offals (by-products of sorghum and wheat malting respectively) as well as cassava peels are being promoted as supplements for livestock (Tewe, 1994; Okafor and Nwabuko, 2004). Despite the availability of these feeds, there has been little interest to utilize these novel feed resources especially cassava peels for animals in large scale.

The work in Cambodia and Vietnam has shown that there is no risk of toxicity when fresh cassava foliage is fed to either goats (Seng and Rodriguen, 2001) or cattle (Ho Van Do *et al*., 2001; Seng Mon *et al*., 2001) provided the animals are adapted gradually to this feed. Even now, in spite of overwhelming evidence from two decades of satisfactory performance of animals fed on cassava chips in the European community countries, arguments still persist in scientific circles as to the safety of cassava for sustainable livestock production (Tewe, 1994). The work of Osuntokun (1970), Delange and Ahiluwalia (1983), Ekpechi (1967), Akin tonwa and Tunwshe (1993), among others, on cassava cyanide toxicity constitute strong circumstantial evidence which influence current thinking on safety of cassava for human and livestock feeding.

To fully understand the biochemical and toxicity effects of cyanide in man and animal so as to determine the total toxic effects of this free radical, the absorption, distribution and toxicity of cyanide in different organs of animal species need to be thoroughly investigated. This forms the basis of this present study.

MATERIALS AND METHODS

Twelve Newzaland white rabbits purchased from Non-ruminant Department of Michael Okpara University of Agriculture, Umudike, Nigeria were used. The animals were kept two per hutch at room temperature (28-30^{o}C) and had free access to drinking water and their diet. The animals were acclimatized to their environment and diet for at least one week before experiments were

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commenced. The animals were starved for 24hours before the feeding experiment. The test animals on high cyanide cassava starch (Group 1) were fed diets compounded as shown in table 1. For low cyanide diet (Group 2) animals, the feed composition is the same as in table 1, but with different concentration of cyanide in the feed. For control animals (Group 3), their feed was purchased from a local livestock feed shop near Michael Okpara University of Agriculture, Umudike.

Treatments
Each of the animals in test and control groups was fed for 28days. Group 1 and 2 received diets (Table 1) containing 110.93 $mgCN^{-}100g^{-1}$ (high cyanide) and 44.03$mgCN^{-}100g^{-1}$ (low cyanide) respectively. The cassava starch for group 1 diets was prepared by oven drying high cyanide cassava root pulp together with the peels. The cassava starch for group 2 was prepared as above but from relatively medium cyanide cassava. The control feed contained 27.53 mgCN 100g.

Table 1. Composition of cassava (*Manihot esculenta Crantz*) starch diet.

Ingredients	Quantity g/kg
Cassava flour	600
Wheat offal	80
Vitamin free casein	180
Vitamin mixture	40
Salt mixture	20
Banana flavour	40
Groundnut Oil	40

*The composition of both high cyanide and low cyanide feeds were the same, but while the cassava flour was prepared from peeled root pulp from medium cyanide cassava for low cyanide diet the high cyanide diet contained unpeeled cassava root pulp from high cyanide cassava.

Sacrificing The Animals/Tissue Preparation
The animals were sacrificed after 28 days and blood collected from each group with 10ml syringe intravenously from the heart. The animals were dissected immediately and their urine collected direct from the bladder. Whole stomach and intestine (tissue+ contents) were dissected out, weighed and the contents separated from the walls. The walls were cut into pieces, homogenized in normal saline and centrifuged for 10min at 10,000g. The supernatant was decanted for analysis. The contents were also centrifuged and the supernatant collected.

Cyanide (CN^{-}) and Thiocyanate (SCN) Determination
Despite some limitations, serum and urinary thiocyanate values are the most useful biomarkers for the estimation of cyanide intake. The distribution of cyanide in animal feeds, urine, blood, small intestinal walls and contents and stomach walls and contents were determined by the method of Esser *et al.* (1993) and thiocyanate by the ferric nitrate reagent method (Sorbo, 1953).

Enzyme assay
The assay of some plasma enzymes as indicator of chronic changes in liver, kidney, and muscle cell damage resulting from cyanide poisoning was carried out. The plasma enzymes were assayed as follows: aspartate aminotranferase (AST) and alanine aminotraferase was as recommended by Reitman and Frankel (1957) and alkaline phosphatase (ALP) as described by Klein *et al.* (1960).

Other biochemical determinations
Glucose was determined using glucose oxidase method (Passey *et al*., 1977), total protein by Biuret method as described by Layne (1957), serum albumin by the dye-binding (broomocresol green) method (Doumas *et al.*, 1971).

Statistics: Students t-test was used for statistical comparison.

Table 2. Some chemical components of test and control diets.

Diet	Cyanide (mg HCN /kg)	Thiocyanate (mg/kg)
High cyanide diet	110. 93	ND
Low cyanide diet	44.03	ND
Commercial feed (for control)	37.14	ND

The values are the mean of 5 separate determinations
ND= Not detected by method of assay.

RESULTS AND DISCUSSION

The results of the mean value for total cyanide in urine, stomach walls and contents are presented in Table 3. The group fed with high cyanide cassava diet had the highest levels of total cyanide in urine, stomach and small intestine, while the least levels of cyanide were found in the control group. Detection of varying levels of total cyanide in urine, stomach and intestine of these animals is evidence of cyanide exposure resulting from consumption of sublethal doses of cyanide in the feeds. Consumption of these cyanide containing products over a long period of time may aggravate iodine deficiency disorder (Ermans *et al*., 1983) as cyanide detoxification product (thiocyanate) competitively interferes with iodine metabolism or may result in chronic cyanide toxicity.

It is interesting to note that commercial feed given to the control animals contained appreciable level of cyanide.

Table 3. Levels of total cyanide in urine, stomach contents and walls and small intestinal walls and contents of rabbits

Sample	Cyanide conc. (µg/ml) or µg/g of tissue)		Control
	High cyanide Group	Low cyanide Group	
Urine	5 ±0.72	3.96±0.11	.08±0.34
Stomach content	2.96±0.31	2.58±0.22	2.0±0.43
Stomach wall	2.70±0.31	2.33±0.10	2.5±0.17
Small intestinal content	7.70±0.84	6.44±1.32	.88±0.21
Small intestinal wall	7.40±0.11	8.0±1.71	7.0±0.14

*The values are the mean of 3 separate determinations.

Table 4. Levels of thiocyanate in urine, serum, stomach contents and walls and small intestinal walls and contents.

Sample	Thiocyanate (µg/ml) or µg/g of tissue)		Control
	High cyanide Group	Low cyanide Group	
Urine	10.2 ±0.91	5.31±0.10	5.01±0.72
Serum	3.47±0.21	2.7±0.74	2.81±0.53
Stomach content	6.25±1.7	5.0±1.31	4.7±.0.81
Stomach wall	1.44±0.30	1.32±0.32	0.9±0.13
Small intestinal wall	0.95±0.11	0.42±0.13	0.37±0.11
Small intestinal content	1.33±0.13	1.02±0.07	1.11± 0.73

*The values are the mean of 3 separate determinations.

Table 5. Biochemical indicators of cyanide toxicity in rats fed cassava diets containing varying concentrations cyanide.

Groups	Total Protein	Albumin	ALP	ALT	AST	TB	Blood glucose
	g/dl	g/dl	U/L	U/L	U/L	mg/dl	mg/dl
High cyanide	5.63	2.82±1.7	152	16	17	1.20	143
Low cyanide	5.65	2.63±1.3	118.5	9.0	8.5	0.6	131.5
Control	5.78	2.82±1.4	102.2	10.5	8.0	0.84	97.

ALP=Alkaline phosphatase, ALT=Alanime aminotrasferase, AST=Aspartale aminotrasferase, TB=Total bilirubin

There was no statistically significant difference ($P>0.05$) between the levels of cyanide in low cyanide feed and that of commercial feed. In this connection, Okafor and Nwabuko (2004) have reported that some of the animal feeds in Umuahia, Nigeria contained some levels of cyanide. The amounts of thiocyanate found in serum, urine, small intestinal walls and contents and stomach walls and contents are shown in Table 4. There was a statistically significant difference ($P<0.05$) between the mean serum thiocyanate (SCN) of the high cyanide group and that of low cyanide group and control. The same is true of their mean urinary thiocyanate. The concentrations of thiocyanate in the serum and urine of these groups of animals indicate the levels of cyanide exposure and were due to the detoxification of cyanide by the enzyme rhodanese. Thiocyanate remains the most useful biomarker for cyanide exposure since it is a stable metabolite. The half-life of serum level is 3days and the level therefore reflects the mean daily load during the last days (Rosling, 1994). The results of this work agree with the reports of Okoh and Pitt (1982) and Okoh (1983) on the metabolism of cyanide into thiocyanate and the excretion of the radical in the urine of rats.

The results of the analysis of the biochemical indicators of cyanide toxicity are presented in Table 5. The serum total protein was found to be within the normal value (5.63-5.78g/dl) for all the groups of animals while the albumin level was very low 2.36-2.82g/dl for all the groups. The low levels of serum albumin of the animals could probably be due to reduced liver synthesis (Walmsley and White, 1994; Rosenthal, 1997). The risk of cyanide toxicity with the above values is high since serum proteins (especially albumin) are known to be involved in cyanide detoxification. Elevated blood glucose levels (above normal) were also observed in the

high cyanide and low cyanide groups being 143mg/dl and 131.5mg/dl respectively. This finding is consistent with an earlier report (Isom, *et al.*, 1975) that cyanide alters glucose metabolism resulting in a 100% increase in conversion of glucose by the pentose-phosphate pathway.

Assay of some clinical important enzymes indicates increases in the activities of aspartate transaminase (AST) by 41.7% and alamine transaminase (ALT) by 33.3% above normal (for high cyanide group) but within the normal range (for low cyanide group and control. The increases in AST and ALT activities may indicate damage to mitochondrial and/or cytoplasmic membranes of liver, since a rise in transaminases is a sensitive indicator of hepatic cytoplasmic and/or mitochondrial membranes damage even if there is no detectable impairment of function. The liver cells are known to contain more AST than ALT and in most conditions damage to both mitochondrial and cytoplasmic membranes leads to greater increase in plasma AST activity than in ALT. (Joan *et al*., 1987). Also, the level of alkaline phosphatate (ALP) in all the groups is within the normal range. The level of total bilirubin is within normal showing that there was no obstruction of the bile duct.

In conclusion, the results from this study showed exposure to cyanide poisoning through ingestion of cassava based ration. The evidence of toxicity is seen in the changes in the biochemical parameters measured. Thus, though the use of cassava as animal feed source is laudable, measures should be taken to ensure adequate processing to avoid cyanide toxicity.

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