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Comparison of the effects of cassava (*Manihot esculenta Crantz*) organic cyanide and inorganic cyanide on muscle and bone development in a Nigerian breed of dog

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Effects of cassava (*Manihot esculenta Crantz*)-borne organic cyanide and inorganic cyanide in the form of sodium cyanide on bone and muscle development were investigated in eighteen dogs of Nigerian breed. After 16 weeks of stabilization in the laboratory from the time of purchase when the dogs were fed on the same diet, they were randomly assigned to three experimental groups of six dogs each. The control group was fed on rice while the other two groups were fed on either cassava (gari) or rice plus cyanide. The three diets were made isoenergetic and isonitrogenous by varying the quantity of meat incorporated into them. The results obtained after 14 weeks of feeding the respective diets indicated that there was retardation of muscle development in the gari-fed dogs. This may have resulted from gluconeogenesis from muscle protein associated with suppression of production of insulin by the pancreas in this group. The results indicated also that the effects of inorganic dietary cyanides on muscle development were different. Both forms of dietary cyanides, however, had no adverse effect on bone development.

Cyanide: Muscle: Bone: Growth: Dogs

Cassava (*Manihot esculenta Crantz*) is extensively grown in the tropics. In the South Eastern part of Nigeria it is a staple food of the human population. It is eaten in the form of 'gari' or 'foofoo'. Its use as a source of energy for animal feeds has also long been recognized (Kok & Robeiro, 1949; Modebe, 1963; Aina *et al.* 1968; Peixoto, 1968; Nestel, 1973; Sonaiya *et al.* 1982; Tewe & Pessu, 1982).

Cassava, however, contains the cyanogenic glycoside linamarin which is hydrolysed to hydrocyanic acid in vivo. The latter is further detoxified using essential amino acids like methionine and lysine (Umoh *et al.* 1979), to thiocyanate (Oke 1969, 1973; Ononogbu & Emole, 1978; Omole & Sonaiya, 1981) which is a potent goitrogen that has been shown to depress thyroid function in rats (Ekpechi *et al.* 1966), pigs (Sihombing *et al.* 1971) and man (Ekpechi 1973; Ermans *et al.* 1973; DeLange *et al.* 1980). In addition, thiocyanate, in association with low protein intake, has been implicated in the aetiology of tropical pancreatitis and diabetes mellitus in man (Abu-Bakare *et al.* 1986). It also causes other pathophysiological conditions like tropical ataxic neuropathy in man (Osuntokun, 1969). The thyroid gland, through its hormone (thyroxine or tetraiodothyronine), is known to influence tissue growth by regulating protein turnover in tissues (Goldberg *et al.* 1980; Brown *et al.* 1981; Brown & Millward, 1983). Similarly, insulin secreted by the pancreas affects protein turnover in tissues (Dubowitz & Brooke, 1973). By depleting the body's reserve of methionine, and by its effect on the thyroid gland and pancreas, it would be

Table 1. *Mean proximate composition (g/kg) of the three experimental diets (from Kamalu, 1991)*

Nutrients	Control diet (rice)	Test diet (gari)	Test diet (rice + cyanide)
Water	709·6	727·3	709·6
Crude protein (nitrogen × 6·25)	129·0	131·3	129·0
Diethyl ether extract	46·0	53·0	46·0
Crude fibre	1·5	5·5	1·5
Energy (KJ/g)	252·7	266·5	252·7

expected that cassava-based diets and other cyanide-containing diets will retard muscle and bone growth. However, only limited information is currently available on the effects of cyanide on muscle and bone growth.

Hick (1950) reported that cyanide had no toxic effect on muscles. Tewe & Maner (1980) reported a slowing of bone development in pigs fed on dietary cyanide but that was only when it was accompanied by protein or iodine deficiency or both. The present study was, therefore, designed to investigate the effects of cassava-borne organic cyanide and inorganic dietary cyanide on muscle and bone development, as well as any differences in the effects of these forms of cyanide on muscle and bone development.

MATERIALS AND METHODS

Animals

Eighteen young dogs of local breed bought from the Orba and Ibagwa market in Nsukka, Anambra State of Nigeria, were used for the study. The dogs were dewormed and treated for ectoparasites immediately on acquisition and housed in individual metabolism cages under the same room conditions. They were aged by dentition (Table 2) as described by Miller *et al.* (1964).

Diet

All eighteen dogs were fed on a diet of boiled rice and meat (pork) at 100 g/kg body-weight per d and given water *ad lib.* for 16 weeks after which they were randomly assigned to three experimental groups each of six dogs. The dogs in group 1 were fed on the control diet of rice, those in group 2 on the test diet of gari, while those in group 3 were fed on the test diet of rice to which inorganic cyanide in the form of sodium cyanide had been added at 10·8 mg/kg diet to approximate the quantity of organic cyanide contained in the gari diet. All three diets were made isoenergetic and isonitrogenous by varying the quantity of meat incorporated in them. Table 1 shows the mean proximate composition of the three diets. The dogs were fed their respective diets at 100 g/kg body-weight per d and given water *ad lib.* for the period of the study.

Quantitative measurements

The body-weight and the approximate ages of each dog were determined at the inception of the study (Table 2). After 14 weeks of feeding the dogs their respective diets, the body-weight of each dog was again determined (Table 2). Each dog was then killed with an overdose of anaesthetic. The relative body growth rate (Table 2) was determined using the final body-weight for each dog divided by its weight at inception of the experiment (Goss, 1964).

Table 2. *Mean age (months) and body-weights (kg) of the dogs in the three experimental groups*

(Mean values with their standard errors)

Group ... Diet* ...	1 Rice		2 Gari		3 Rice + cyanicde		Statistical significance of difference
	Mean	SE	Mean	SE	Mean	SE	$P <$
Age at inception of study (months)	7·30	0·26	7·30	0·26	7·42	0·33	0·05
Initial body-wt (kg)	4·55	0·41	5·01	0·31	5·22	0·79	0·05
Final body-wt (kg)	8·28	0·45	8·63	0·55	7·63	0·57	0·05
Relative body growth rate	1·73	0·40	1·83	0·22	1·51	0·19	0·05

* For details, see Table 1 and p. 484.

The following muscles were carefully dissected out and the wet weight determined: biceps brachii and common digital extensor muscles from the fore-limbs, and the pectineus and cranial tibial muscles from the hind-limbs. The mean of the weights of each muscle from the right and left limbs was determined and used as the weight for the particular muscle. The muscle mass index or relative muscle weight (g muscle weight/kg body-weight) was also calculated for each muscle. This allometric variable was chosen because it yields a size-independent dimensional constant and, thus, allows comparison of organ weights in individuals of varying body-weights, both within and among mammalian groups, over a wide range of body sizes (Stahl, 1965).

The humerus, radius and ulna bones from the fore-limbs, and the femur, tibia and fibula bones from the hind-limbs were also dissected and cleaned of all muscles and tendinous attachments and their weights, lengths and diameters (maximum diameter at the centre) determined. The mean of the values for the right and left limb bones was used as the value for each bone in each dog.

Statistical analysis

Means with their standard errors were calculated for initial and final body-weights, relative body growth rate, the muscle weight, the muscle mass index (relative muscle weight) and the bone weight, length and diameter for the three experimental groups. Analysis of variance (F ratio) was used to evaluate the existence of statistically significant differences between the means of the three groups (Snedecor, 1965; Fisher & Yates, 1967). Fisher's t test was then employed to determine the pairs that differed significantly.

RESULTS

Animals

The dogs appeared clinically healthy for the duration of the study. The subcutaneous fat on the carcasses of the gari-fed dogs was observed visually to be much greater than those of the control (rice-fed) and rice plus cyanide-fed dogs.

Age and body-weight

The means of the ages of the dogs in each of the three groups at the inception of the study as well as their initial and final body-weights are presented in Table 2. These were similar for all the groups. The relative body growth rates were also similar for the three groups (Table 2).

Muscle analysis

The absolute wet weights of the four muscles studied were not significantly different for the three groups (Table 3). However, comparison using the muscle mass index revealed that the biceps brachii ($P < 0{\cdot}01$), common digital extensor ($P < 0{\cdot}01$) and pectineus ($P < 0{\cdot}05$) muscles of the gari-fed dogs were significantly smaller than those of rice plus cyanide-fed dogs. The muscle mass index of the pectineus ($P < 0{\cdot}02$) and cranial tibial ($P < 0{\cdot}05$) muscles of gari-fed dogs were also significantly smaller than those of control (rice-fed) dogs. Although the muscle mass index was also smaller in the biceps brachii and common digital extensor muscle of gari-fed dogs when compared with control dogs, the differences were not statistically significant (Table 3).

Bone analysis

There were no statistically significant differences in the bone weights, lengths and diameters in the three experimental groups (Table 4).

DISCUSSION

Although cassava is a very good source of energy and minerals, it is severely deficient in proteins and amino acids (Oke, 1966, 1968). This necessitates the supplementation of cassava-based diets with essential amino acids like methionine and lysine to help meet the body's need for both protein synthesis and cyanide detoxification through the rhodanese (thiosulphate sulphurtransferase; *EC* 2.8.1.1) pathway. Cassava-based diet is commonly fed with animal proteins like fish meal and meat. These have been found very suitable in single-stomached animals because they contain vitamin B_{12} which is helpful in the detoxification of cyanide (Oke, 1969), thus sparing methionine for other body uses. In the present study, an attempt has been made to minimize the effects of the low protein content of the gari diet by making the three diets isoenergetic and isonitrogenous by the addition of meat. This is confirmed by the lack of statistically significant differences in the relative body growth rates and the final body-weights of dogs in the three groups.

However, although differences between the final body-weights of the three groups were not statistically significant, the gari-fed dogs tended to be heavier than dogs in the other two groups. This apparent greater weight of the gari-fed dogs was associated with greater subcutaneous fat deposition. Similar findings of increased subcutaneous fat deposition have been reported in pigs fed on cassava-based diets (Kok & Robeiro, 1949; Modebe, 1963; Peixoto, 1968).

Judging by the absolute wet weight of the muscles, the three diets appeared to have produced similar effects on muscle development. However, comparison using the muscle mass index indicated that development was retarded in some muscles of the gari-fed dogs relative to either the rice-fed (control) or the rice plus cyanide-fed dogs (Table 3). Cyanide-containing diets cause an increase in serum and tissue thiocyanate levels as well as a decrease in serum thyroid hormone level (DeLange *et al.* 1980; Tewe & Maner, 1980; Ayangade *et al.* 1982). In another aspect of study involving these dogs, Kamalu & Agharanya (1991) observed evidence of hypothyroidism and goitre in the rice plus cyanide-fed but not in the gari-fed dogs. But the hypothyroidism observed in the rice plus cyanide-fed dogs was not associated with a reduction in muscle mass. Instead, and surprisingly, reduction of muscle mass was observed in the gari-fed dogs which showed no evidence of hypothyroidism (Kamalu & Agharanya, 1991), but rather had hypoinsulinaemia associated with low plasma concentrations of free amino acids, increased gluconeogenesis from protein, and histopathological evidence of pancreatic injury. Gluconeogenesis from protein

Table 3. *Effects of cassava (gari)-borne organic cyanide and inorganic cyanide on absolute muscle weight and muscle mass index of Nigerian dogs*

(Mean values with their standard errors)

Group …	1		2		3			Statistical significance of difference†: *P*			
Diet* …	Rice		Gari		Rice + cyanide		'F'	Group 1 *v.* group 2	Group 1 *v.* group 3	Group 2 *v.* group 3	
	Mean	SE	Mean	SE	Mean	SE	ratio				df
Muscle wt (g)											
Biceps brachii	10·43	0·71	10·46	0·89	10·73	0·91	0·04	> 0·05	> 0·05	> 0·05	10
Common digital extensor	3·28	0·39	3·29	0·21	3·65	0·15	0·65	> 0·05	> 0·05	> 0·05	10
Pectineus	3·02	0·20	2·80	0·23	3·07	0·24	0·39	> 0·05	> 0·05	> 0·05	10
Cranial tibial	10·04	0·63	8·72	0·97	8·45	0·45	1·41	> 0·05	> 0·05	> 0·05	10
Muscle mass index (g muscle/kg body-wt)											
Biceps brachii	1·36	0·06	1·21	0·04	1·42	0·05	4·99	> 0·05	> 0·05	< 0·01	10
Common digital extensor	0·42	0·03	0·39	0·02	0·49	0·03	4·65	> 0·05	> 0·05	< 0·01	10
Pectineus	0·40	0·02	0·33	0·01	0·41	0·04	3·77	< 0·02	> 0·05	< 0·05	10
Cranial tibial	1·34	0·14	1·01	0·05	1·13	0·07	3·82	< 0·05	> 0·05	> 0·05	10

* For details, see Table 1 and p. 484.
† Fisher's *t* test.

Table 4. *Effects of cassava (gari)-borne organic cyanide and inorganic cyanide on bone weights, lengths and diameters of Nigerian dogs*

(Mean values with their standard errors)

Group...	1		2		3		
Diet*...	Rice		Gari		Rice + Cyanide		Statistical significance of difference: *P*
	Mean	SE	Mean	SE	Mean	SE	
Bone wt (g)							
Humerus	26·16	1·54	24·61	1·33	27·26	1·52	> 0·05
Radius	11·39	0·89	10·40	0·71	10·97	0·87	> 0·05
Ulna	10·33	0·86	10·60	0·69	10·80	0·74	> 0·05
Femur	28·64	2·07	27·10	2·32	30·90	2·32	> 0·05
Tibia/fibula	24·75	2·34	22·74	2·34	25·14	1·90	> 0·05
Bone length (mm)							
Humerus	121·1	2·9	116·0	2·5	120·9	2·2	> 0·05
Radius	130·9	4·0	121·5	3·9	127·5	1·9	> 0·05
Ulna	155·1	5·0	143·7	4·0	151·3	3·0	> 0·05
Femur	137·9	3·3	132·2	3·2	139·2	2·7	> 0·05
Tibia/fibula	140·1	4·2	132·2	3·9	140·3	2·9	> 0·05
Bone diameter (mm)							
Humerus	11·1	0·4	11·4	0·2	11·9	0·4	> 0·05
Radius	8·5	0·3	8·4	0·3	8·1	0·4	> 0·05
Ulna	6·9	0·4	7·2	0·3	7·0	0·5	> 0·05
Femur	10·2	0·3	10·2	0·2	10·6	0·6	> 0·05
Tibia/fibula	8·6	0·4	8·7	0·3	8·6	0·3	> 0·05

* For details, see Table 1 and p. 484.

was five and two times greater in the gari-fed and rice plus cyanide-fed dogs respectively, relative to the rice-fed control dogs (Kamalu, 1991).

Although the reasons for the differences observed in the responses of the different muscles to either the gari or rice plus cyanide diets are not known for sure, the present findings, as well as the biochemical and histopathological differences previously reported in the gari- and rice plus cyanide-fed dogs (Kamalu, 1991; Kamalu & Agharanya, 1991), suggest that the metabolic fate and effects on muscle development of organic and inorganic dietary cyanide in the dog may differ. This agrees with the observations of Tewe & Maner (1981) on the effects of organic and inorganic cyanide on muscle development, but is contrary to the observations of Ermans *et al.* (1973) who reported that the metabolic fate of hydrocyanic acid closely resembles that of inorganic cyanide in single-stomached animals.

Cyanide-containing diets may be expected to depress protein turnover in tissues in at least two ways. First, the use of methionine for the detoxification of hydrocyanic acid to thiocyanate will deprive the body of this essential amino acid which is the body's source of methyl group, hence depressing protein turnover. Second, the thiocyanate produced is goitrogenic and decreases thyroid hormone production resulting in marked decreases in both protein synthesis and degradation, with the fall in synthesis being proportionally greater than the fall in degradation, leading to depression of protein turnover (Goldberg & Griffin, 1977; Goldberg *et al.* 1980; Brown & Millward, 1983; Hayase *et al.* 1987). A decrease in thyroid hormone level is also associated with a decrease in the levels of various lysosomal proteases like cathepsin B (*EC* 3.4.22.1) and D (*EC* 3.4.23.5) in the liver and muscles (DeMartino & Goldberg, 1978). However, the hypothyroidism observed in the rice plus cyanide-fed dogs was not associated with reduction in muscle mass suggesting that the rice plus cyanide diet probably contained enough dietary protein for both cyanide detoxification and other body needs as indicated by findings of the highest level of free plasma amino acids (phenylalanine and tyrosine) in this group (Kamalu & Agharanya, 1991). In addition, Goldberg *et al.* (1980) reported that an alteration of thyroid hormone level is usually associated with synchronous 2–3-fold alteration in the levels of a variety of lysosomal enzymes, and suggested that this may be responsible for the various unexplained effects of the thyroid hormone in disease conditions.

On the other hand, insulin normally depresses gluconeogenesis, and low insulin secretion decreases protein synthesis and increases catabolism and gluconeogenesis from muscle protein (Wright *et al.* 1956; Felig *et al.* 1977). The smaller muscle mass of gari-fed dogs may, therefore, have resulted from the increased gluconeogenesis sequelae to hypo-insulinaemia. Atrophy of muscle fibres has also been reported in diabetic amyotrophy in man (Garland, 1955; Dubowitz & Brooke, 1973). Increased gluconeogenesis from protein may also have contributed, at least partially, to the increased subcutaneous fat deposition observed in the gari-fed dogs. Maynard *et al.* (1979) reported that the ability of the liver and other tissues to store sugar as glycogen is limited and that when the carbohydrate available exceeds the current need of the body for energy purposes sugar is transformed into fat.

It was also observed in the present study that the effect of the test diets varied between muscles of both the fore- and hind-limbs. The gari diet retarded development in the pectineus and cranial tibial but not in the biceps brachii and extensor digitorum longus muscles relative to the control rice diet, while the rice plus cyanide diet did not. However, comparing the gari and rice plus cyanide diets, the former retarded development in all the muscles studied except in the cranial tibial. The reasons for these differences are not clear. In the rat, Brown *et al.* (1981) reported that the plantaris and gastrocnemius muscles were more responsive to the hypothyroid state than the soleus muscle which was more responsive to the hyperthyroid state. They attributed this to the muscle fibre types predominating in these muscles; the gastrocnemius and plantaris being composed predominantly of fast-twitch glycolytic fibres and the soleus of slow-twitch oxidative fibres. The four muscles studied, the biceps brachii, common digital extensor, pectineus and cranial tibial, are all composed predominantly of fast-twitch oxidative glycolytic fibres (Armstrong *et al.* 1982). It is, therefore, difficult to relate the present findings in the gari-fed and rice plus cyanide-fed dogs to the fibre-type composition of these muscles. However, these differences may, probably, not be attributable to the fibre-type composition of individual muscles as is the case for either hypothyroidism or hyperthyroidism in rats, because the retardation of muscle mass in the gari-fed dogs observed in the present study was associated, not with hypothyroidism, but with hypoinsulinism. Second, Dubowitz & Brooke (1973) reported that atrophy of muscles in diabetes amyotrophy affected all muscle fibre types. Although the muscle fibre sizes were not determined in the present study, the observations made tend to resemble those in Duchenne dystrophy or myotonic dystrophy where there was no simple correlation between the fibre-type composition of individual skeletal muscles and their reaction to a disease, as observed in the present study.

Bone development as indicated by absolute bone weight, length and diameter was not significantly different in the three experimental groups. This indicated that the cassava-borne organic cyanide and the dietary inorganic cyanide did not have any deleterious effects on bone development. This agrees with the findings of Tewe & Maner (1980) in pigs that cyanide did not affect bone development unless there was concurrent iodine or protein deficiency, or both, at which time retardation of bone development was observed.

In conclusion, the present study has shown that in the dog and in the presence of adequate protein supplementation a gari diet caused retardation of muscle development, possibly by depressing insulin production and increasing gluconeogenesis from muscle protein. The diet of rice containing an equivalent quantity of inorganic cyanide in the form of sodium cyanide did not retard muscle development relative to the control diet of rice. The difference in muscle development was only apparent when the allometric variable of muscle mass index was used for comparison. The cassava-borne organic cyanide and dietary inorganic cyanide had the same effect on bone development as the control diet.

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