Iron bioavailability and utilization in rats fed cassava-based complementary diets

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Abstract
The study evaluated the iron bioavailability and utilization in rats fed cassava-based complementary diets. Bioavailability and utilization were determined in Sprague-Dawley rats using the iron balance and hemoglobin depletion-repletion methods in a 6 x 8 randomized block design. Rats were depleted by feeding them a low iron casein diet for 14 days. During the depletion period, the rats were fed four composite diets formulated using iron improved cassava varieties as the base ingredients. Hemoglobin was determined at the end of depletion and repletion periods. Iron bioavailability expressed as hemoglobin regeneration efficiency (HRE) was higher in the rats that consumed the positive control diet than in those fed test (improved with iron) diets. The present study also found an inverse correlation between diet iron content and bioavailability \( r = -0.88, P < 0.05 \), and a direct relationship between the gain in hemoglobin and iron utilization \( r = 0.95, P < 0.05 \). Further, most of the rats recovered their hemoglobin and packed cell volume status after repletion \( (Hb > 12 \text{ g/dl}) \) except for rat fed the negative control suggesting that iron was poorly utilized. The study confirmed that the overall iron bioavailability expressed as hemoglobin regeneration efficiency (HRE) from composite flour formulated from cassava is low.

Key words: Iron bioavailability, iron utilization, complementary food, improved cassava, composite flours, rats.

Introduction
Iron deficiency anemia is still a major nutritional problem in the world, affecting primarily infants, children and fertile women in the developing world but also in industrialized countries 1. Iron deficiency anemia in infants is particularly important because it can lead to negative changes in psychomotor and mental development, some of which are irreversible 2. Breast-fed infants generally have an adequate iron status during the first 4-6 mo of life but after this time, when their iron stores have been depleted, additional dietary iron needs to be supplied to meet the infant’s requirement 3. During this period of early life, complementary foods carefully formulated from locally available staples can supply iron.

The introduction of staple crop genotypes bred for high amounts of minerals and vitamins can complement existing nutrition interventions and provide a sustainable and low-cost way of reaching the resource poor people who cannot afford animal products 4. Cassava-based diets provide a large proportion of the daily intake of energy and micronutrients for poor populations in many areas of sub-Saharan Africa 5.

The importance of cassava to the livelihoods of many millions of poor people has made the commodity a target for interventions. New Partnership for Africa Development (NEPAD) has adopted the slogan “Cassava: A Powerful Poverty Fighter in Africa” for its Pan-African Cassava Initiative. Harvest Plus Germplasm Development (Africa Component) intends to develop cassava varieties with increased levels of iron in the storage roots.

Bioavailability of trace elements especially iron from foods has always been of interest to nutritionists, food scientists and clinicians. It has been shown that iron deficiency disorder is not exclusively as a result of inadequate dietary intake but by a result of interacting factors. According to Forbes and Erdman 6, these factors include intake level of the mineral and its chemical form; promoters such as ascorbic acid and other chelators, inhibitors, such as phytic acid, fiber and amino acid or interaction between mineral elements. Therefore, the overall aim of this study was to evaluate the bioavailability and utilization of iron from cassava-based composite diets using rats as a model.

Materials and Methods
Experimental design and composition of the diets: Cassava and soybean used in the study were produced in the Experimental Farm of the International Institute of Tropical Agriculture, Ibadan, Nigeria. Freshly harvested cassava roots were peeled manually with stainless steel knife, washed thoroughly with portable water to remove all dirt and adhering sand particles. The washed peeled cassava roots were then grated into mash using a petrol engine driven stainless steel grater, packed into Hessian sack and dewatered immediately using a hydraulic press to express liquid from the mash as well as prevent fermentation. The pressed grated cassava mash was then sieved manually with stainless steel sieve to pulverize the pressed cake and separate fibrous materials. The resulting flour was dried in an oven dryer at 40°C and milled...
using a stainless steel milling machine before being packaged into polyethylene bags and stored at a cool dry room at -20°C. Soybean and groundnut were processed by roasting the grains in a preheated stainless steel pot on an electric stove for 15 minutes, stirring vigorously with a wooden spatula and then peeling the skin after roasting. The peeled grains were ground into fine flour with a domestic grinding machine. Cassava leaves flour was obtained by cleaning fresh leaves in water and pressed between the palms to remove excess water. The leaves were heat-treated in an empty dry pot, placed over a source of heat. The leaves were tossed constantly with a wooden spoon to ensure even distribution of heat. This was done for 5 minutes. The heat-treated leaves were pounded in a wooden mortar for 30 minutes. The pounded leaves were put in twice its weight of water, gradually brought to a boil and cooked for 1 hour 30 minutes. Without discarding the water used for cooking, processed leaves were dried at 60°C for 48 hours, milled and stored in air-tight zip-lock bags until analysis. The carrot powder was obtained by washing the carrot roots with water and then slicing and drying at 40°C. It was later milled into flour with a stainless steel milling machine. These were formulated by blending the ingredients in different proportions to form composite flours using the Owl Tech Wizard Software™ Version 3 to develop and optimize the different formulations. The flour was collected in polythene bags and stored in a deep-freezer (at -4°C) until ready for use. The iron bioavailability of the composite flours formulated from different improved cassava varieties was determined as described by Forbes et al. in 48 Sprague-Dawley weaning rats obtained from Biological Sciences Department, University of Agriculture, Abeokuta, Nigeria, and weighing 30–35 g (21-25 days old). The animals were individually housed in stainless steel cages with metal grid floors in a temperature and light-controlled environment. The hemoglobin (Hb) depletion-repletion method with hemoglobin gain as an indicator in a rat model with 10 Sprague-Dawley weaning rats (Blue Spruce Farms) was used to determine iron bioavailability and utilization. Iron balance and the percentage of iron absorption were evaluated during the repletion phase. Rats were fed the casein diet (90% protein) for 14 days. The animals also received deionized water ad libitum throughout the experiment period (28 days). During the 14 days depletion period, anemia was induced by obtaining blood from the tail vein of the rats and by feeding them a low iron diet. The diet was modified to exclude iron (Sigma, St. Louis MO) and contained in a g/kg diet of casein (90% protein) 100.0, vegetable oil 80.0, mineral mixture 58.0, water 42.0 and cellulose 10.0. Levels of iron in the experimental diets were confirmed by atomic absorption spectrometry.

After 14 days of dietary depletion period, the rats were weighed and blood samples were taken from the tail vein until sufficient blood was collected for the determination of hemoglobin (initial Hb). Hemoglobin concentration was measured with 0.02 ml blood samples diluted in 4 ml of Drabkin’s reagent. Absorbance readings were taken after 10 minutes at 540 nm. The anemic rats were assigned to 6 groups using completely randomized block design (n = 6) of approximately equal mean body weights and hemoglobin concentrations.

During this period, rats were fed four composite diets formulated from the improved cassava varieties. The other two diets consisted of a positive control diet that was formulated to include iron in the mineral mixture and the negative control formulated without (depleted diet) iron. During this period, the weight and food intake of the rats were recorded daily considering food spills. Deionized water was provided ad libitum during the repletion phase and fresh food was weighed daily for each animal. The feces were collected daily and separated from split food with a nylon sieve; urine was not collected because it contains negligible iron. The feces were dried, weighed, ground and analyzed for Fe according to the same methodology used for the diets. At the end of the repletion period, a blood sample was obtained from the tail vein to determine Hb concentration (final Hb).

The study was approved by the University of Agriculture, Animal Committee, and animals were maintained in accordance with the guidelines and principles of laboratory care and use of laboratory animals of University of Agriculture, Abeokuta, Ogun State, Nigeria.

**Determination of iron bioavailability and utilization indices:**

Hemoglobin and iron determination were used to estimate the following variables:

1. Percentage of bioavailability, calculated as hemoglobin regeneration efficiency (HRE). HRE is a measure of dietary iron incorporated into Hb. The calculation of Hb iron was based on the assumption that 6.7% of body weight is blood and Hb contains 0.335 mg of iron per gram. The formula is as follows: %HRE = (mg Fe\text{Hb})(final) – mg Fe\text{Hb}(initial) x 100/mg Fe consumed.
2. Iron content of Hb was calculated assuming a total blood volume of 6.7% of the rat body weight and an average iron content of Hb of 0.335 mg Fe/L. mg Fe\text{Hb} = (Body weight (g) x Hb (g/L) x 0.7 x 0.335)/10000. 3) Apparent iron absorption: % Fe Absorption = [(mg Fe intake – mg Fe in feces)/mg Fe intake]. 4) Iron balance = mg Fe intake – mg Fe in feces. 5) Iron utilization (mg) = (%HRE x % iron in the diet)/100.

**Statistical analysis:** The analysis of variance (ANOVA) and Duncan’s multiple range test were used to test the significance of differences between means (p<0.05) by procedure of SAS. A correlation between the iron content of diets and iron bioavailability was determined with Pearson correlation analysis. Data presented are means ± SD, n = 6.

**Results**

Table 1 shows the iron intake and excretion, balance and absorption % from the rat fed the depleted and replete diets. There were no differences in food intake among groups fed the test diets (P>0.05). Rats fed diets with the positive control excreted less (1.16±0.33 mg) iron but rats fed Diet 3 had the lowest iron balance (1.28± 0.44 mg) among the groups (P<0.05). However, their iron balance was still positive (Table 1). The negative control had no values for iron intake, excretion, balance and absorption because they had no iron in their diet. The percentage of iron absorbed was higher for the rats fed diet with positive control than for the test diets. In the rats fed the test diets, the highest absorption was found in the rats fed with Diet 2 (23.30±3.0%) followed by rats fed Diet 1 (21.31±2.2%). There was a significant difference among the groups fed test diets in their percent iron absorption (P < 0.05).

Table 2 presents the initial and final body weight, hemoglobin (Hb), iron in hemoglobin (Fe\text{Hb}), packed cell volume (PCV) and
plasma iron (µg/dl). The mean weight, Hb concentration, FeHb, % PCV and Fe in plasma after depletion was 34.37±3.77 g, 7.90±7.77 g/dl, 0.61±0.22 mg, 23.07±4.44% and 69.17±3.0 µg/dl respectively. After repletion, rats fed with the positive control gained more weight (21.50±3.0 g) and FeHb (1.11±2.2 mg) than rats in other groups, while rats fed with Diet 1 showed more gain (P<0.05) in hemoglobin (5.9±0.1 g/l) and PCV (18.70±4.5 g/dl) (Table 3). After repletion rats in Diet 3 gained more iron (16.03 µg/dl) than rats in the other groups. The percentage of iron bioavailability, expressed as HRE, was highest in the rats fed the positive control (41.85±3.3%) and lowest in rats fed the Diet 3 (9.85±1.0 %). There is an inverse correlation between diet iron content and bioavailability (r = -0.88, P < 0.05). Rats in the negative control had zero bioavailability because their diet was iron free. In general, iron utilization was lowest in rats fed Diet 3 (0.73±0.1 mg) and highest in rats fed the positive control diet (1.05±0.1 mg). There was a direct relationship between the gain in Hb and iron utilization (r = 0.95, P < 0.05).

**Discussion**

The nutritional adequacy of micronutrients depends on their amount and bioavailability in the complementary foods. Dietary components affecting the bioavailability of Fe and Zn in foods are well documented 14, 15. Hence, complementary foods can be classified as having high, moderate or low bioavailability for iron and zinc based on certain criteria set by the Food and Agricultural Organization and/or the World Health Organization 16, 17. Estimates for both iron and zinc bioavailability depend first on the content in a meal of animal and fish protein relative to plant-based foods. Secondly, bioavailability of iron also depends on the content of ascorbic acid, and, for some models, on the consumption of tea or coffee at the same time. For plant-based complementary foods with a low fat content, bioavailability of fat-soluble vitamins A, D, E, K and carotenoids may be compromised when breastfeeding ceases. Fiber, specifically pectin, impairs β-carotene absorption by interfering with gastric emptied and mixed-micelle formation.

Iron nutrition during the weaning period is of particular concern because the timing of the introduction of complementary foods usually coincides with increased iron requirements due to rapid growth at <4–6 mo of age 1. Thus, it is very important to ensure adequate quantities of bioavailable iron in weanlings’ diets 18. In this study, iron bioavailability expressed as hemoglobin regeneration efficiency (HRE) was higher in the rats that consumed the positive control diet than in those fed test (fortified with iron) diets. Similar results were reported by Cook et al. 19, who found in humans that those fed bread fortified with increasing amounts of iron (1, 3 and 5 mg) had lower percentage of iron absorbed, but their absolute absorption increased in response to increasing iron intakes. Also, Hernandez and colleagues 20 found iron bioavailability to be higher in the rats that consumed unfortified diets than in those fed diets fortified with iron and the percent iron absorption reported by this study are within the ranges reported by these authors. The findings of this study are contradictory to those reported by some authors who found a better iron bioavailability from diets with high iron content 21. According to Hernandez et al. 20, the differences may be explained by the manner in which iron bioavailability was evaluated. As suggested by Layrisse and Garcia-Casal 22, the percentage of iron absorbed decreases as iron intake increases, but the total iron absorbed is higher. The present study also find an inverse correlation between diet iron content and bioavailability (r = -0.88, P < 0.05), and a direct relationship between the gain in Hb and iron utilization (r = 0.94, P < 0.05). Further, most of the rats recovered their Hb and PCV status after repletion (Hb > 12 g/dl) except for rat fed the negative control suggesting that iron was poorly utilized. The study found a direct relationship between the weight gain and gain in hemoglobin and hematocrit concentrations (r = 0.95, P < 0.05).

**Conclusions**

The study confirmed that the overall iron bioavailability expressed as hemoglobin regeneration efficiency (HRE) from composite flour formulated from cassava is low.

**Acknowledgements**

The authors are grateful to the International Institute of Tropical Agriculture, Ibadan, Nigeria, for support to this research.

**Table 1.** Iron balance and % apparent iron absorption in rats fed diets based on formulated diets for 2 wks.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fe in diet (mg/100)</th>
<th>Fe intake (mg)</th>
<th>Fe excreted (mg)</th>
<th>Fe balance2 (mg)</th>
<th>% Apparent Fe absorption3</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve control</td>
<td>2.52</td>
<td>2.65±0.40a</td>
<td>1.16±0.33b</td>
<td>1.49±0.65b</td>
<td>56.23±5.6a</td>
</tr>
<tr>
<td>-ve control</td>
<td>0.00</td>
<td>0.00±0.00f</td>
<td>0.00±0.00f</td>
<td>0.00±0.00f</td>
<td>0.00±0.00f</td>
</tr>
<tr>
<td>Diet 1</td>
<td>7.54</td>
<td>8.87±0.44a</td>
<td>6.98±0.22a</td>
<td>1.89±0.21a</td>
<td>21.31±2.2a</td>
</tr>
<tr>
<td>Diet 2</td>
<td>7.38</td>
<td>8.67±0.44a</td>
<td>6.65±0.29a</td>
<td>2.02±0.06a</td>
<td>23.30±3.0b</td>
</tr>
<tr>
<td>Diet 3</td>
<td>7.42</td>
<td>8.15±0.34a</td>
<td>6.87±0.14a</td>
<td>1.28±0.14a</td>
<td>15.71±5.0e</td>
</tr>
<tr>
<td>Diet 4</td>
<td>7.39</td>
<td>8.42±0.47a</td>
<td>6.63±0.09a</td>
<td>1.79±0.16a</td>
<td>21.26±2.3b</td>
</tr>
<tr>
<td>Mean</td>
<td>5.38</td>
<td>6.13±0.11</td>
<td>4.72±0.01</td>
<td>1.41±0.54</td>
<td>22.97±7.2</td>
</tr>
</tbody>
</table>

1. Values are mean ± SE, n = 6, (P<0.05) 2. Iron balance = g iron intake – g iron feces. 3. % Fe absorption = (mg Fe intake – mg Fe in feces) x 100/ mg Fe intake 4. Values in a column not sharing a superscript letter are significantly different (P<0.05).
### Table 2. Body weight, hemoglobin (Hb), hemoglobin iron and plasma iron content in rats fed formulated diets for 2wks.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight (g)</th>
<th>Hb (g/dl)</th>
<th>Fe in Hb (mg)</th>
<th>% PCV</th>
<th>Fe in plasma (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>+ve control</td>
<td>34.25±2.50</td>
<td>55.75±8.09</td>
<td>8.20±1.50</td>
<td>13.90±0.90</td>
<td>0.63±0.15</td>
</tr>
<tr>
<td>-ve control</td>
<td>33.22±1.23</td>
<td>44.98±3.09</td>
<td>7.50±3.00</td>
<td>10.40±2.01</td>
<td>0.56±0.19</td>
</tr>
<tr>
<td>Diet 1</td>
<td>33.75±4.78</td>
<td>53.75±4.78</td>
<td>8.40±3.60</td>
<td>14.30±0.80</td>
<td>0.64±0.17</td>
</tr>
<tr>
<td>Diet 2</td>
<td>33.75±4.79</td>
<td>53.25±7.89</td>
<td>8.10±1.50</td>
<td>13.30±1.90</td>
<td>0.61±0.12</td>
</tr>
<tr>
<td>Diet 3</td>
<td>32.50±5.00</td>
<td>50.00±8.17</td>
<td>7.30±2.10</td>
<td>11.90±1.80</td>
<td>0.53±0.25</td>
</tr>
<tr>
<td>Diet 4</td>
<td>38.75±4.79</td>
<td>54.50±5.26</td>
<td>7.90±0.90</td>
<td>12.80±0.90</td>
<td>0.69±0.13</td>
</tr>
<tr>
<td>Mean</td>
<td>34.37±3.77</td>
<td>52.04±3.99</td>
<td>7.90±7.77</td>
<td>12.77±5.50</td>
<td>0.61±0.22</td>
</tr>
</tbody>
</table>

1. Values are mean ± SD, n = 6
2. Fe in hemoglobin: mg Fe = (body weight (g) x Hb (g/dl) x 6.7 x 0.335)/10000.
References