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Cassava, Protein and Iodine Interaction in the Thyroid Status of Rats

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The possible synergistic role of dietary cyanide from cassava, low iodine intake and protein deficiency and/or imbalance on the thyroid status and goiter endemia in Akungba and Oke-Agbe villages in Akoko area of south-western Nigeria was investigated using rats as model. Thirty (30) weaning albino rats were fed on corn starch or cassava as the major source of carbohydrates, 3.5 and 10.0% levels of protein supplied by either casein or *Amaranthus viridis* and a salt mixture with or without iodine for 42 days. Cassava with a total hydrocyanic acid (HCN) content of 25 mg kg^{-1} DW did not cause growth depression when fed to rats with casein at 10% protein level. Iodine deficiency resulted in slightly higher weight gain while protein deficiency especially the vegetable rations caused significant growth reductions. Interaction between cassava/iodine deficiency and protein imbalance, especially in the vegetable rations, caused a significant increase in the weight of the thyroid gland when expressed as percent of body weight. Dietary cyanide caused a significant increase in serum and urinary thiocyanate levels while iodine deficiency caused a significant reduction in the plasma protein-bound iodine (PBI). These results would partially explain the goiter endemia in Akungba and Oke-Agbe villages whose nutritional status was mimicked in these experiments.

Key words: Dietary cyanide, Iodine, Thyroid status, Goiter endemia, Proteins, Cassava.

Introduction

It is well established that iodine deficiency plays a significant role in the etiology of goiter (Koutras *et al* 1970) but the existence of other contributory factors cannot be overruled (Chesney *et al* 1928). The goitrogenic role of cassava has been established by studies carried out in Eastern Nigeria (Ekpech 1967) and in severe endemic goiter areas of Idjwi Island, Kivu and Ubangi (Delange and Ahluwalia 1983). The role of cassava in goitrogenesis is due to the presence of linamarin and lotaustralin-cyanogenic glucosides - which on hydrolysis by the enzyme linamarase (E.C.3.2.1.21) liberate cyanide. The cyanide is then detoxified *in vivo* mainly through the enzyme rhodanese (E.C.2.8.1.1) in the presence of labile sulfur (from methionine) to yield thiocyanate (SCN) which inhibits iodine uptake by the thyroid gland thereby causing thyroid enlargement.

Edible plants, especially the Cruciferae, contain glucosin olates which on hydrolysis also produce intermediate products including thiocyanate, isothiocyanate and goitrin, all of which are invariably goitrogenic (Dietz 1989). In addition to the goitrogenic activities of dietary cassava and glucosinolates, epidemiological data among goitrous patients in the southern part of Senegal suggests that low social status and malnutrition may have deleterious effects on thyroid function (Ingenbleek and Visscher 1979; Ingenbleek *et al* 1980). On the other hand, protein malnutrition has been shown in pigs to result in thyroid gland atrophy (Delange and Ahluwalia 1983) and general protection against the antithyroid action of cassava by reducing the quantity of SCN arising from the HCN detoxification. The effect of the interaction between cyanide, iodine deficiency and protein malnutrition on thyroid status was investigated by Maner and Gomas (1973) and Tewe (1982) using KCN at levels between 480 and 1480 mg kg^{-1} diet. They observed growth depression at a cyanide level of 480 and death at 1480 mg kg^{-1} diet, with some effects on thyroid status between these two extreme values.

During our investigations of the chemical and nutritional causes of goiter in Akungba and Oke-Agbe, we surveyed food habits, collected and analysed food and water samples from the two villages and found them to be deficient in both protein and iodine (Akindahunsi *et al* 1983) but the total HCN content of cassava products consumed in these areas varied only between 5.2 and 25.0 mg kg^{-1} dry weight (DW) with the fresh tuber containing just 100.8 mg kg^{-1} wet weight (WW). The question, therefore, was if there would still be a synergistic interaction between the low dietary cyanide, iodine and protein intake to account for the goiter endemia observed in the region.

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The aim of this investigation, therefore, was to mimic the dietary regime of this goitrous population in a rat bioassay to test the hypothesis that low dietary protein and iodine would interact with dietary cyanide (however small) causing synergistic effect on thyroid function and result in goiter. It may offer an explanation for the goiter endemia in Akungba and Oke-Agbe.

Materials and Methods

Fresh cassava roots were purchased from the mass markets in Akoko area, peeled and cut into small pieces. The chips were sun dried and milled. *Amaranthus viridis,* identified as one of the commonest vegetables in Akoko was also purchased from that area, dried in the oven at 50°C for 24 h and milled to powder (Siebtechnik milling machine).

Bioassay. Thirty female albino rats of the Wister strain weighing between 55 and 65g were obtained from the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife. They were housed individually in stainless steel metabolic cages (Associated Crates Ltd., Stockport, England), randomly alloted to six treatment group and provided food and water *ad libitum.* The group on iodine deficient treatment was given distilled water while the other groups were given ordinary tap water which contained about 5.8$\mu g l^{-1}$ iodine (Akindahunsi 1992).

Table 1 gives the gross composition of the diets fed to animals for 42 days after a 3-day acclimatization period. During the acclimatization period, all the animals were fed the same corn starch based nitrogen free basal diet.

Body weight was recorded at the beginning, weekly and at the end of the experiment. Urine of 24h was collected once weekly and frozen until analysed. At the end of the experiment, the rats were anaesthetized with diethyl ether and blood was collected by heart puncture into plastic tubes containing lithium heparin. The thyroid was removed and weighed fresh. Five animals were sacrificed at the beginning of the experiment to give a baseline data for the weight of the organ.

Analysis. Cyanide content of fresh and sun-dried cassava was determined by the method of Lambert *et al* (1975) after initial enzymic hydrolysis. Nitrogen (N) content of the amarnath was determined by the semi micro-Kjeldahl's method (AOAC 1984). Crude protein was obtained by multiplying the N content by 6.25. Creatinine content of the 24h urine was determined by the Jaffe reaction (Visiliades 1976). Thiocyanate content of the urine and plasma was analysed by the method of Pettigrew and Fell (1972). Plasma protein bound iodine (PBI) was analysed by the method of Henry *et al* (1974) and soluble protein according to the method of Peterson (1977).

Statistical analysis. Mean and standard error of mean (SEM), analysis of variance, Duncan's new multiple range test and level of significance at $P \leq 0.05$ were analyzed by the method of Zar (1984).

Results and Discussion

For clarity and easy reference, each treatment group is described by the percentage protein of the diet contributed by casein or *A. viridis,* the percentage carbohydrate (cassava or corn starch) and iodine content (+ or -).

Table 2 shows the levels of the physicochemical parameters determined. Body weight gain was highest in rats fed 73.4% corn starch (Diet 1) and 78.4% cassava (Diet 2) supplemented with 10% casein protein diet and iodine. The most interesting finding in this study was that the thyroid gland/body weight ratio was greatest (0.64) in rats fed 77.5% cassava-3.5% vegetable protein (Diet 5) followed by those on Diet 6-54.3% cassava-10% vegatable protein ration (0.57) as shown in Fig 1.

Table 1
Gross composition of experimental diets (g $100\,g^{-1}$ feed dry weight)

	Treatment groups					
Ingredients	1	2	3	4	5	6
Corn starch	73.4	0.0	0.0	0.0	0.0	0.0
Cassava	0.0	78.4	85.9	85.9	77.5	54.3
Non-nutritive cellulose	5.0	0.0	0.0	0.0	0.0	0.0
Casein	11.6	11.6	4.1	4.1	0.0	0.0
Amaranthus viridis	0.0	0.0	0.0	0.0	12.5	12.5
Palm oil	5.0	5.0	5.0	5.0	5.0	5.0
*Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0
**Mineral premix	4.0	4.0	4.0	4.0	4.0	4.0
Total	100	100	100	100	100	100

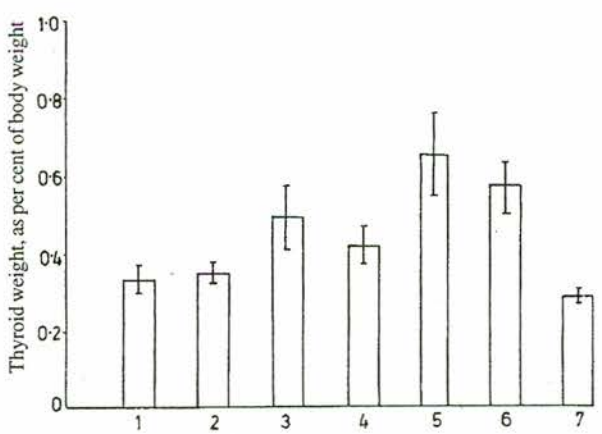
*Vitamin premix is a commercial product from Nutritional Biochemicals, Ohio, USA; **mineral premix contained per kg mixture: $CaHPO_4$, 735.0g; K_2HPO_4, 81. 0g; K_2SO_4, 68.0g; NaCl, 30.6g; $CaCO_3$, 21.0g; Na_2HP0_4, 21.4g; MgO, 25.0g; and trace metal mixture, 18.0g. The trace metal mixture contained ferric citrate, 31.0g; $ZnCO_3$, 4.5g; $MnCO_3$, 23.4g; $CuCO_3$, 1.85g; KI, 0.04g; and citric acid to make 100g. All diets contained iodine (0.04g KI $100g^{-1}$ mineral premix) except diet 3.

Table 2

Physicochemical parameters of 42-day growth study of rats on varying dietary cassava, protein and iodine rations

Parameters	Treatment groups					
	1	2	3	4	5	6
Weight of rats *(g)	123.8±5.2^{a}	119.1±4.1^{a}	83.8±13.3^{b}	73.1±4.4^{b}	41.3±5.2^{c}	56.9±6.9^{c}
Daily weight gain (g)	1.49±0.13^{a}	1.43±0.12^{a}	0.54±0.30^{b}	0.36±0.14^{b}	-0.36±0.20^{b}	-0.30±0.09^{bc}
% (thyroid/body**) weight	0.33±0.04	0.35±0.01	0.49±0.08	0.42±0.05	0.64±0.14	0.57±0.07
Plasma protein (g dl^{-1})	4.80±0.06^{a}	4.85±0.10^{a}	4.45±0.09^{b}	4.30±0.12^{b}	3.35±0.12^{c}	4.05±0.12^{b}
Plasma PBI (µg dl^{-1})	6.15±0.22^{a}	6.50±0.27^{a}	4.15±0.25^{b}	6.10±0.19^{a}	6.30±0.21^{a}	5.90±0.16^{a}
Plasma SCN (mg dl^{-1})	0.12±0.01^{f}	0.90±0.01^{b}	0.96±0.01^{a}	0.78±0.01^{c}	0.55±0.01^{d}	0.23±0.01^{e}
Urinary SCN (mg dl^{-1})	0.35±0.06^{c}	1.07±0.13^{a}	1.10±0.14^{a}	0.57±0.05^{b}	0.65±0.03^{b}	0.52±0.02^{b}
Urinary creatinine (g dl^{-1})	0.03±0.01^{b}	0.07±0.01^{a}	0.06±0.01^{ab}	0.03±0.01^{b}	0.04±0.01^{b}	0.04±0.01^{b}

a, each value represents mean of five rats per treatment ± SEM: means of the same superscript letter(s) along the same row are not significantly different at $P \leq 0.05$;*,initial (start of experiment) body weight was 59.9±5.8g;**% thyroid weights of rats was 0.28±0.01% at the start of the experiment.



1. Com/10% casein protein/KI
2. Cassava/10% casein protein/KI
3. Cassava/3.5% casein protein/KI
4. /cassava/3.5% casein protein/KI
5. Cassava/3.5% vegetable protein/KI
6. Cassava/10% vegetable protein/KI
7. Start of experiment

Fig 1. Thyroid weights of rats given different treatments indicating mean of four values ± SEM

The plasma protein content was the highest in rats fed 10% casein protein in diets 1 & 2 and was significantly higher than that in animals fed 3.5% casein. Plasma thiocyanate (SCN) level was the highest in animals fed iodine deficient diet (0.96) and was significantly different from that of animals fed cassava-10% casein diet (0.90). Plasma SCN was low in rats fed cassava-10% vegetable diet (0.23) and lowest as expected in corn starch-casein fed animals (0.12 mg 100 ml^{-1}). The thiocyanate level in the urine was generally higher in rats fed cassava-based diets compared to those on the corn starch diet.

The total cyanide (HCN) content in the unfermented sundried cassava was 25 mg kg^{-1} dry weight. In cassava based diets, the HCN content corresponded to 13.6-21.5 mg kg^{-1} dry weight of the diet. The cassava products in the diets of the goitrous population of Akungba and Oke-Agbe villages in Akoko division of south-western Nigeria contained between 5.2 and 17.6 and 17.6 mg kg^{-1}. The HCN level used in this experiment therefore mimics to a large extent the human intake of HCN in these villages.

When cassava, at 78.4% corresponding to 19.6 mg HCN kg^{-1} diet, was supplemented with 10% casein protein and fed to rats, there was no significant difference in growth rate compared to the control corn starch diet (Table 2). Maner and Gomez (1973) observed a slight growth depression when KCN was fed to rats at 480 mg kg^{-1} diet. At 960 mg kg^{-1} diet, there was a significant growth reduction and death at 1480 mg kg^{-1} diet. Tewe (1982) also observed a slight depression

in growth rate when rats were fed 750 mg KCN kg^{-1} diet. The HCN levels used by these earlier investigators were 20-60 times the concentration used in the present experiment and could easily account for the differences observed in the results. In addition to this 60% of the total 25 mg HCN kg^{-1} dry cassava used in the present experiment was still in the bound form which would at least be partly excreted intact in the urine (Adewusi and Oke 1984) and the hydrolysis of which would also depend on the microflora content of the large intestine (Carter *et al* 1980). The deleterious effect of cassava ingestion by animals has been attributed to the detoxification of HCN (the hydrolysis product of linamarin) by rhodanese which makes use of labile sulfur supplied mainly by methionine *in vivo*. This, in effect, depletes the level of this essential amino acid in the liver and other tissues where it is required for protein synthesis (Oke 1978). Therefore, growth depression consequent upon cyanide ingestion was not observed in animals on the cassava-casein based diet probably due to the low cyanide level in the diet and the possibility that part of the linamarin could have been excreted intact.

A dietary survey of Akungba and Oke-Agbe and the analysis of the typical breakfast, lunch and dinner of people showed that the energy content was 3.8-4.1 $kcal^{-1}$ g diet and the protein level was 2.4-3.4%,contributed substantially by vegetable proteins. *A viridis* was also found to be the predominant vegetable eaten by these communities (Akindahunsi *et al* 1993). The cassava-3.5% vegetable protein diet was therefore formulated to mirror the life situations in these villages. In this experiment, the source of carbohydrate did not seem to affect weight gain since animals fed with casein-corn starch or cassava based diets gained about the same weight. A similar result had been reported by Adewusi and Oke (1980).

Cassava seemed to marginally increase the weight of the thyroid gland compared to the control as reflected by the (10%) higher values obtained for cassava fed rats in absolute values or as percent of total body weight. Body weight gain and the thyroid gland were smaller in animals fed with cassava-3.5% cassein protein compared to those fed 10% casein diet. However, when the weight of the thyroid gland was expressed as percent of body weight, it was baout 15% higher in rats fed 3.5% than those on 10% casein protein diets.

The fact that animals fed with this diet lost weight showed that *A. viridis* at this level was inadequate for body maintenance and would therefore represent a condition of gross malnutrition. Animals fed with cassava-10% vegetable protein lost weight marginally showing that the vegetable even at this level was barely adequate for body maintenance when eaten over a long period without adequate supplementation by other protein sources. Mild but not gross malmutrition was evident in both Akungba and Oke-Agbe probably because mixed protein sources are eaten and even a poor quality protein if taken in a large quantity could satisfy nutrient requirements. The overall loss in weight by animals fed with 10% vegetable protein could probably be due in part to the reduced caloric intake, increased fibre content from the *A. viridis,* decreased protein digestibility due to antinutritional factors (Bostid, 1984) and probable imb alance in the amino acid content. Though the animals on 3.5 or 10% vegetable protein lost weight, their thyroid gland gained almost 100% additional weight making the ratio of this gland to their total body weight the highest among all the treatments. This finding did not support the observation of Tewe (1982) that malnutrition caused atrophy of the thyroid gland nor that of Delange and Ahluwalia (1983) that protein malnutrition will generally protect against the antithyroid action of cassava. Indeed the present result indicates that in situations of gross protein malnutriton, the thyroid gland weight would increase in the presence of exacerbating factors such as low iodine and high cassava (thiocyanate) intake. This would be consistent with the observation of Ingenbleek and Visscher (1979) and Ingenbleek *et al* (1980)that protein malnutrition could increase the risk of goiter endemia as well as the size of existing ones in communities with a high cassava/thiocyanate and low iodine intake.

Plasma protein bound iodine in often used to monitor iodine availability when T3 and T4 levels cannot be quantitfied. Despite its limitations, PBI level was significantly lower in rats fed with iodine deficient cassava-casein diet, highest in cassava-10% casein fed animals but generally not significantly different from the other treatments.

Ermans *et al* (1980), T Adewusi and Akindahunsi (1994) and Delange and Ahluwalia (1983) observed that the thyroid function is critically related to the balance between the dietary supplies of iodine and thiocyanate. Thiocyanate concentration in the urine of rats fed with 3.5% protein (casein and vegetable rations with adequate iodine) were similar and the iodine content may not differe significantly. Using the PBI content (6.1 and 6.3 mg dl^{-1}) and the thiocyanate levels monitored in this experiment (0.57 and 0.65 mg dl^{-1} for casein and amaranth diets, respectively), the iodine/thiocyanate ratio in animals fed with cassava-3.5% protein level either from casein or vegetable would be expected to be similar. Yet the animals fed with the vegetable protein had a higher thyroid

gland weight when expressed as a percentage of total body weight. These observations seemed to indicate a major role for protein malnutrition in the etiology of goiter in the presence of a low iodine/thiocyanate ratio. This conclusion had also been drawn by Ingenbleek and Visscher (1979) and Ingenbleek *et al* (1980) based on epidemiological investigations in the goitrous areas of Senegal. Protein deficiency, aggravated by methionine depletion as a result of cyanide detoxification, is expected to substantially reduce the production of enzymes and proteins involved in the thyroid hormone synthesis as earlier observed by Donati *et al* (1963).

Conclusion

It has been indicated earlier that this experiment was designed to mimic the life situation in Akungba and Oke-Agbe where the incidence of goiter has been established to be high (Akindahunsi 1992). The results of this animal model tend to indicate a major synergistic relation between protein malnutrition and low iodine/thiocyanate ratio in the etiology of goiter and would therefore explain, at least in part, the goiter endemia in these two villages.

Though the gross composition of the diets in the present study was adequately documented, the relationship between calorific content of diets and performance of animals would be the focus of a future investigation.

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