

INFLUENCE OF DIETARY INORGANIC CYANIDE (KCN) ON PLACENTAL THIOCYANATE TRANSFER, TISSUE RHODANESE ACTIVITY AND PERFORMANCE OF RATS DURING GESTATION

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ABSTRACT

Thirty two bred rats were allotted to two diets to investigate the influence of non-addition or addition of 1000 ppm KCN to cassava — soybean diets on thiocyanate concentrations, tissue rhodanese activity and performance parameters. KCN caused a non-significant ($P > 0.05$) depression in body weight gain but had no effect on weights of liver, kidney and placenta, nor on number and weight of 19 day old foetus.

High (1000 ppm) KCN level caused a significant ($P < 0.05$) increase in urinary thiocyanate excretion of thiocyanate. It however had no marked effect on serum protein bound iodine, amniotic fluid thiocyanate, rhodanese activity of foetal and maternal tissues nor on foetal thiocyanate concentration.

INTRODUCTION

The noxious agent — hydrocyanic acid which is present in cassava in form of the cyanogenic glucosides linamarin and lotaustralin (Nartey, 1968) has been implicated in a number of chronic pathological conditions observed in man (Ekpechi, 1973; Osuntokun, 1968). The use of cassava and its products as livestock feeds has risen tremendously

within the last decade (Nestle, 1973).

Quantitative estimations on the hydrocyanic acid content of cassava reported in literature varies widely. Rogers (1963) reported that values as low as (10mg/kg) and as high as 2,000mg/kg KCN have been estimated in various strains of fresh and dried cassava samples. Oven dried cassava peels also contain 1,320mg/kg HCN (Tewe, 1975).

As the foetal-dam relationship in domestic animals is gaining considerable interest in recent times due to economic emphasis on foetal mortality and performance, a study was carried out by Tewe, Maner and Gomez (1977) which demonstrated that there was a placental barrier against transfer of thiocyanate into the foetus when pregnant rats were fed fresh and dried cassava containing 173 ppm and 92 ppm HCN respectively.

The finding has motivated this study which was aimed at quantifying the thiocyanate transfer into the foetus when a higher level of cyanide (added as KCN) than that encountered in the fresh cassava is fed to rats during gestation.

MATERIALS AND METHODS

Thirty-two 100 days old female albino rats of the sprague Dawley strain, weighing 252g on average were bred by

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pairing them in individual cages with male rats until vaginal plugs were detected on a collecting tray positioned below the mating cages. They were then separated and the females randomly allotted to two treatment groups. The control group received a basal diet prepared from the cassava meal of a low HCN variety while the other received the basal diet with 1000 ppm cyanide, added as KCN. The gross composition is shown in table 1.

TABLE 1

Gross composition of experimental diets based on low HCN variety a cassava meal

Ingredient	%
Cassava meal.....	60.3
Soybean meal.....	28.7
Corn oil.....	5.0
Minerals ^b	4.0
Vitamins ^b	2.0

- a. A sweet cassava cultivar-Venezuela variety was used for preparing the diets.
- b. The mineral and vitamin premixes were obtained from the Nutritional Biochemicals Corporation Cleveland, Ohio, U.S.A.
- c. 1000 ppm cyanide (2.5g. KCN/kg diet) was first mixed with the minerals in the treatment 2 of the experiment before its aggregation with other feed component.
- d. Crude protein content for control and experimental ration were 14.7% and 15.8% respectively.

Eight rats from each treatment were maintained individually in stainless steel metabolic cages while the rest were individually housed in wire cages. Feed intake was controlled daily to ensure an equal consumption in the two groups. Demineralized water was given *ad libitum* throughout the period of the study. Rats were weighed twice weekly. Urine was col-

lected daily on the rats kept in metabolic cages. The weekly samples were pooled for urinary thiocyanate analysis by the Bowler (1944) method which was modified by using activated charcoal to remove the interference of the yellow colour of urine. Subsequent checks on the used activated charcoal showed negligible absorption of thiocyanate.

The rats were anaesthetized with chloroform on the 19th day of gestation. Blood was obtained by heart puncture for serum thiocyanate (Bowler, 1944) and serum protein bound iodine (Barker, Humphrey and Soley, 1955). The uterus was dissected, the amniotic sac pierced with a hypodermic needle and the amniotic fluid drawn off for thiocyanate determination by Bowler's method. The foetus was removed and frozen for thiocyanate determinations and rhodanese activity by the Sorbo (1951) technique as modified by Tewe (1975). Maternal liver and kidneys were also weighed and frozen for rhodanese activity determinations.

Variance and covariance analysis were carried out by the method of Snedecor and Cochran (1967). The Bayes exact test for symmetrical comparisons (BET) of Waller and Duncan (1969) was employed in evaluating significance between treatment means.

RESULTS

Performance and weight of Organs: The effects of the two dietary cyanide levels on the performance of gestating rats is presented in Table 2. The average daily feed intake of all rats was similar as this was controlled.

Addition of 1000 ppm of cyanide caused a non-significant ($P > 0.05$) depression in average weight gain during the 19 day gestation period. Weight of organs (liver, kidney and placenta) were not affected by high cyanide intake.

The number of foetuses obtained from the gestating rats at slaughter were similar. Covariance analysis used to correct for differences due to litter size between treatment groups also confirmed that foetal weights were not statistically

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TABLE 2

Performance of gestating rats fed cassava meal diets containing levels of added cyanide

<i>Parameters</i>	<i>Experimental variable</i>	
	<i>0 ppm added cyanide</i>	<i>1000 ppm added cyanide</i>
Number of does per treatment	14	15
Daily feed intake (g)	14.0 ± 0.14	14.2 ± 0.08
Total weight gain (g)	56.0 ± 3.1	49.6 ± 3.4
Fresh weight of tissues (g):		
Liver	10.2 ± 0.3	10.1 ± 0.3
Kidney	1.5 ± 0.03	1.4 ± 0.03
Placenta	0.49 ± 0.01	0.49 ± 0.02
Number of foetus per gestating rat	11	12
Fresh weight of foetus (g)	2.3 ± 0.05	2.3 ± 0.06

± Standard error of mean.

different ($P < 0.05$). Maternal serum and urinary thiocyanate, protein bound iodine; Changes in chemical components are shown in tables 3 and 4. The addition of 1000 ppm KCN had no significant effect on serum thiocyanate and a significant ($P < 0.05$) increase in urinary thiocyanate was recorded on the 1000 ppm KCN. Maternal Rhodanese Activity: Rhodanese activity determinations of

liver, kidney and placenta indicate that the added cyanide had no significant effect on the activity of this enzyme in any of the tissues. Thiocyanate and Rhodanese activity in foetus and Amniotic fluid: Neither foetal thiocyanate nor rhodanese activity was significantly affected by the 1000 ppm KCN diet. Amniotic fluid thiocyanate was also unaffected by treatments.

TABLE 3

Metabolic changes in gestating rats fed cassava meal diets containing two levels of cyanide (KCN)

<i>Parameters</i>	<i>Experimental variable</i>	
	<i>0 ppm. added cyanide</i>	<i>1000 ppm added cyanide</i>
Serum protein bound iodine (mg/100ml.)	2.80 ± 0.21	3.10 ± 0.12
Serum thiocyanate (mg/100ml.)	2.31 ± 0.06	2.82 ± 0.35
Urinary thiocyanate (mg excreted per 100g feed intake)	2.8 ± 0.15 ^b	9.90 ± 0.86 ^a
Rhodanese activity (mg thiocyanate/min/g protein):		
Liver	29.9 ± 1.14	29.3 ± 1.12
Kidney	22.5 ± 0.84	23.6 ± 1.19
Protein content (mg % fresh tissue):		
Liver	21.9 ± 0.58	21.9 ± 0.56
Kidney	17.1 ± 0.56	16.4 ± 0.55

a,b. = Values without common superscript in horizontal row are significantly different ($P < 0.05$).

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TABLE 4

Changes in placenta, foetus and amniotic fluid thiocyanate concentration of gestating rats fed cassava meal diets containing two levels of cyanide (KCN)

<i>Parameters</i>	<i>Experimental variable</i>	
	<i>0 ppm added cyanide</i>	<i>1000 ppm added cyanide</i>
Foetal thiocyanate (mg/100ml.)	2.79 ± 0.34	2.65 ± 0.36
Amniotic fluid thiocyanate (mg/100ml.)	0.99 ± 0.14	0.79 ± 0.04
Phodanase activity (mg thiocyanate/min/g protein):		
Placenta	0.72 ± 0.06	0.69 ± 0.10
Foetus	4.9 ± 0.17	4.9 ± 0.16
Protein content (mg % of fresh tissues):		
Placenta	13.9 ± 0.32	14.1 ± 0.22
Foetus	3.8 ± 0.08	3.7 ± 0.09

± = Standard error of mean.

DISCUSSION

Total weight gain was lower in the gestating rats consuming diets with the higher level of cyanide. This is in consonance with earlier studies reported by Tewe *et al* (1977) using high HCN cassava diets. The high urinary thiocyanate excretion on the high cyanide ration also suggests utilization of more sulphur sources — especially the sulphur amino acid for detoxification of cyanide at the expense of tissue synthesis (Maner and Gomez 1973).

As shown by rhodanase activity determinations, the level of cyanide employed in this trial (1000 ppm) does not inhibit the conversion of cyanide to thiocyanate through the sulphur-transferase pathway, in the kidney and liver of the gestating rats.

Rhodanase activity determinations as shown in Table 4 confirms that the placenta is not a cyanide detoxification tissue. Also the claim by Tewe (1975) that the foetus possesses the ability to detoxify some quantity of cyanide is confirmed by the turn over rate of the rhodanase enzyme in the foetal tissue. The lower values in the foetus than those observed in the liver and kidney might be largely due to the sampling procedure for foetal rhodanase activity determinations. For

this analysis, whole foetus samples were homogenized as opposed to the homogenization of the liver and kidney samples of the gestating rat. Other foetal tissues present in the homogenate may therefore cause a dilution effect on the observed rhodanase activity.

While precautions were taken to ensure that there is no appreciable cyanide loss from the 1000 ppm KCN diet by keeping in properly sealed polythene bags, it may be advisable to use cassava products of high HCN content for subsequent studies. A handy product which can be effectively used in these studies is the dried cassava peel. Studies on the dried peels show that it contained HCN of up to 1,320 ppm in some samples. This will ensure that glucosides from cassava are used rather than inorganic cyanide. The potency of these two forms may differ in animals.

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