INFLUENCE OF CYANOGENIC GLUCOSIDE FRACTION OF CASSAVA ON PERFORMANCE, THIOCYANATE CONCENTRATION AND RHODANESE ACTIVITY OF RATS DURING GROWTH AND REPRODUCTION

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SUMMARY

FEEDING of fresh and dried cassava (CMC 84 variety) containing 172.6 and 90.6mg HCN/Kg fresh material respectively caused a non-significant ($P > 0.05$) increase in daily feed intake as compared to a corn starch control diet. Growth rate of suckling rats was least on the fresh cassava diet and intermediate on the dried cassava diet. Post weaning feed consumption, growth rate and feed utilization were also considerably reduced by the cassava diets.

The fresh cassava diet caused a significant ($P < 0.01$) increase in serum thiocyanate of Weaning Rats. At the end of the post-weaning growth study period, serum thiocyanate was significantly ($P < 0.01$) higher on both the fresh and dried cassava diets. Rhodanese activity changes did not show any consistent treatment effect.

INTRODUCTION

THE presence of cyanogenic glucosides in cassava has been receiving considerable attention from nutritionists and livestock feed manufacturers in recent times. Clinical evidence of chronic influences of the cyanogenic glucosides of cassava as initially provided by Ekpechi (1967) and Osuntokun (1970), has prompted investigations into the implications of cassava diets in livestock performance. To this end, a number of rat screening tests have been run to provide more concrete evidence than that inferred from human populace with a diversity in their food habits.

While Osuntokun (1970) has established that Wistar rats fed a cassava product called 'purupuru' showed increased plasma thiocyanate concentration, Maner and Gomez (1973) have demonstrated that rats fed fresh chopped cassava containing 150mg HCN/Kg gained weight at a significantly faster rate than those fed sucrose or a sun-dried cassava meal based diet, even when the quantity of protein and dry matter was controlled for equal consumption by all groups. The question then remains whether growth depressions observed in rats fed cassava diet is due to the metabolic effects of the cyanogenic glucosides or is a result of other factors which were not controlled in these experiments.

In the present study, experiments were carried out with rats to elucidate the role of a bitter variety of cassava in its fresh and dried forms on their performance during the gestation, lactation and post-weaning growth phases. Quantitative estimations of the major metabolic product of cyanide (thiocyanate), and the cyanide-thiocyanate converting enzyme (rhodanese), were also carried out in their offspring during weaning and post-weaning periods.

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Fig. 1 GROWTH CURVE OF SUCKLING AND GROWING RATS FED CORN STARCH, FRESH AND DRIED CASSAVA (CMC 24) DIETS DURING LACTATION AND POST WEANING GROWTH PHASES.

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MATERIALS AND METHODS

Gestation Period:

Thirty-six female albino rats of the Sprague-Dawley strain having an average body weight of 230g and age of about 100 days were randomly allotted to three treatment groups on the basis of body weight. There were three replicates of each treatment, each consisting of four rats. Each replicate was maintained in metallic breeding cages of a 64cm by 20cm dimension. Two male rats were alternated daily for mating each group of 4 rats.

The treatments consisted of a corn starch based control diet, a fresh 'bitter' cassava (CMC 84) and a 'bitter' cassava meal (CMC 84) based diet. Their gross

and proximate compositions are shown in Tables 1 and 2.

The rats on the fresh cassava diet were fed daily by grating a known quantity of freshly harvested cassava and thoroughly mixing it with a definite proportion of supplement. Rats on the dried cassava and corn starch diets were also fed *ad libitum*. Feed intake and body weights were recorded. The male rats were kept in individual metallic growth cages of 20 sq. cm dimension. The resting male rats were fed the respective diet of the treatment group for which they were being used for mating. All rats were given water *ad libitum*. Pregnant rats were identified visually and individually maintained in plastic cages in the last week of gestation and were continued on their respective diets.

TABLE 1

Gross Composition of Experimental Diets based on Bitter (CMC 84) Fresh and Oven-dried Cassava

INGREDIENT	EXPERIMENTAL DIETS		
	Control %	Fresh Cassava %	Cassava Meal %
Corn Starch	39.3	—	—
Fresh Cassava	—	62.9	—
Cassava Meal	—	—	62.9
Soyabean Meal	29.7	26.1	26.1
Corn oil	5.0	5.0	5.0
Minerals ³	4.0	4.0	4.0
Vitamins ³	2.0	2.0	2.0

- 1 Diest were calculated to supply 15% true protein on air dry (approx 90% dry matter) basis.
- 2 Fresh cassava was fed by mixing it with a supplement containing, in percentage: Soyabean meal, 70.4; Minerals, 10.8; Vitamins, 5.4; and Corn oil, 13.5. A weight ratio of 4.4 parts of fresh cassava and 1 part of the supplement was calculated to supply a 15% protein diet.
- 3 Mineral and Vitamin premixes were obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, U.S.A.

TABLE 2

Proximate Composition of Experimental Diets Based on Corn Starch, Fresh and Oven-dried Cassava (CMC 84 Variety)

Experimental diet	Dry Matter %	Crude Protein %	Ether Extract %	Crude Fibre %	N-free Extract %	Ash %
Corn Starch 1	91.2	14.8	6.3	1.8	71.9	5.2
Fresh Cassava ²	90.2	17.3	6.1	3.3	67.1	6.2
Dried Cassava 1	90.6	16.2	6.7	3.3	67.2	6.6

- 1 Proximate composition recorded on air-dry bases.
- 2 Proximate composition recorded is the mean value of oven-dried (60°C) samples of the mixed fresh cassava diet.

Lactation period:

On parturition, the does (mother rats) were continued on their respective diets and water was supplied *ad libitum*. Weekly weights of the nursing dams, offspring, feed intake, litter size and mortality throughout the lactating period of 21 days were recorded.

At 21 days of age, the offspring were weaned, separated by sex, and one male and one female, nearest the mean weight of their respective sexes, were selected from each litter and used in a 28-day growth study. The remaining offspring from each litter were anaesthetized with chloroform and blood obtained by heart puncture for individual serum thiocyanate determinations (Bowler, 1944). The liver and kidneys were removed for rhodanese activity determinations, using the Sorbo (1951) method as modified by Tewe (1975).

Post-Weaning growth period:

The selected offspring were fed the same diets as that consumed by the respective nursing dams. Feed and water were offered *ad libitum* throughout the 28-day growing period. Feed intake and body weights were recorded twice weekly. At the end of the study, the rats were anaesthetized and blood was obtained by heart puncture for serum thiocyanate determinations as previously described. The liver and the kidneys of each rat were weighed and frozen for rhodanese activity determinations.

Statistical analysis:

Data were subjected to variance analysis by the method of Snedecor and Cochran (1967). Differences between treatment means were evaluated by the Baye's exact test for symmetrical comparisons (BET) as described by Waller and Duncan (1969).

RESULTS AND DISCUSSIONS**Performance in gestation and lactation:**

Table 3 shows the feed intake of the pregnant and nursing dams and lactation respectively. Feed intake was highest in the dried cassava group during both periods.

As shown in Table 4, average number and weight of offspring at birth were not affected by dietary treatment. Both litter size and weight were similar in all groups. This confirms observations in gestation studies by Tewe *et al* (1976) that fresh and dried cassava (CMC 84 variety) diets do not affect birth weight in first litter offspring of rats.

Although not statistically significant ($P > 0.05$), both litter size and body weight of offspring were reduced when the nursing dam was continued on the fresh cassava diet during lactation. The average weight gain of offspring from the dried cassava diet was intermediate between that of the control and the fresh cassava treatment. Mortality was highest in the group that consumed the fresh

TABLE 3

Performance of Female Rats fed Corn Starch, Fresh and Oven-dried Bitter Cassava (CMC 84) Based Diets During Gestation and Lactation

Parameters	Diets		
	Corn Starch	Fresh Cassava	Cassava meal
Average daily feed intake during gestation, g.	16.4 $\pm$ 0.7	18.2 $\pm$ 0.3	17.5 $\pm$ 1.0
Average daily feed intake during lactation, g.	35.0 $\pm$ 0.8	37.3 $\pm$ 0.9	36.7 $\pm$ 1.4
Average body weight change during lactation, g.	-11.1	-6.2	-6.5

1 The feed intake in the fresh cassava group is expressed on 90% dry matter basis.
 $\pm$ Standard error of mean.

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TABLE 4

Performance of Suckling Rats whose Does were offered Corn Starch and Cassava Diets during Gestation and Lactation

Parameters	Diets		
	Corn Starch	Fresh Cassava	Cassava meal
Average number of offspring per litter at birth	10	9	10
Mortality at birth, % (still birth)	0	3.6	0
Mortality at weaning, %	9.6	22.3	12.1
Birth weight, g.	6.2	6.3	6.0
Weaning weight, g	34.2	28.7	30.1
Average weight gain per weaning rat, g	28.0 ± 1.8	22.4 ± 1.3	24.1 ± 1.3

cassava diet. Figure 1 illustrates the body weight change of offspring during lactation and post-weaning. It can be observed that growth of offspring on corn starch diet was superior to that on other treatments by the end of the first week of trial. This superiority was maintained to the end of the lactation period. It has been reported that suckling rats depend solely on milk from their mothers until about the fourteenth day of life. Consumption of diets offered to their dams by the suckling rats can be appreciable after this period (N.R.C. 1972). This evidence will therefore suggest that the lower growth rate observed on the cassava diets in the early part of lactation is not explained by consumption of HCN-containing diets, but can result from the composition of the milk suckled from their mothers on the different treatment groups. Honour, Myant and Rowlands (1952) indicated that when offered cyanide containing diets, lactating rats transfer thiocyanate to their young through their milk. Although the quantity of this

goitrogenic substance transferred through the milk is not known, it is well established that during the first few days of post-natal development in any animal species, a delicacy exists in the physiological state of the newly born. A quantity of thiocyanate which may appear to be of no toxicological importance to the adult animal may therefore have noticeable influences on the performance of the suckling rat. It is also remarkable that reduced weight gain was higher on the fresh cassava diet which had the highest HCN content.

Post-weaning Performance:

Post-weaning growth performance was remarkably affected by dietary treatment as shown in Table 5. The same trend as that observed in the lactation phase was witnessed in this period of growth as illustrated in Figure 1. Rats on the fresh cassava diet consumed significantly ($P < 0.01$) less and grew at a significantly ($P < 0.01$) slower rate when compared

TABLE 5

Performance of Offspring on Corn Starch and Cassava Diets During Post-weaning Growth

Parameters	DIETS		
	Corn Starch	Fresh Cassava	Cassava Meal
Average daily feed intake, g ¹	13.4 ± 0.5a	11.6 ± 0.4b	12.6 ± 0.4b
Average daily weight gain, g	4.5a ± 0.2	2.2c ± 0.2	3.9b ± 0.1
Feed/gain	3.0c	5.3a	3.3b
Protein efficiency ratio	2.3a	1.2c	2.0b
Fresh weight of tissues, % of body weight:	Liver	4.7	4.6
	Kidney	0.7c	0.8b

¹ The feed intake in the fresh cassava group is expressed on 90% dry matter basis.

a,b,c. Values without common superscripts in horizontal rows are significantly different ($P < 0.05$).

± Standard error of mean.

to the average performance of the control group. Growth rate of rats on the dried cassava group was also significantly ($P = 0.01$) lower than those on the control diet. The lower weight gain observed on the cassava diet may have been due to the lower feed intake, as a calculation of the feed efficiency shows that the feed was poorly utilized on the fresh cassava diet. Indeed, the dried cassava diet also had a significantly ($P < 0.05$) higher feed/gain ratio than the control diet. Protein efficiency also followed same trend. These observations may therefore indicate that the protein quality of the fresh and dried cassava diets are lower than that of corn starch diet. As soya-bean meal provided the bulk of the protein in the three experimental diets as shown in Table 1, the hydrocyanic acid of the cassava diets may be the culpable factor in the utilization of dietary protein in these diets.

Fresh Weights of liver and kidney of offspring obtained after 28 days growth study did not show any significant difference. Slightly higher values of 4.7 and 4.6% of total body weight were however obtained on the fresh and dried cassava diets as compared to 4.4 of total

body weight on the corn starch diet. Weight of kidney was significantly higher ($P < 0.01$) on the fresh cassava treatment. This may suggest a stress effect on the excretory organs where the detoxification products of cyanide are passed out of the body.

Thiocyanate Concentrations and Rhodanese Activity:

Table 6 shows the serum thiocyanate concentration and rhodanese activity of the liver and kidney of weaning rats. The serum thiocyanate concentration was significantly ($P < 0.01$) higher on fresh cassava than in the other two groups at weaning. Rhodanese activity in the weaning rats was significantly higher ($P < 0.05$) in the corn starch group than on the cassava diets.

After 28 days on the post-weaning growth trial as shown in Table 7, serum thiocyanate values were not only significantly higher in the fresh cassava group, but also significantly higher ($P < 0.01$) in the dried cassava group than on the control diet. Values of the sulphur-transferase (rhodanese) activity obtained in the liver was highest in the

TABLE 6

Mean values for serum thiocyanate and tissue rhodanese activity of weaning rats whose mother (Does) were fed corn starch and cassava diets

	Corn Starch	Fresh Cassava	Dried Cassava
Serum thiocyanate, mg/100ml.	1.39 $\pm$ 0.1b	2.34 $\pm$ 0.1a	1.26 $\pm$ 0.1b
Rhodanese activity of fresh tissues, mg thiocyanate/ min/g protein: Liver	28.2 $\pm$ 2.0	35.9 $\pm$ 1.2	28.9 $\pm$ 0.9
Kidney	25.2 $\pm$ 0.4a	23.5 $\pm$ 0.5b	23.0 $\pm$ 0.6b

a,b. Values without common superscripts in horizontal rows are significantly different ($P < 0.05$).
 $\pm$ Standard error of mean.

TABLE 7

Mean values for serum thiocyanate and tissue rhodanese activity of rats fed corn starch and cassava diets during the post-weaning growth phase

	Corn Starch	Fresh Cassava	Dried Cassava
Serum thiocyanate, mg/100ml	1.32 $\pm$ 0.04c	3.06 $\pm$ 0.08a	2.01 $\pm$ 0.03b
Rhodanese activity of fresh tissues, mg thiocyanate/ min/g protein: Liver	33.1 $\pm$ 0.9c	36.7 $\pm$ 1.2b	40.6 $\pm$ 0.4a
Kidney	28.2 $\pm$ 0.6a	25.2 $\pm$ 0.8b	28.9 $\pm$ 0.6a

a,b,c. Values without common superscripts in horizontal rows are significantly different ($P < 0.05$).
 $\pm$ Standard error of mean.

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dried cassava group. Values were least on the corn starch diets. That of the kidney was also significantly ($P < 0.01$) reduced by the fresh cassava diet as compared to the corn starch ration.

The results indicate that serum thiocyanate concentrations in rats have remarkable relationships with the performance of rats both at the lactation and post-weaning growth phase.

It is worth mentioning that the hydrocyanic acid of the fresh and dried cassava used in this experiment contained 172.2mg HCN/Kg fresh material and 92.1mg HCN/Kg air dry material respectively as determined by the method of Wood (1966). The differences in the pattern of serum thiocyanate therefore reflects both the concentration of hydrocyanic acid in the different diets and the quantity of each diet consumed in the two periods studied.

The activity of the rhodanese enzyme does not have any consistent effect on the liver and kidney. Indeed, Osuntokun (1969) did show that the rhodanese enzyme is abundantly distributed in many tissues in the rat. Himwich and Saunders (1968) also confirmed this in different animal species.

The results obtained in this study does not appear to be in consonance with that reported by Maner and Gomez (1973). When it is however noted that these workers fed sun-dried cassava meal as opposed to oven-dried cassava meal used in this study, it can be appreciated that growth depressions can also be caused by fungal proliferation on sun-dried cassava chips as reported by Clerk and Caurie (1968). Indeed, work carried out with fresh and sun-dried cassava by Tewe *et al* (1976) in Nigeria also followed the same trend as that of Maner and Gomez (1973).

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