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Implications of Cyanide Toxicity in Animal Feeding Studies Using High Cassava Rations

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Abstract Studies on the chronic toxicity of cassava and/or added cyanide have been performed with rats and pigs. Methionine supplementation significantly improved body growth and feed conversion of animals fed cassava-based diets with or without added cyanide and led to an increased urinary excretion of thiocyanate. The improvement of the protein quality and the utilization of the methionine-sulfur in the detoxification processes appear to be the main reasons for the response to methionine supplementation. No gross thyroid lesions (goiter) have been observed on any of the rats fed either the control diet or cassava containing 150 mg of hydrocyanic acid per kilogram. A study on the methionine and iodine interaction is under progress and partial results are presented.

Résumé Les auteurs ont poursuivi, sur des rats et des procs, des études de toxicité chronique du manioc seule ou additionnée de cyanure. Un supplément de méthionine améliore sensiblement la croissance corporelle et l'efficacité de conversion des aliments chez des animaux soumis à un régime à base du manioc, avec ou sans addition du manioc, et entraîne l'élimination d'une plus grande quantité de thiocyanate dans l'urine. L'amélioration de la qualité des protéines et l'utilisation du soufre-méthionine dans le processus de désintoxication semblent être les raisons principales de la réponse au supplément de méthionine. Aucune lésion macroscopique de la thyroïde (goitre) n'a été observée chez les rats soumis soit au régime témoin soit à le manioc contenant 150 mg d'acide cyanhydrique par kilogramme. Les auteurs donnent les résultats préliminaires d'une étude en cours sur l'interaction de la méthionine et de l'iode.

CASSAVA (*Manihot* spp.) comprises a major source of food for a large portion of the world population and especially for those located in the tropical belt. Besides its important role in human nutrition, cassava also has been used as a feedstuff for livestock, and especially as an energy source for swine in the Philippines, Africa, and many areas of Latin America. Much of the published data on swine feeding has been reviewed (Maner 1972). Indications are that cassava is a good source of energy if used at levels of less than 30–40% in well-balanced diets. At higher levels slight growth depression and a decrease in efficiency of feed utilization are observed. However, this depression in pig performance can be overcome by the utilization of high-quality protein and the supplementation of the diets with adequate methionine.

Protein Quality

Studies carried out by Portela and Maner (1971 unpublished data) clearly demonstrate the effect of methionine supplementation to growing pig diets based on cassava meal and soybean meal. Two replications of five pigs each per treatment were used to study the effect of adding 0.1% or 0.2% DL-methionine to cassava-based diets containing 15.9% crude protein (Table 1). During the 42-day study the addition of methionine improved both growth and feed conversion (Table 2) although the differences in response were not statistically significant ($P > 0.05$).

TABLE 1. Composition of cassava diets for growing pigs (Portela and Maner 1971 unpublished data).

Diet	Control group	Cassava-fed group
	%	%
Cassava meal	–	56.0
Soybean meal	15.7	26.5
Yellow maize	79.3	12.5
Bone meal	3.5	3.5
Vitamin Premix	1.0	1.0
Mineral Premix	0.5	0.5
Calculated protein (%)	15.8	15.9

TABLE 2. Performance of growing pigs fed cassava-based diets with and without methionine supplementation (Portela and Maner 1971 unpublished data). Values with the same superscript are not statistically different ($P > 0.05$).

Diet	Average daily gain[a] (*kg*)	Average daily gain (*kg*)	Feed/gain
Corn–soybean	0.74[c]	2.07	2.81
Cassava–soybean	0.76[bc]	2.01	2.65[ab]
Cassava–soybean–0.1% methionine	0.83[ab]	2.03	2.46[a]
Cassava–soybean–0.2% methionine	0.82[ab]	2.03	2.49[ab]

[a]Two replications of five pigs each per treatment, 42-day experiment.

Similar results (Maner and Mesa 1971 unpublished data) were obtained with rats fed diets based on dried cassava meal and supplemented with casein. Supplemental levels of 0, 0.12, 0.17, 0.22, and 0.27% methionine were compared. A quadratic response in gain, feed efficiency and protein efficiency ratio (Table 3) was obtained. Performance was improved as the level of supplemental methionine was increased to 0.17% and decreased progressively at higher levels of supplementation.

A repetition of this study (Calderón and Maner 1972 unpublished data) produced similar results in that maximum performance during the 28-day trial was obtained when the level of methionine supplementation to the cassava meal based diet reached 0.17%. However, in contrast to the first trial, a depression in performance was not observed at higher levels of supplementation. Supplemental levels of 0.22 and 0.27% methionine supported performance not statistically different from that obtained with 0.17% methionine (Table 4).

Cyanide Toxicity

Reports (Clark 1939; Montgomery 1965; Ekpechi et al. 1966; Oke 1968, 1969; Ermans et al. 1969; Osuntokun 1970; Osuntokun and Aladetoyinbo 1970; Delange and Ermans 1972; Thilly et al. 1972) have indicated that long-term consumption of cassava containing low levels of hydrocyanic acid (HCN) produce a chronic cy-

TABLE 3. Protein value of cassava protein with varying levels of methionine supplementation compared to casein protein. Five rats were used per treatment in this 16-day experiment (Mesa and Maner 1972 unpublished data).

Diets[a]	Average gain (*g*)	Feed/gain (*g*)	Protein efficiency ratio
Casein + Cassava	28.5	5.1	2.35
" " + 0.12% methionine	33.7	4.4	2.63
" " + 0.17% "	41.9	3.9	2.91
" " + 0.22% "	39.9	4.2	2.73
" " + 0.27% "	31.6	4.8	2.52

[a]All diets contained 8.73% crude protein: 6.00% from casein and 2.73% from cassava.

TABLE 4. Effect of methionine supplementation to cassava–casein diets fed to growing rats (Calderón, Maner, and Gómez 1972 unpublished data).

Per cent protein	Source of protein	Average[a] gain (g)	Feed/gain	Protein efficiency ratio
6.00	Casein + Cassava starch	13.3	10.9	1.52
6.00	" " " + 0.12% DL-methionine	47.0	5.6	2.95
8.63	" " meal	37.1	5.7	2.04
8.63	" " " + 0.12% "	56.4	4.7	2.49
8.63	" " " + 0.17% "	66.9	4.3	2.69
8.63	" " " + 0.22% "	67.3	4.2	2.76
8.63	" " " + 0.27% "	69.9	4.3	2.72
8.63	" " starch	53.4	4.5	2.60
8.63	" " " + 0.18% "	91.2	3.6	3.24
8.63	" " " + 0.18% "	56.7	4.1	2.79

[a]Twenty-eight-day feeding trial.

TABLE 5. Effect of HCN in cassava meal and fresh cassava on growth and urinary excretion of thiocyanate in rats; each value represents the average of four rats (Maner 1972 unpublished data).

Days on expt	Diet	Average weight (g)	Thiocyanate excreted (mg/day)
9	Sucrose	146	0.11
	Cassava meal	154	0.47
	Fresh cassava	177	3.69
	Fresh cassava + $FeCl_2$(0.3%)	174	3.72
14	Sucrose	174	0.12
	Cassava meal	194	0.49
	Fresh cassava	221	2.80
	Fresh cassava + $FeCl_2$(0.3%)	218	3.86
29	Sucrose	220	0.16
	Cassava meal	238	0.56
	Fresh cassava	271	4.50
	Fresh cassava + $FeCl_2$(0.3%)	288	5.02
70	Sucrose	344	0.20
	Cassava meal	333	0.56
	Fresh cassava	405	3.58
	Fresh cassava + $FeCl_2$(0.3%)	405	4.06

anide toxicity with resulting ataxic neuropathy and/or goiter. However, the effect appears to be complicated by other factors in addition to HCN and these factors appear to be predisposing for the development of the previously mentioned conditions.

Maner (1972 unpublished data) fed fresh chopped cassava containing 150 mg HCN/kg to growing rats for a period of 4 months to measure the effect HCN would have on performance, thyroid development, and the urinary excretion of thiocyanate (SCN). These parameters were compared to those of rats fed either sucrose or sun-dried cassava meal containing only 5 mg HCN/kg. Rats fed a protein supplement and fresh cassava gained weight at a significantly faster rate than those fed either the sucrose or the cassava meal-based diets (Table 5), even when the quantity of protein and dry matter was controlled for equal consumption by all groups. Average excretion of

TABLE 6. Effect of cyanide on body growth of rats in an 18-day experimental period (Calderón, Maner, and Gómez 1972 unpublished data).

	Added cyanide (as KCN), *ppm*							
	0	480	960	1600	2400	3200	4800	8000
Initial no. rats	5	5	5	5	5	5	4	4
Final no. rats	5	5	4	4	5	2	1	1
Total wt gain, *g*	27.9	24.4	18.5	14.6	11.5	2.6	4.1	−9.1
Feed consumed, *g*	127.2	109.5	92.3	87.6	84.4	72.9	81.6	61.5

SCN in the urine was proportional to the quantity of HCN consumed. Significantly larger quantities (37-fold increase) were excreted by the rats consuming fresh cassava than by those fed sucrose-based diets, and those consuming dried cassava meal excreted five times as much SCN/day as those receiving the control sucrose diet. An autopsy showed no differences in thyroid size in any of the rats on the four different treatments.

Since no detrimental effects were observed when the fresh cassava containing 150 mg HCN was fed as part of a well-balanced and adequate diet, additional studies were undertaken to determine the effect higher levels of cyanide (–CN), added to the diet as potassium cyanide (KCN), would have on rat performance, serum and urinary SCN, methionine requirement, and goiter production.

Preliminary observations (Calderón, Maner, and Gómez 1972 unpublished data) suggest that 3200-ppm dosage or higher of KCN added to the diets was lethal for growing rats. One out of four rats survived in the groups fed a basal cassava starch–casein diet supplemented with 4800 and 8000 ppm cyanide, respectively, and two out of five when 3200 ppm was added. The total body weight gains in an 18-day experimental period declined as the levels of added cyanide increased from 0 to 8000 ppm (Table 6). Urine samples collected during the last 8 days of the experimental period were analyzed for SCN (Bowler 1944) and the results clearly showed an almost linear increase of urinary SCN excretion as the level of added cyanide was increased (Fig. 1). Plasma SCN concentration was also higher for rats fed the cyanide-supplemented diets (Fig. 2) than for rats fed the control diet (0 ppm CN^-), but the differences were not as pronounced as those observed in the SCN urinary excretion.

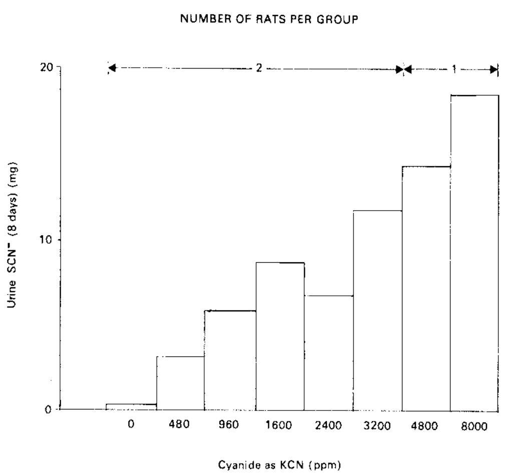

FIG. 1. Effect of cyanide on urinary thiocyanate excretion in rats.

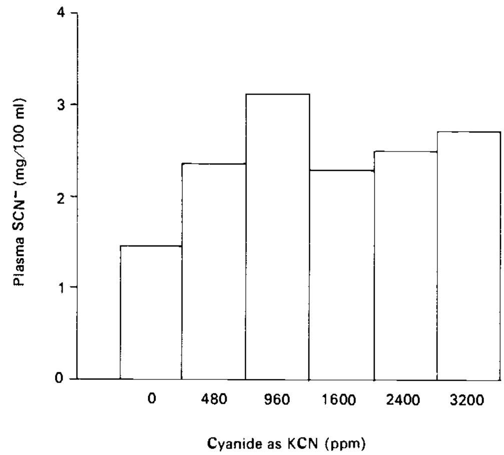

FIG. 2. Plasma thiocyanate concentration in rats fed varying levels of cyanide.

Although there is a great deal of information on the acute toxicity of HCN, relatively little is

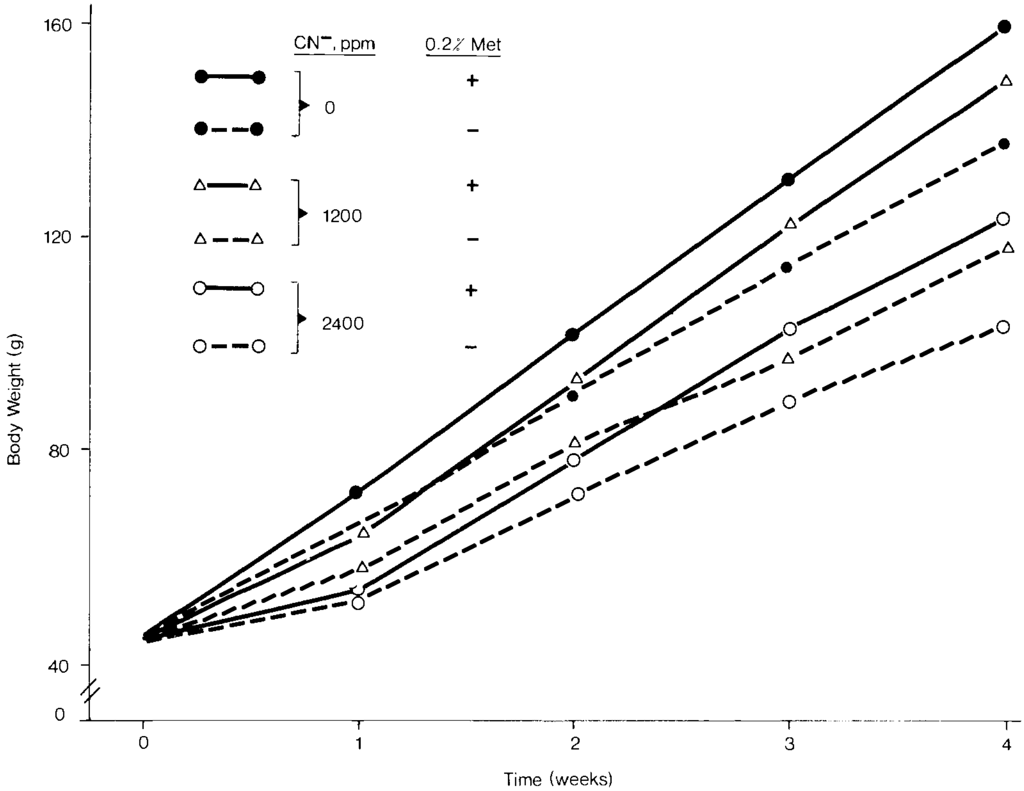

FIG. 3. Methionine and cyanide interaction on the body growth of rats (six rats per group).

known about the chronic effects derived from the continuous ingestion of sublethal amounts of cyanide. Our preliminary observations suggest that levels of added cyanide below 2400 ppm could be considered sublethal for rats. Previously cited (unpublished) observations with rats (Maner and Mesa) and pigs (Portela and Maner) have also demonstrated the beneficial effect of methionine supplementation to cassava diets, which was at first considered as the correction of a methionine deficiency per se occurring in the dietary protein but which more probably exerts its effect both by improving protein quality and as a readily available source of sulfur for cyanide detoxication through the thiosulfate–rhodanase system. Therefore, our subsequent studies (Calderón et al. unpublished data) using sublethal doses of added cyanide considered methionine supplementation as an additional variable. Since whole cassava roots (peel and pulp) are normally used in practical animal feeding, the experimental diets were formulated using cassava meal rather than cassava starch.

At all levels of added cyanide, rats fed the methionine-supplemented diets exhibited a significantly ($P < 0.01$) faster rate of growth than those fed unsupplemented diets (Fig. 3). Methionine supplementation improved feed consumption, and led to an increased urinary excretion of SCN as the level of added cyanide was augmented from 0 to 1200 and 2400 ppm, respectively (Table 7). Plasma SCN concentration did not follow the same pattern as the urinary excretion of SCN which increased as the level of cyanide and methionine were increased (Table 7). On autopsy none of the rats showed any indications of enlarged thyroids.

Reports in the literature (Delange et al., 1968; Ermans et al., 1969; Delange and Ermans, 1971; and Thilly et al., 1972) indicate that endemic goiter in the Congo and New Guinea cannot be explained solely on the basis of an iodine deficiency but may be complicated by dietary goitrogenic factors and especially by the large quantity of cassava consumed in the goitrous areas. The

TABLE 7. Interaction of methionine and cyanide supplementation to cassava meal diets in rats; six rats per treatment, 28-day experimental period (Calderón, Maner, and Gómez 1972 unpublished data).

	Dietary variables					
	0.2% methionine			0% methionine		
CN^- ppm[3]	0	1200	2400	0	1200	2400
Total weight gain, *g*	111.7	103.3	76.1	91.5	73.4	56.0
Food consumed, *g*	362.8	360.9	276.5	348.7	285.5	236.1
Feed conversion	3.3	3.4	3.7	3.8	3.9	4.2
Total urine SCN^-, *mg*[a]	68.1[b]	87.5	116.0	43.7[b]	62.3	63.5
Plasma SCN^-, *mg/100 g*	2.05[b]	3.24	2.72	2.34[b]	2.45	2.81

[a]CN^- = cyanide as KCN; SCN^- = thiocyanate.
[b]Mean of two rats; remaining values on the same lines represent means of three rats per group.

TABLE 8. Effect of methionine and/or iodine supplementation to cassava meal–casein diets fed to rats; partial results over 2-week experimental period using four rats per experiment. Average body weight of rats was 88 g (Gómez, Calderón, and Maner 1972 unpublished data).

	Dietary variables			
	0.2% methionine		0% methionine	
	Iodine	No iodine	Iodine	No iodine
Avg total wt gain, *g*	65.5	64.6	37.0	27.5
Avg food intake, *g*	196.1	201.6	168.8	154.7
Avg total SCN urinary excretion				
Pre-experimental period (6 days), *mg*	0.62	0.58	0.61	0.59
First week experiment, *mg*	4.31	3.55	2.61	2.41
Second week experiment, *mg*	4.76	4.25	3.62	2.82

goitrogenic activity of cassava was experimentally demonstrated in rats (Ekpechi et al. 1966), and further experimental evidence in humans (Delange and Ermans 1971) suggested that the ingestion of cassava grown in the goitrous area of Idjwi Island (Republic of Zaïre) reduces the thyroid iodine intake and increases the renal iodine excretion.

The prevalence of iodine deficiency in the tropics, and the wide use of cassava as a staple food in these areas, have led us to approach this problem in animal experiments. An experiment with rats was designed to study the interaction of supplemental methionine (0 and 0.2%) and iodine (addition of potassium iodide (KI) to, or its withdrawal from, the mineral mixture) on a cassava meal–casein diet. The partial results of the first 2 weeks of the experiment in four groups of rats kept in metabolic cages for urine collection are shown in Table 8. The effects of methionine supplementation on body growth of rats fed cassava meal-based diet are confirmed again. During a 6-day pre-experimental period, the rats were fed a pelleted-laboratory diet (Purina chows) and daily urine collections were analyzed for SCN. The daily urinary SCN excretion was consistently low throughout the entire pre-experimental period but the ingestion of the experimental diets (cassava meal–casein) brought about a rapid increase in the quantity of SCN excreted in the urine (Table 8). Despite the day-to-day variation, daily excretion of SCN in the urine followed a similar pattern for all the experimental groups. The total weekly SCN excretion (Table 8) was consistently lower in the absence of added methionine, and increased from the first to the second week in all the experimental groups, possibly as a consequence of the higher

TABLE 9. Analysis of the amino acid content of two samples of cassava, "Llanera" variety (Maner 1972 unpublished data).

	% of crude protein	
Amino acid	Sample 1	Sample 2
Arginine	17.10	12.90
Histidine	.60	.53
Isoleucine	.77	1.04
Leucine	1.24	1.52
Lysine	1.54	1.56
Methionine	–[a]	.33
Cystine	.51	–[a]
Threonine	.86	1.00
Phenylalanine	.78	.94
Valine	1.23	1.32
Tryptophan	.50	.50

[a]Non-detectable level.

food consumption. Further observations on thyroid and blood and tissue enzyme activities will be obtained at the end of the experimental period and published at a later date.

Discussion

At least two reasons exist for the response to methionine supplementation of diets containing high levels of cassava. Cassava protein contains a very small quantity of sulfur-containing amino acids (Table 9) and when fed with casein or soybean meal, both deficient in methionine, there is a methionine deficiency per se which results from the combination of the two ingredients. The protein quality, therefore, is improved by the addition of crystalline DL-methionine. However, the methionine deficiency may be complicated by the utilization of the methionine-sulfur in the detoxication of HCN present in the cyanogenic glucosides of cassava. Since thiosulfate, and not methionine per se, is the required substrate for the formation of SCN by rhodanese (Himwich and Saunders 1948), methionine has to be metabolized so that methionine-sulfur would be available for detoxication purposes. However, SCN may be formed by a different mechanism such as the reaction of a persulfide group with cyanide, as proposed for the mechanism of xanthine oxidase inhibition by cyanide (Massey and Edmondson 1970).

Thiocyanate may be responsible for the goitrogenic activity of cassava as suggested by Delange and Ermans (1971). Pigs fed a corn–soybean meal diet supplemented with 0.5% potassium thiocyanate showed lower protein-bound iodine levels and heavier thyroids; however, higher incorporation of radioiodine in the thyroids was observed as compared to the parameters of pigs fed the same basal diet without SCN addition (Sihombing et al. 1971).

It therefore appears that methionine serves both to overcome a dietary deficiency of sulfur-containing amino acids and as a readily available source of labile sulfur for cyanide detoxication. The detoxication produces SCN that exerts a goitrogenic effect on the body that can cause thyroid hypertrophy especially in the absence of adequate dietary iodine. However, in the presence of adequate methionine and iodine, cyanide is without measurable effect on goiter production or nerve degeneration.

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