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BIOAVAILABILITY OF ZINC AND COPPER IN RATS FED ON NIGERIA PEASANT DIETS.

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ABSTRACT

Bioavailability studies on zinc and copper from three different rural Nigerian peasant diets in Wistar albino rats were carried out. Serum levels of minerals were used to assess bioavailablity. The mineral composition of diets studied ranged between 0.94 and 1.79mg/100g for zinc and between 2.19 and 1.62mg/100g for copper. The serum zinc levels ranged between 1.90 and 3.54mg/l for the test groups and 3.67 and 5.54mg/l for the control groups. On the analysis of the effect of the diets on zinc bioavailability there was no significant difference ($p>0.05$) between group 2 fed on "foofoo and edikang ikong" which had the lowest level and group 3 fed on "ekpan nkukwo" which had the highest serum zinc value. There was a significant difference ($p<0.05$) between the test groups and the control groups fed on semi-synthetic basal diet. The serum copper level (6.54mg/l) was highest in the animals fed on "foofoo and edikang ikong" soup while those fed on "ekpan nkukwo" had the least (2.68mg/l) serum copper level. The values for the control groups ranged between 1.92 and 3.84mg/l. There was a significant difference in copper bioavailability between the test groups and the control groups using the serum copper level. In general when compared with the zinc and copper from the control diets, the uptake of zinc and copper in most of the diets was marginal although copper was more available.

KEY WORDS: Zinc, Copper, Bioavailability, Diet

INTRODUCTION

Diet is a primary source of zinc and copper for man. Several regulating systems affect the digestion and absorption of zinc and copper in animals and also their levels in the blood and other tissues. For instance, the rate of absorption which may vary from one species to another (Underwood, 1977) is dependent on the diet and its protein and amino acid contents (Stuart, *et al* 1986).

Individuals on low income diet are at risk from marginal or inadequate zinc nutrition because the best dietary sources of the mineral are the more expensive foods especially meat (Sanstead, 1973).

Unlike in developed countries where diets rich in meat are readily available, developing countries thrive more on diets of plant origin. These diets are poor sources of zinc and copper. Even when the elements are present in the diets such antinutrient as phytate which is also present, interact with mineral elements and reduce the availability. Bioavailability of zinc and copper from local foods in Nigeria has not been studied. It therefore becomes necessary to study Nigerian peasant diets to determine the bioavailability of zinc and copper from them.

MATERIALS AND METHODS

Collection and preparation of diets

The different foodstuffs used in preparing the meals were purchased from the local market in Calabar Municipality, in Cross River State, Nigeria. Most of the root crops, tubers and vegetables were grown in villages around Calabar, the capital city of the Cross River State of Nigeria. Garria (fried cassava flour) was bought as sold in the market.

The weights of the various condiments for the preparation of the meals were taken using triple balance (Ohaus, 2610g capacity, USA).

The methods of Umoh (1972) were used for the preparation of the different diets. Three diets commonly eaten by the people in the South Eastern Nigeria (Akwa Ibom and Cross River States) were prepared and used in the studies. These included:

Diet 1: Garri, (fried cassava flour) (*Manihot esculenta)* and "afang" (*Gnetum africanum*) soup with smoked fish.

Diet 2: Cassava "foofoo" (cooked fermented cassava) (*Manihot esculenta*) and "edikang ikong (thick vegetable) (*Telfaria spp*) soup.

Diet 3: Ekpan nkukwo (grated cocoyam)

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(*xanthosoma mafaffa shoot*) and dried shrimps.

Each diet was separately mixed and dried at 60°C in air drought oven (Gallenkamp) for 24 hours. The dried materials were ground into powder using a steel bladed grinder and kept in plastic containers. The containers were then stored in the refrigerator from where representative portions of samples were taken for various analyses.

Selection and grouping of animals

Thirty weanling albino rats of Wistar strain bred in the animal house of the Department of Biochemistry, University of Calabar, Nigeria, were used. These were divided into six groups of five rats each. The animals were all housed in Perspex cages (North Kent Plastic cages Ltd., England) with stainless steel top and bottom grid.

Feeding of animals

Three of the groups of the animals were fed on the test diets. Three control diets prepared from ingredients (Table 1) viz: complete basal diet, a zinc deficient basal diet and a copper deficient basal diet. The zinc deficient basal diet was formulated by modifying the mineral mix of Seal and Heaton (1983) while the copper low basal diet was formulated by modifying the mineral mix of Cunnane *et al* (1990). Deionized water was given *ad libitum*.

Food intakes were measured and faecal collections made for 10 days (Fairweather-Tait, 1987). Faeces were analyzed for zinc and copper contents. At the end of the experiment, the animals were sacrificed using chloroform anaesthesia.

Collection procedures

Faeces and spilled diets were collected from trays under the cage of each group for 3 days ie days 5,6,7. Feaces collected were rinsed with deionized water, air dried and kept in polythene bag and stored in a freezer, until analyzed. Blood sample (about 5ml) each were collected from the right heart venticle of each animal for determination of the serum zinc and copper.

Proximate and antinutrient composition

Crude protein, crude fat, moisture, ash and crude fibre of diets were determined according to AOAC (1985). The carbohydrate was calculated as 100-(fat + protein + ash + crude fibre). The method of McCance and Widdowson (1953) was used to determine the phytic acid composition of diet while the method of Burns (1971) was used for the determination of the tannin.

Mineral determination

Zinc and copper contents were determined using Pye Unicam atomic absorption

Table 1:Composition of the basal diet

Ingredient	Composition in g/kg of diet
Casein	200
Sucrose	600
Cellulose fibre	50
Vegetable oil	100
Vitamin mixture[1]	10
Mineral mixture[2]	40

[1] Vitamin mix contained vitamin A concentrate (200,000 units/g) → 4.5g/kg: Vitamin D concentrate (400,000 units/g) → 0.25g/kg) Tocopherol → 5.00g/kg; Ascorbic acid → 45.00g/kg; Inositol → 5.0g/kg, Choline Chloride → 75g/kg, Menadione → 2.25g/kg, Para amino benzoic acid → 5.00g/kg, Niacin → 4.50g/kg, Riboflavin → 1.00g; Pyridoxine hydrochloride → 1.00g/kg, Thiamine → 1.00g/kg, Calcium Pantothenate → 3.00g/kg, Biotin → 20.00g/kg, Folic acid → 90.00g/kg and Vitamin B_2 → 1.35g/kg.

[2] Mineral mix contained NaCl → 143.808g/kg, $KHPO_4$ → 400.608g/kg, $MgSO_4$ → 59.578g/kg, $CaCO_3$ → 390.336/kg, Manganese Sulphate → 4.1088g/kg KI → 0.8276g/kg, Zn SO_4 → 0.5648g/kg, Copper Sulphate → 0.5136g/kg, Cobalt Chloride → 0.02568g/kg, $FeSO_4$ 7H2O → 0.01275g/kg.

Table 2: Proximate composition, antinutrient, zinc and copper contents of the Nigerian peasant diets

	Diet 1	Diet 2	Diet 3
Moisture content (%)	66.53 ± 0.03	73.85 ± 0.02	64.00 ± 0.02
Ash content (g/100g)	6.51 ± 0.08	5.00 ± 0.02	11.00 ± 0.01
Crude protein (g/100g)	15.31 ± 0.02	9.63 ± 0.02	13.13 ± 0.01
Crude fat (g/100g)	19.40 ± 0.10	18.00 ± 0.15	38.00 ± 0.01
Crude fibre (g/100g)	3.60 ± 0.10	4.20 ± 0.10	4.76 ± 0.05
Carbohydrate (g/100g)	55.17 ± 0.06	63.14 ± 0.21	33.30 ± 0.50
Energy value (kcal/100g)	456.68 ± 0.98	453.20 ± 0.45	528.94 ± 2.52
Zinc (mg/100g)	1.37 ± 0.01	0.94 ± 0.00	1.79 ± 0.01
Copper (mg/100g)	1.62 ± 0.00	1.46 ± 0.00	2.19 ± 0.01
Phytate (mg/100g)	13.17 ± 0.74	7.69 ± 0.81	19.21 ± 0.72
Tannin (mg/100g)	0.40 ± 0.09	0.44 ± 0.06	0.41 ± 0.05

Mean of 3 determinations ± SD

NB: Diet 1: Garri and "afang" soup with smoke fish

Diet 2: Foofoo and "edikang ikong" soup

Diet 3: Ekpan nkukwo and dried shrimps

Table 3: Zinc balance in the rats fed with the different diets for 10 days

Groups	Average zinc intake (mg)	Average feacal zinc excreted	% Excreted	Zn retention	% Zn retention
1.	1.37 ± 0.01	0.46 ± 0.01	33.57%	0.91 ± 0.01	66.42%
2.	0.94 ± 0.00	0.51 ± 0.00	54.26%	0.43 ± 0.01	45.74%
3.	1.79 ± 0.01	0.77 ± 0.01	43.02%	1.02 ± 0.01	56.98%
4.	1.72 ± 0.01	0.69 ± 0.01	40.12%	1.03 ± 0.01	59.88%
5.	1.66 ± 0.00	0.71 ± 0.01	42.77%	0.95 ± 0.01	57.23%
6.	1.26 ± 0.01	0.48 ± 0.01	38.10%	0.78 ± 0.01	61.90%

Groups

1: Rats fed on garri and afang soup with smoked fish

2: Rats fed on foofoo and edikang ikong soup

3: Rats fed on ekpan nkukwo with shrimps

4: Rats fed on the complete basal diet

5: Rats fed on the copper deficient basal diet

6: Rats fed on the zinc deficient basal diet

composition, zinc and copper contents of the diets were determined and are presented in Table 2. The protein contents were generally low. The fat content of "ekpan nkukwo" (diet 3) was much higher, 38.00g/100g than in the other diets studied. The carbohydrate content was higher for the other two diets. The antinutrients composition was generally low. The zinc and copper contents were moderately high.

Animal experiment

The balance experiment showed that the animals fed on diet 1, (garri and 'afang' soup with smoked fish) group 1, showed the highest percentage of zinc retention, 66.42%, while the lowest value was from the group fed on diet 2, (foofoo and edikang ikong' soup) group 2, with a value of 45.74%. The percentage retention of zinc by animals fed on diet 3 ('ekpan nkukwo') group 3, spectrophotometer. The test diets and the faeces were digested using perchloric acid (25:5 w/v) and concentrated nitric acid at 130°C for 8 hours. The serum was treated with perchloric acid (25:5 w/v) and concentrated nitric acid left to stand over night. Zinc and copper were determined by atomic absorption spectrophotometry (Pye Unicam) in all cases.

Table 4:Copper balance in the rats fed with the different diets for 10 days

Groups	Average copper intake (mg)	Average feacal copper excreted	% Excreted	Cu retention	% Cu retention
1.	1.62 ± 0.00	0.67 ± 0.001	41.36	0.95 ± 0.00	58.64%
2.	1.46 ± 0.00	0.60 ± 0.01	41.09	0.86 ± 0.01	58.90%
3.	2.18 ± 0.01	0.71 ± 0.01	32.56	1.47 ± 0.01	67.43%
4.	2.54 ± 0.01	1.01 ± 0.01	39.76	1.53 ± 0.01	60.24%
5.	1.83 ± 0.01	0.64 ± 0.01	34.79	1.19 ± 0.01	65.03%
6.	2.50 ± 0.00	0.94 ± 0.01	37.60	1.56 ± 0.01	62.40%

Groups

1: Rats fed on garri and afang soup with smoked fish
2: Rats fed on foofoo and edikang ikong soup
3: Rats fed on ekpan nkukwo with shrimps
4: Rats fed on the complete basal diet
5: Rats fed on the copper deficient basal diet
6: Rats fed on the zinc deficient basal diet

Statistical analysis

Single factor Analysis of Variance and Fisher's Least Square Difference were performed to ascertain if there was a significant difference in parameters between pairs of experimental groups. Significance was accepted at $p<0.05$.

RESULTS

The proximate analyses antinutrient was 56.98%. For the control groups, the values ranged between 57.23 and 61.90%.

For copper, diet 3, ('ekpan nkukwo'), had the highest percentage of copper retention, 67.43% while the least was 58.64% observed in diet 1, garri and 'afang' soup with smoked fish. For the control groups the values ranged between 60.24 and 65.03%. Table 3 and 4 show these observations.

Difference in serum contents of zinc and copper

Serum zinc and copper levels (mg/l) of the experimental animals are summarized on Table 5. The highest value for serum zinc for the test animals was from the group fed on 'ekpan nkukwo' (group 3) with a value of 3.54 ± 0.41mg/l while the least was from those fed on 'foofoo and edikang ikong' soup (group 2) with a value of 1.90 ± 0.22mg/l. For the control groups the highest value for serum zinc was from the group fed on copper deficient basal diet (group 5) with a value of 5.45 ± 0.13mg/l. Among the control animals, the least serum zinc was observed from the group fed on the zinc deficient basal diet (group 6) with a value of 3.67 ± 0.15mg/l.

A value of 6.54 ± 0.18mg/l which was the highest value for serum copper was from the test group fed on 'foofoo and edikang ikong' soup (group 2). The least value from the test group of 2.68 ± 0.45mg/l was recorded for the group fed on 'ekpan nkukwo'. The control groups had values ranging between 1.92 ± 0.41mg/l and 3.84 ± 0.21mg/l.

DISCUSSION

The bioavailability of zinc and copper from the diets were studied by comparing the serum levels of zinc and copper of the rats fed with the

Table 5: Serum zinc and copper levels of the experimental rats (mg/l)

Groups	Serum zinc	Serum copper
1.	2.66 ± 0.09	4.54 ± 0.04
2.	1.90 ± 0.22	6.54 ± 0.18
3.	3.54 ± 0.41	2.68 ± 0.45
4.	4.11 ± 0.08	1.92 ± 0.41
5.	5.45 ± 0.13	3.84 ± 0.21
6.	3.67 ± 0.15	2.92 ± 0.31

Groups

1: Rats fed on garri and afang soup with smoked fish
2: Rats fed on foofoo and edikang ikong soup
3: Rats fed on ekpan nkukwo with shrimps
4: Rats fed on the complete basal diet
5: Rats fed on the copper deficient basal diet
6: Rats fed on the zinc deficient basal diet

test diets with those fed on the control diets (semi synthetic diets) formulated as shown on Table 1. Garri and 'afang' soup with smoked fish (diet 1) and 'ekpan nkukwo' with shrimps (diet 3) showed a much higher bioavailability of zinc (serum response). These two diets contained high protein and low fibre levels compared with the other diet. This may explain the observed high serum zinc levels. The report of this study is in good agreement with the observation of other researchers (Sandstrom and Ceberbald 1980, Rao and Rao, 1980) and supports their similar observation that high levels of protein and low levels of fibre, in the diet enhance the bioavailability of zinc in such diets. Serum zinc content of the group fed on the zinc deficient basal diet was still more than those of test groups with a higher zinc availability. Although there is a dearth of information on the bioavailability of copper, from Nigerian peasant diets, which would have formed a basis for comparism, copper bioavailability was highest from foofoo and 'edikang ikong' soup. Copper was more available from garri and 'afang' soup with smoked fish and foofoo and 'edikang ikong' soup than the control diets.

The zinc retained from the meals ranged between 66.42% and 45.74%. These observations were in agreement with those of another worker (Fairweather-Tait, 1997) who observed that the absorption of zinc from diets was less than 15 – 50% (<15–50%) depending on the diets. However, this high retention rate is not enough to indicate high bioavailability of zinc. Lee *et al* (1989) had shown that when the body is at risk of zinc deprivation the intestinal absorption of exogenous zinc is increased and there is a reduction in intestinal absorption of endogenous zinc arising from intestinal conservation.

The values observed for copper, between 67.43% and 58.90% showed a high absorption of copper.

CONCLUSION

The assessment of bioavailability of these minerals showed that while copper was much more readily available in these diets this was not the case with zinc. Zinc bioavailability was marginal in all the diets studied. These observations might account for the rare incidence of copper deficiency and a higher rate of zinc deficiency in this country. The variations observed in the bioavailability among diets could be due to the chemical forms of the minerals in the diets, individual difference among the animals and the amount of fed intake by the animals.

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