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Research Article

Zinc Bioavailability in Selected Cereals Grown in Nasarawa State, Nigeria

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Abstract

The aim of the study was to determine the concentrations of Zinc in selected cereal crops from various Local Government Areas in Nasarawa State, Nigeria and also to determine its bioavailability using albino rats. The dried cereals; *Oryza sativa, Zea maize* and *Sorghum bicolor* were collected from farms in seven local government areas of Nasarawa State, Nigeria. The cereals were processed and Zn concentrations of the samples from each local government determined using standard methods. Afterwards, the rats were divided into two groups of ten rats each, a control group and a test group. The cereals were then processed and fed the experimental group of the albino rats for seven days before blood samples were collected and the plasma separated, then zinc concentration determined. Results showed that Karu LGA had the highest Zn concentration in *O. Sativa* (10.34 mg/g), Nasarawa-Eggon had the highest Zn concentration of 16.72mg/g in *Z. mayz*, and the highest Zn concentration of 17.98mg/g in *Sorghum bicolor*. Statistical analysis of the bioavailable Zn however, showed a non-significant ($p > 0.05$) decrease in Zn bioavailability in the experimental group when compared to the control group. The results of this research therefore indicates that the Zn concentrations in the various cereals obtained from the selected LGAs of Nasarawa State was not bioavalable when the cereals were consumed by the rats hence the need to up Zn concentrations and bioavailability through supplementation to avoid its deficiency among the populace.

Keywords: Zinc; Bioavailability; *Oryza sativa; Zea maize; Sorghum bicolor*

Introduction

Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis of and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. It plays a central role in the immune system, affecting a number of aspects of cellular and hormonal immunity [1]. It also plays an important biochemical roles as a component of zinc metalloenzymes and proteins such as DNA polymerase, carbonic anhydrase, lactate dehydrogenase, and protein chain elongation factor [2]. Some zinc metalloenzymes are; Carboxypeptidase (hydrolysis of C-terminal residues), leucineaminopeptidase (Hydrolysis of N- terminal residues), DNA-polymerase (DNA replication), peptidases (hydrolysis of peptides), superoxide dismutase (antioxidant activity), phospholipase C (hydrolysis of phospholipids), alkaline phosphatase (hydrolysis of phosphate esters), carbonic anhydrase (hydration of CO_2/maintenance of acid-base balance), α-amylase (hydrolysis of glucosides), alcohol dehydrogenase (hydride transfer from alcohol to NAD^+ in alcohol metabolism), lactate dehydrogenase (irreversible reaction of pyruvic acid to lactic acid) [3]. Although zinc-dependent biochemical mechanisms in physiologic functions have received extensive study, clear relationships have not been fully defined. Zinc is ubiquitous

within cells in contrast to iron, which is contained in defined cellular components and has defined physiological roles. At the cellular level, the functions of zinc can be categorized into three general functional classes, namely catalytic, structural and regulatory functions [4,5].

Zinc deficiency disease is prevalent in many parts of Nigeria [6] including nasarawa state some of the examples are acrodermatitis enteropathica, dwarfism and hypogonodism. The effects of marginal or mild zinc deficiency are less clear. A reduced growth rate and impairments of immune defense are so far the only clearly demonstrated signs of mild zinc deficiency in humans. Other effects such as impaired taste and wound healing, which have been claimed to result from a low zinc intake, are less consistently observed [7]. The general causes of zinc deficiency include inadequate intake, increased requirements, malabsorption, increased losses and impaired utilization [8]. Primary zinc deficiency syndromes may be due to diets poor in zinc [9]. Acrodermatitis enteropathica, characterized by the triad of dermatitis, diarrhea, and alopecia occur as autosomal recessive disorder due to mutations in the gene (SLC39A4) that codes the zinc transporter protein, ZIP4. It also can occur in an acquired form in exclusively breastfed infants due to mutation in the zinc transporter gene SLC30A2 (ZincT-2), responsible for transfer of zinc from serum to breast milk, in their mothers [10,11]. Secondary zinc deficiency occurs in various conditions like malabsorption syndrome, cirrhosis of liver, chronic renal disease, total parenteral nutrition, sickle cell disease, diabetes, malignancies, other chronic disorders and drug therapy with penicillamine, anticonvulsants and ethambutol [12]. This may result from low dietary intake or heavy reliance on foods with little or poorly absorbable zinc. Inadequate dietary zinc intake is common in many parts of the world. It is often exacerbated by physiologic conditions associated with elevated zinc requirements [9,13].

Zinc deficiency diseases can be prevented by eating Zn rich foods such as eggs, liver, and kidney of animal, seafood, milk, cheese, nuts, meat, poultry, whole grain cereal (Rice, wheat, maize and millet among others) and refined cereals grains. However, the staple diets are rich in anti-zinc constituents [14], sources other than staple foodstuffs may be important alternative sources. Onions, pumpkin seeds, dark chocolate, garlic, watermelon seeds are also rich sources of zinc, nuts spinach, seafood such as cooked oysters, mushrooms, legumes and grain cereals (especially bran and germ) have relatively high zinc content, while tubers, refined cereals, fruits and vegetables have less [15]. Most cereals contain vitamins and minerals with all the essential amino acids needed by man except for glycine and tryptophan, and when consumed with other food items can supplements for the nutrients or even those lacking in the cereals [16]. Zinc from foods of animal origin has higher bioavailability than from food of plant origin due to the presence of fibre and phytate that inhibit zinc uptake by the intestine [17].

Bioavailability refers to the fraction of intake that can be absorbed into the blood system and used for physiologic functions of the body. For zinc, in healthy individuals, it is determined by three factors: the individual's zinc status, the total zinc content of the diet, and the availability of soluble zinc from the diet's food components [18]. Various dietary factors can influence zinc absorption. Phytic acid (inositol hexa- and pentaphosphate) is the principal dietary factor known to limit zinc bioavailability by strongly binding zinc in the gastrointestinal tract [19].

Materials and Methods

Materials

Cereal crops

Dried healthy samples (cereals) were harvested on farmlands in various Local Government Areas of Nasarawa state from the month of February to May 2018, the cereal foodstuffs used are *Zea maize, oryza sativa* and *Sorghum bicolar* from seven different local governments in Nasarawa state: Doma, Nasarawa, Obi, Karu, Nasarawa-Eggon, Wamba and Kokona. From each local government listed, the three different species of the cereals was obtained from the local farmers and then taken to Chemistry Laboratory, Sheda science and technology complex (SHETSCO), Abuja, for quantitative determination of zinc (*in vitro*). The results obtained was used to select the cereals with highest zinc concentration and then grounded (in the same ratio) using cereals grinding machine (model 9F320) into the size consumable by the rats, was used as the experimental feeds for the rats while the normal feeds obtained from grand cereals and oil mills limited was used as the control and both the two feeds (experimental and the control) were fed for ten days. The blood of the two groups obtained by decapitation was then taken to the federal collage of animal health and production technology, Vom, Jos for identification of the bioavailable zinc. The zinc was identified by Salisu Mohammed of the department of biochemistry. A sample of each crop was deposited at the departmental herbarium for reference purposes.

Experimental animals

Twenty white albino rats were purchased from the Animal House of the University of Jos were used for the experiment. The rats were randomly distributed into two groups (ten per group), weighing between 38 to 55g and taken to the biochemistry and molecular biology departmental animal house, NSUK. They were caged in clean metabolic cages and acclimatized for thirty days after which they were starved for twelve hours before the feeding with the experimental sample commenced; the rats were given feeds and water ad libitum during the acclimatization period.

The faeces and urine were collected separately to avoid mixing with the feeds. Sawdust was also used which helps in the absorption of the urine, the cage was sanitized at least once in a week and the sawdust was changed during the period.

Instruments/Equipment's

The instrument/ equipment used for the experiment include; surgical blade(s), serviette, EDTA-coated collection tube (Ethylene diamine tetra acetic acid), centrifuge, micro pipette, refrigerator, electric hot plate or equivalent-adjustable and capable of maintaining a temperature of 90.95oC, and glass wares such as Griffin or Pyrex beaker, watch glasses, qualitative filter paper and filter funnel, graduated cylinder. Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer (Model AE 1661000) (AAS) was used in the analysis of zinc. MSC Mistral 2L refrigerated Ultra centrifuge and MSE benchtop centrifuge were employed in centrifugation procedures. Shuanghe Hot sale corn Hammer mill/cereals grinding machine (model 9F320) was used to process the cereal samples into a size consumable by the rats.

Chemicals/reagents

All the chemicals and reagents used for the experiment were of analytical grades. They include; diethyl ether was purchased from Thomas Baker (Chemicals) Ltd., Bombay. Nitric acid and hydrochloric acid purchased from British Drug House (BDH) Chemical Limited, Poole, England, perchloric acid from May and Baker(M&B) Laboratory Chemicals, Dagenham, England.

Methods

Processing of the Cereal Crops

Each sample was thrashed or de-husked to obtain the seed kernel, and then taken in a different polyethene bags until further processing. For the zinc chemical analyses, all collected samples were transported to the department of biochemistry and molecular biology laboratory, Nasarawa state university keffi, where they were grounded to a fine homogenous powder using a locally made (wooden) motar and pestle. The grounded cereals were air dried and taken to the chemistry laboratorysheda science and technology complex (SHETSCO) at kwali, Abuja for quantitative determination of zinc using atomic absorption spectrophotometer UNICAM969, Shimdazu instrument (shimdazu corporation, chiyodaku, Tokyo, Japan).

Experimental design

A total of twenty (20) rats were used for the experiment and the animals were divided into two groups, ten in each group. The first group was the experimental (test) group and the animals in the group were fed with the cereals (*Oriza sativa* from karu, *Zea maize* and *Sorghum bicolor* from Nasarawa-Eggon) in the same ratio and grounded using cereals grinder into a size consumable by the rats. And the second group is the control which grand cereals and oil mills was used as their feed. They rats were acclimatized for thirty days before the experimental feeding period commenced. The experimental feeding period lasts for ten days, the rats were sacrificed by decapitation at the end of the feeding period. The blood samples was collected for each rat in a separate container (EDTA-coated bottle), and centrifuged to obtain plasma. The bioavailable zinc was quantified at the Federal College of Animal Health and Production Technology, Vom, Jos-Plateau, State, Nigeria.

In vitro Zinc analysis

The cereal samples were prepared according to the method by Kheiljazkor and Nelson (1996). 3g of the grounded cereals each were weighed on the balance and then transferred into a conical flask and 20ml of nitric acid was added and placed on a hot plate inside a fume hood and boiled until the solution became clearer (complete digestion). The conical flask was then dropped from the hot plate and allowed to cool, the solution is then filtered into a 50ml volumetric flask and made up to volume with de ionized distilled water. The resultant ash was cooled was cooled to room temperature and digested to a clear solution with 5ml of HNO3 in a digestion flask. The residue was then filtered into a calibrated 50ml volumetric flask using whatman No.41 filter paper. The solution was made up to the volume with de ionized water (i.e 50ml). The total concentration of the metal in the digest was determined by aspiring the solution into atomic absorption spectrophotometer (Buck model 210A).

Collection of blood sample

Eight hours prior to the termination of the feeding experiments, the feeds were removed at the end of day ten. The rats were anesthetized with diethyl ether and the blood was collected from each rat into a trace element free EDTA-coated collection tube. The plasma was obtained for each sample collected by centrifugation at 5000rpm for 15minutes in a refrigerated centrifuge. The aliquots (plasma) measuring about 1.5ml, was carefully transferred using micropipette into clean, dry labeled sample container (plain tube) and stored frozen in a refrigerator until analysis.

In vivo zinc analysis

Plasma sample were prepared for atomic absorption Spectrophotometry (AAS) analysis according to a procedure based on the method of Price (1972), Wilson and Walker (1995), and Williams and Wilson (1979). The instrumental flame conditions for the element analyzed is at 213.8nm wavelength, 1.3 slit widths, 10mA lamp current and oxidant (air) of 9.4 L/min. All atomic absorption spectrophotometric measurements were carried out with a Hitachi AE 166100 spectrophotometer which is equipped with data processing unit, a strip chart automatic recorder, a hollow cathode lamp, graphite furnace heating system, and argon gas was used as nebulizer gas. The calibration curve was prepared with standard solutions for atomic absorption. Zero reference reading was prepared for all reagents with the same procedures as for sample digestion. The linearity of the curve for zinc indicated outstanding precision. This model of the atomic absorption spectrophotometer (AAS) has provisions for flame and flameless procedures of elemental concentration determinations. The flame technique was used for the determination of Na, Mg, Zn, Ca and Fe which were the cations of interest. The flameless method can be used for cations that are usually found in very low concentrations in biological fluids eg Cd, Pb, Cu and Co.

In the two techniques, the flame and the graphite atomizer (air-acetylene flame) are subjected to a strong magnetic field during atomization of the element of interest. This produces azee man effect on the atomic vapor of the element. The energy emitted from a hollow cathode lamp is thus split into two arrays-one parallel (P//) and the other perpendicular (P⊥) to the magnetic field. The two beams are affected by the lights cattering and broad band molecular absorption while the beam parallel is affected additionally by sample absorption. Electronic subtraction of P⊥ from P// gives the true absorption of the sample. Thus zinc was determined by direct aspiration of the sample into an air-acetylene flame.

Procedure

An aliquot of plasma sample (1.0ml) was accurately measured and transferred into 100ml labeled dry pyrex beakers. The sample is acidified with the digestion mixture (conc. Nitirc acid: Perchloric acid, 6:1v/v mixture) allowed for 3-4mins before adding 2ml of sulfuric acid and allowed for 1-2 hrs so that the colour observed can become colorless, covered with a ribbed watch glass or other suitable covers. The resulting mixture was heated on a steam bath, hot plate or other heating source at 90-95^{0}C until the volume has been reduced to 15-20 ml(in a fume cupboard and heated slowly at first until frothing ceased). Heating continued at 600^{0}C, with the addition of more of the digestion mixture until white residue was obtained. After the ashing, it is allowed to one to two hours. The beaker containing the white residue (ash) was allowed removed and allowed to cool, then re-dissolved in 5ml of distilled water and transferred accurately into quantitatively dry labeled trace element-free tubes with a cap, more distilled water (5ml) was used to rinse the beaker free of all traces of ashes and to make up 10ml.this solution is stored as stock solution of each digested sample at 10^{0}C until AAS analysis. The sample is then filtered through a 0.45µm filtered and incubated at 37^{0}C and allowed to cool. The sample is spinned with a centrifuge to obtain the decadent. 5ml is used and calibrated with 100ml/l. The zinc was obtained by direct aspiration of the sample into an air-acetylene flame. With the Hitachi model AE-166100, the concentration of the zinc was calculated directly in parts per million (PPM) using a 180-0205 data processing unit. Results obtained in ppm were converted to µg/dl samples.

Statistical analysis

The data obtained were reported as mean ± standard deviation (SD) with the aid of software known as SPSS (statistical product and service solution) version 23.0. Test for levels of significance, was further performed using LSD (least significant difference) and Duncan tests, all the levels of significance were set at $p< 0.05$.

Results and Discussion

Results

Zinc concentration in cereal food stuffs grown in Nasarawa state

Table 1 below shows the highest Zn concentration in *Oryza sativa* from Karu local government area (10.34), followed by *Oryza sativa* from Kokona (5.74 mg/g), Nasarawa-Eggon (5.58 mg/g) and Doma (5.58 mg/g), Wamba (3.92 mg/g), Toto (3.85 mg/g) and Obi (3.02 mg/g). For *Zea maize*, the highest concentration was ob-

served in Nasarawa-Eggon (16.72) followed by Obi LGA (12.64), Toto (12.63 mg/g), Doma (12.51 mg/g), Wamba (10.59 mg/g), Karu (9.06 mg/g) and Kokona (5.74 mg/g). The concentration of Zn in *Sorghum bicolor* was observed to be highest in Nasarawa-Eggon (17.80) followed by Karu (17.73 mg/g), Wamba (17.35 mg/g), Toto (14.57 mg/g), Kokona (14.08 mg/g), Obi (12.21 mg/g) and Doma (10.38 mg/g).

Local government	*Oryza sativa* (mg/g)	*Zea maize* (mg/g)	*Sorghum bicolor* (mg/g)
Doma	5.58	12.51	10.38
Toto	3.85	12.63	14.57
Obi	3.02	12.64	12.21
Karu	10.34	9.06	17.73
Nasarawa-Eggom	5.58	16.72	17.80
Wamba	3.92	10.59	17.35
Kokona	5.74	5.74	14.08

Table 1: Zinc concentration in cereal food stuffs grown in Nasarawa state.

Bioavailability of Zinc in Rats

As shown in table 2 below, the mean plasma Zn concentration of rats in the experimental group was lower than that of the control group

S/N	Plasma Zn conc. (µg/dl) Experimental	Plasma Zn conc. (µg/dl) Control
1	0.137 ± 0.006	0.183 ± 0.006
2	0.143 ± 0.116	0.173 ± 0.015
3	0.160 ± 0.010	0.163 ± 0.006
4	0.153 ± 0.058	0.183 ± 0.015
5	0.190 ± 0.010	0.143 ± 0.006

Table 2: Bioavailability of Zinc in experimental and control group of Rats.

Results are means of three replicate values per sample (n=3).

Comparison of mean plasma Zn concentration between the experimental and control group in Rat

Statistical comparison of the mean plasma Zn concentration in experimental and control group rats showed that the mean plasma Zn concentration of the control group which were not fed with the experimental cereals was non-significantly ($p < 0.05$) higher than the experimental group rats that were fed with the experimental cereals.

	Plasma Zn Conc. (µg/dl) Experimental	plasma Zn conc. (µg/dl) Control	P-Value	Remark
MEAN	0.16 ± 0.21	0.17 ± 0.02	0.0792	$p > 0.05$

Table 3: Comparative mean plasma zinc concentration between the experimental and control group in Rat.

$P > 0.05$ not significant.

Discussion

From the table 1 above, there is for each crop a wide variation in zinc concentration. The zinc concentration in cereal foodstuffs grown on farmlands in the various part of Nasarawa state, Nigeria showed that the *Oriza sativa* from karu, *Sorghum bicolor* and *Zea maize* from Nasarawa-eggon has the highest zinc concentration. The propensity for plants to accumulate and translocate these essential elements to edible and harvested parts depends to a large extent on soil and climatic factors, plant genotype and agronomic management. Mineral contents of crops is known to be related directly to the minerals in the soil on which they are grown Bioavailability of minerals is, of a normal and essential process enabling the organism to have access to micromineral reserve for use as metalloproteins or cofactors. In terms of zinc concentration, *Sorghum bicolor* and followed by *Zea maize* from Nasarawa-eggon has the highest of 17.81mg/g and 16.72mg/g, and then *oryza sativa* from Karu has the lowest with the value of 10.34mg/g. The results agrees closely with that of kiri., *et al*. 2016, who found zinc in rice to be 0.0074 ± 0.0006mg/100g, maize to be 0.0192 ± 0.0006mg/100g and sorghum to be 0.3690 ± 0.0007mg/100g respectively. There is much literature on the relationships between micronutrient availability in soils and associated yields, but little information on crop micronutrient concentration in the edible parts of crops i.e reports on trace mineral levels in Nigerian foods are very limited.

Table 4.3 shows that the plasma zinc analysis of the experimental group has low bioavailable zinc of 0.16 ± 0.21 in comparison with the grand cereals and oil mills limited 0.17 ± 0.02. The results are in close agreement with Kiri., *et al*. 2017, who found zinc in run-off water (experimental) to be 0.15 ± 0.08 and tap water (control) to be 0.25 ± 0.11 respectively. The concentration of zinc in blood plasma is currently the best available biomarker of the risk of zinc deficiency in a population (Roohani., *et al*. 2013). However, the bioavailability of zinc in plasma may differ due to other dietary factors like phytate and oxalate (Lonnerdal, 2000). Pooled dose responds relationship between zinc intake increased the serum/ plasma zinc status by 9%, this evidence can be utilized together with currently used balance studies and repletion/depletion studies, when setting zinc recommendation as a basis for nutrition policies [20]. Therefore, this project research is consistent and in line with other research on plasma zinc level and bioavailability of zinc. Consequently, there are also limited data for the contents of trace elements in foods. Therefore, the International Institute of Tropical Agriculture (IITA), conducted a survey in 2001-2003, to create awareness on the micronutrient deficiencies in Nigeria with emphasis on the trace elements zinc, iron and iodine, and the vitamins, A and D among others (Maziya-Dixon., *et al*. 2004). International Food Composition Tables, such as that of the United States Department of Agriculture, USDA, provides a comprehensive database for trace elements and vitamins [21]. Mineral contents of foods differ by region and the rate of mineral utilization (consumption of foods) differs according to ethnic pattern processing methods. Hence it will not be wise to rely totally on foreign data to evaluate mineral contents of local foods.

Conclusion

This study has generated Zn data on the major basic foodstuffs and composite meals consumed in a rural and semi urban community Nasarawa state province. It will allow the estimation of Zn intake and bioavailability in the communities and provides useful information on the Zn content on common Nigerian foods. Wide variation exist in the mineral composition of the cereals analysed in this study. Assuming an average daily consumption of 250g, each of the samples analyzed can hardly provide the populace RDA for the trace element concerned. With proper selection and combination, it is possible to use local household foodstuff to formulate multimixes that can be used as home-based complementary foods. The blends formulated in this study are strongly recommended for used particularly by rural and poor urban mothers to feed their infants and children and even adults being during the complementary feeding period. Fortification and/ or supplementation maybe necessary to meet upthe recommended daily allowance. This will ensure availability and affordability as well as help in alleviating some economic and time-related constraints faced in child feeding practices. However, the cereals in Nasarawa state is a good source of bioavailable zinc for the populace, because statistical analysis of the experimental groups indicates there is no significant difference ($p>0.05$) between the two groups (experimental sample and the control).

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Volume 3 Issue 9 September 2019