Study of Iron Bioavailability in a Native Nigerian Grain Amaranth Cereal for Young Children, Using a Rat Model[1,2]

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ABSTRACT

Iron bioavailability in Nigerian grain amaranth cereal fortified by two iron compounds, sodium ferric ethylenediaminetetraacetate (NaFeEDTA) and ferrous fumarate ($FeC_4H_2O_4$), was compared with that in cereal fortified with ferrous sulfate ($FeSO_4$). Grain amaranth is important because of its potential as a cereal for young children in Nigeria and other third world countries. Although hemoglobin gain in all three groups fed fortified cereal was significantly higher than that in the group fed cereal with no added iron, hemoglobin gain was highest in animals fed amaranth cereal with ferrous fumarate. Relative biological values for animals receiving unfortified amaranth cereal or cereal fortified with NaFeEDTA, ferrous fumarate, or $FeSO_4$ were 0.78, 0.93, 1.05, and 1.00, respectively. Body weight gain, hemoglobin gain, and concentrations of phytate and tannin as well as the protein efficiency ratio of fortified amaranth cereal were compared with the same parameters from a previous study of iron bioavailability in fortified Egyptian balady bread prepared with high-extraction wheat. Protein efficiency ratio of fortified amaranth cereal was approximately 1.6 as compared with 0.9 for the Eyptian bread. High relative biological values and expected body weight gain indicated optimum iron absorption from the amaranth cereal. This study indicates that ferrous fumarate is the iron fortifier of choice for grain amaranth cereal.

Grain amaranth (*Amaranthus caudatus*), a hardy plant indigenous to the tropics, may become a future primary staple cereal crop upon which millions of people in the developing countries in Central Africa and South America will depend. It can be grown inexpensively with minimal cultivation on marginal agricultural land. As part of an effort to popularize its consumption on the West African coast, improved germ plasms were obtained from Rodale Research Inc., Kutztown, PA, and taken to Nigeria for agronomic trial plantings. Amaranth seeds have been chemically analyzed and found to contain approximately 18% protein and 8% seed oil, which indicates that amaranth is a good source of plant protein for humans (Becker et al 1981, Ologunde, *unpublished data*). The analysis of 35 test samples of grain amaranth from lots collected primarily from Guatemala, but also from Peru and Mexico by Bressani et al (1987a), showed an average protein content of 15% (12.8–17.4%), a net protein ratio (NPR) of 2.20, protein digestibility of approximately 80%, and a crude fiber content of 6.4%. Grain amaranth is also a rich source of minerals: 22.2 mg/100 g calcium, 47.4 mg/100 g potassium, and 249 mg/100 g phosphorus (Ologunde, *unpublished data*). Proteins found in most cereals, including those prepared from wheat or corn, are generally considered incomplete because they lack the essential amino acid lysine. The relatively high percentage of lysine in proteins found in grain amaranth (Marx 1977), however, makes it an effective cereal choice in developing countries where protein deficiency is a major concern.

In Peruvian maize cereal supplemented with amaranth, Morales et al (1988) found that the high protein and lipid contents of amaranth provided 9–10% of total dietary energy as fat, and 6.4–6.7% as protein, while providing only 50% of total dietary energy. In contrast, in order to provide 6.4% of protein of total dietary energy, maize had to provide 70% of total dietary energy. Particularly in the absence of dairy products in the diet, grain amaranth as a supplement or complement to common cereals represents a uniquely beneficial protein source (Morales et al 1988).

Because of the prevalence of iron deficiency anemia in infants and young children in third world countries, the presence of bioavailable dietary iron is highly desirable. Both phytate and tannin, which interfere with iron absorption, were determined in the amaranth cereal used in this study. Dietary phytate, which has been shown to chelate metal ions (Hallberg et al 1987, Hallberg et al 1989), may also bind minerals in the gastrointestinal tract and further inhibit iron absorption (Oberleas 1983). Gillooly et al (1983) showed a significant inverse correlation between the polyphenol content of vegetables and iron absorption and observed that the presence of an individual vegetable with a strong enhancing or inhibiting effect profoundly influenced the bioavailability of nonheme iron in a mixed diet.

Although Bressani et al (1987a) investigated inhibitors and found that 33 raw Guatemalan grain amaranth test samples averaged 0.417% tannic acid and 2.05 units of trypsin inhibitor/ml of reactive mixture, the bioavailability of iron in cooked Nigerian grain amaranth has not previously been evaluated. Processing may affect the chemical form of innate and fortification iron, and the presence of ascorbic acid in the grain itself may also influence iron bioavailability. Information provided by the study is necessary to plan an intervention program among Nigerian children.

Considerable research has confirmed that iron deficiency within population groups is not only caused by inadequate iron intake but also by interactive factors that adversely affect dietary iron bioavailability (Latunde-Dada and Neale 1986). We therefore compared the phytic and tannic acid levels determined in this study with those of an earlier study conducted before the initiation of a nutritional intervention program that used fortified, high-extraction (82%) wheat in Egyptian balady bread (Whittaker and Vanderveen 1990).

MATERIALS AND METHODS

Grain used in the study was produced in agronomy field trials conducted on the teaching and research farm of Obafemi Awolowo University, Ile-Ife, Nigeria. Harvested grain was cleaned and cooked 7 min in four parts boiling water at atmospheric pressure with constant manual stirring in borosilicate tall-form beakers. Cooked grains were drained on soft cloth and spread thinly on absorbent paper at room temperature for about 1 hr to allow excess water to evaporate. The seeds were then transferred to a forced convection oven and maintained at 60°C for about 3 hr or until they attained a moisture content of 5%. Dried grain was ground in a motorized mortar and pestle (model MG2,

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Torsion Balance Co., Clifton, NJ) until fine enough to pass through a 60-mesh sieve. The flour was collected in polyethylene bags and stored in a freezer.

Variability in phytate and tannin content caused by processing was evaluated. The cereal was heated for 20 min at 40, 60, or 100°C, and phytate and tannin concentrations were determined and compared with those of the raw cereal. Phytate concentration was determined by a modified ion-exchange method (Harland and Oberleas 1986), and tannin content was determined by spectroscopy (Price and Butler 1977).

The relative iron bioavailability of grain amaranth cereal supplemented with one of three iron fortification compounds—sodium ferric ethylenediaminetetraacetate (NaFeEDTA) (Hampene NaFe Purified Grade, W.R. Grace & Co., Nashua, NH), ferrous fumarate ($FeC_4H_2O_4$) (Sigma Chemical Co., St. Louis, MO), or ferrous sulfate ($FeSO_4 \cdot 7H_2O$) (Mallinckrodt Inc., Paris, KY)—was compared with that of the cereal alone in a hemoglobin repletion study with hemoglobin gain as an indicator in a rat model (Forbes et al 1989). Four groups of 10 weanling male rats of the Sprague-Dawley strain (Blue Spruce Farms, Inc., Altamont, NY) were individually housed in stainless steel cages and maintained under light- and temperature-controlled conditions. Animals received deionized, distilled water ad libitum.

Anemia was induced by phlebotomy (Tim 1979) and a low-iron diet during a seven-day depletion period. The depletion diet was the AIN 76A semipurified rat diet modified to exclude ferric citrate (American Institute of Nutrition 1980), which contained, in grams per kilogram of diet: casein 200.0, DL-methionine 3.0, cornstarch 150.0, glucose 500.0, fiber Celufil 50.0, corn oil 50.0, AIN 76 mineral mix (without ferric citrate) 35.0, AIN 76A vitamin mix 10.0, and choline bitartrate 2.0 (U.S. Biochemical Co., Cleveland, OH).

Following depletion, the animals were weighed and hematocrit and hemoglobin concentration were determined in duplicate from a specimen of fresh blood by the cyanmethemoglobin method (Crosby and Munn 1954). Animals were then randomized into four groups of approximately equal mean hemoglobin concentration and body weight.

Equal amounts of four grain amaranth-based repletion diets were prepared. Three contained approximately 35 mg Fe/kg of diet added from one of the three experimental fortification sources; the fourth consisted of unfortified grain amaranth cereal, which contained 69 mg intrinsic Fe/kg of diet. AIN 76 mineral mix (excluding ferric citrate) and AIN 76A vitamin mix were added to all four cereal diets in proportions identical to those in the depletion diet. Iron concentrations of experimental meals were determined by atomic absorption spectroscopy (Boline and Shrenk 1977). Fresh food was weighed daily for each animal.

Body weight, hemoglobin concentration, and hematocrit percentage were determined after repletion. At the conclusion of the experiment, animals were euthanized, and the excised livers were frozen for subsequent nonheme iron determination by a slightly modified bathophenanthroline method (Torrance and Bothwell 1968, Whittaker and Vanderveen 1990). Results were expressed as both μg Fe/g of liver and total μg Fe/liver. Serum iron and total iron-binding capacity (TIBC) were measured by an electrochemical technique using a Ferrochem II serum iron/TIBC analyzer (ESA, Inc. Bedford, MA) (Skikne 1987).

Relative biological value (RBV) was determined by comparing the hemoglobin gain in animals fed the amaranth cereal with and without fortification iron with that in animals fed cereal with added reference $FeSO_4$.

Data were analyzed by one-way analysis of variance. Means were compared by the least significant difference (LSD) method when statistically significant ($P < 0.05$) (Snedecor and Cochran 1967).

RESULTS

Table I summarizes the comparative bioavailability of iron in fortified and unfortified grain amaranth cereals. There were no significant differences in the initial body weight, hemoglobin concentration, serum iron, TIBC, or hematocrit percentage among the four groups. Hemoglobin gain was highest in animals fed amaranth cereal with added ferrous fumarate, although hemoglobin gain in all three groups fed fortified cereal was significantly higher than in the group fed grain amaranth with no added iron. The reason that final serum iron values are low in comparison to initial values may be because all initial blood specimens were taken from unfasted animals, whereas final blood

TABLE I
Comparison of Bioavailability of Iron in Fortified Grain Amaranth Cereals[a]

Variable	Fortification Compound				Significance	
	Unfortified	NaFeEDTA	Ferrous Fumarate	$FeSO_4$	*P*	LSD[b]
Body wt, initial (g)	96 ± 2[c]	97 ± 3	97 ± 3	96 ± 1	0.976	NS
Body wt, final (g)	122 ± 4	122 ± 3	124 ± 3	121 ± 2	0.889	NS
Body wt gain (g)	26 ± 2	25 ± 1	27 ± 1	25 ± 1	0.680	NS
Hb,[d] initial (g/dl)	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	0.997	NS
Hb, final (g/dl)	13.7 ± 0.3	15.2 ± 0.2	16.4 ± 0.4	15.9 ± 0.3	<0.001	0.9
Hb gain (g/dl)	7.8 ± 0.2	9.3 ± 0.4	10.5 ± 0.5	10.0 ± 0.3	<0.001	1.0
Serum Fe, initial (μg/dl)	36 ± 3	38 ± 3	39 ± 3	44 ± 3	0.345	NS
Serum Fe, final (μg/dl)	23 ± 5	36 ± 5	84 ± 15	41 ± 9	<0.001	27
TIBC,[e] initial (mg/dl)	862 ± 26	777 ± 25	782 ± 25	830 ± 12	0.067	NS
TIBC, final (mg/dl)	567 ± 21	502 ± 12	504 ± 15	535 ± 15	0.024	47
Hct,[f] initial (%)	26 ± 1	27 ± 1	26 ± 1	26 ± 1	0.797	NS
Hct, final (%)	47 ± 1	53 ± 1	53 ± 1	54 ± 1	<0.001	2
Hct gain (%)	21 ± 1	27 ± 1	27 ± 1	28 ± 1	<0.001	3
Liver wt (g)	4.09 ± 0.15	4.00 ± 0.09	4.09 ± 0.09	3.84 ± 0.07	0.313	NS
Nonheme liver Fe (μg/g)	25.4 ± 1.5	43.1 ± 6.3	54.7 ± 7.8	31.2 ± 3.6	0.002	15.5
Nonheme liver Fe (μg)	105 ± 8	172 ± 25	220 ± 30	121 ± 15	0.002	61
RBV[g]	0.78 ± 0.02	0.93 ± 0.04	1.05 ± 0.05	1.00 ± 0.03	<0.001	0.10
PER[h]	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	0.467	NS

[a]Ten animals per group were fed the test diets containing either 35 mg of fortification Fe/kg of diet or no fortification during the seven-day repletion period.
[b]Mean differences must equal or exceed the least significant difference value to be significantly diffferent at $P = 0.05$. NS = not significantly different.
[c]Mean ± SEM ($n = 10$).
[d]Hemoglobin.
[e]Total iron-binding capacity.
[f]Hematocrit.
[g]Relative biological value = Hb gain of fortification compound divided by mean Hb gain of $FeSO_4$.
[h]Protein efficiency ratio = Body weight gain (g)/dietary protein (g) for seven days.

TABLE II
Comparison of Protein Efficiency Ratio (PER) Values in Egyptian Balady Bread, Grain Amaranth Cereal, and Casein-Based AIN 76A Diet

Variable	Egyptian Bread[a]			Casein Diet (AIN 76A)[a]			Grain Amaranth Cereal[b]			
	$FeSO_4$	$FeSO_4$ + Na_2EDTA	NaFeEDTA	$FeSO_4$	$FeSO_4$ + Na_2EDTA	NaFeEDTA	Without Fortification[c]	NaFeEDTA	Ferrous Fumarate	$FeSO_4$
Body wt gain (g)	10 ± 2[d]	11 ± 2	15 ± 1	52 ± 2	50 ± 1	53 ± 1	26 ± 2	25 ± 1	27 ± 1	25 ± 1
Hb[e] gain (g/dl)	5.4 ± 0.5	8.7 ± 0.3	8.7 ± 0.3	6.0 ± 0.2	5.3 ± 0.2	8.4 ± 0.4	7.8 ± 0.2	9.3 ± 0.4	10.5 ± 0.5	10.0 ± 0.3
PER	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1

[a]Ten animals per group were fed test diets containing 30 mg of fortification Fe/kg during the 10-day repletion period (Whittaker and Vanderveen 1990).
[b]Ten animals per group were fed test diets containing either 35 mg of fortification Fe/kg of diet or no fortification during the seven-day repletion period.
[c]Iron level of 69 mg/kg for amaranth cereal was determined by atomic absorption spectroscopy.
[d]Mean ± SEM.
[e]Hemoglobin.

specimens were drawn after an 18-hr fast. Nevertheless, final serum iron values were significantly higher in animals receiving ferrous fumarate than in any other group. TIBC varied inversely with iron status during the repletion period as expected. Although serum ferritin level indicates human iron status, liver nonheme iron level is the most reliable indicator of iron status in the rat. It was of special interest, therefore, that the nonheme iron level was significantly higher in animals fed cereal fortified with ferrous fumarate than in those fed grain fortified with $FeSO_4$. The nonheme iron levels of the three groups fed cereals with fortification iron were all significantly higher ($P = 0.002$) than that of the group receiving unfortified grain amaranth cereal.

The RBVs were 0.78, 0.93, 1.05, and 1.00 for animals receiving unfortified amaranth cereal, and those fed cereal fortified with NaFeEDTA, ferrous fumarate, and $FeSO_4$, respectively. The availability of intrinsic iron in the unfortified cereal was sufficient to produce an RBV of 0.78 and a weight gain approximately that of the other groups.

Table II compares the body weight gain, hemoglobin gain, and protein efficiency ratio values obtained in this study with those of a previous study of fortified Egyptian balady bread (Whittaker and Vanderveen 1990). That study tested the efficiency of NaFeEDTA and $FeSO_4$+Na_2EDTA as potential fortifiers for a traditional Egyptian flat bread that has high bran and phytate contents and is typically baked at an extraordinarily high temperature (approximately 500°C), factors that may inhibit iron bioavailability (El Guindi et al 1988). The hemoglobin gain of the $FeSO_4$ reference animals is 5.4 g/dl for animals fed ground Egyptian bread and 10.0 g/dl for those receiving cereal. Fortification of the bread with NaFeEDTA produced a hemoglobin gain of 8.7 g/dl, which is 62% higher than that produced by $FeSO_4$ fortification. However, addition of NaFeEDTA to the cereal diet produced a 7% decline compared with the $FeSO_4$-fortified cereal. El Guindi et al (1988) reported in a human study that NaFeEDTA seems to counteract the inhibitory effect of phytate in $FeSO_4$-fortified bread. Thus, NaFeEDTA was chosen as an effective fortifier for Egyptian balady bread. The present study, however, indicated that the tannin and phytate in grain amaranth cereal did not inhibit iron absorption as profoundly as in the high-extraction (82%) wheat flour used in the Egyptian bread. Thus, EDTA fortification produced quite different results.

The phytate and tannin concentrations in ground grain amaranth processed in water at 40, 60, and 100°C for 20 min were compared with their contents in raw cereal (Table III). Processing the grain at 40°C for 20 min affected neither phytate nor tannin concentration. At 60°C, however, phytate concentration was reduced slightly, from 7.92 to 7.58 mg/g of cereal, and tannin from 0.22 to 0.15 mg catechin equivalents (CE)/100 g of cereal. Processing at 100°C reduced phytate and tannin to 3.01 mg/g and 0.06 mg CE/100 g, respectively.

Table IV represents phytate and tannin contents of amaranth cereal processed at 100°C for 7 min compared with that of dried, ground traditional Egyptian balady bread. Phytate concentrations (3.47 and 3.45 mg/g, respectively) were similar. The tannin content

TABLE III
Effect of Processing on Phytate and Tannin Concentrations in Grain Amaranth Cereal

Treatment	Phytate (mg/g)	Tannin (mg CE/100 g)[a]
Raw cereal	7.92 ± 0.08[b]	0.22 ± 0.01
Processed at 40°C for 20 min	7.92 ± 0.11	0.22 ± 0.01
Processed at 60°C for 20 min	7.58 ± 0.05	0.15 ± 0.01
Processed at 100°C for 20 min	3.01 ± 0.02	0.06 ± 0.01

[a]CE = catechin equivalent.
[b]Mean ± SEM of triplicate determinations.

TABLE IV
Comparison of Phytate and Tannin Contents in Grain Amaranth Cereal and Egyptian Balady Bread

Component	Amaranth Cereal	Egyptian Bread
Phytate (mg/g)	3.47 ± 0.02[a]	3.45 ± 0.02
Tannin (mg CE/100 g)[b]	0.08 ± 0.01	0.17 ± 0.01

[a]Mean ± SEM of triplicate determinations.
[b]CE = Catechin equivalent.

of amaranth cereal, however, was less than half that found in Egyptian bread: 0.08 mg CE/100 g as compared with 0.17 mg CE/100 g. These results suggest that tannic acid may influence iron bioavailability differences in the two food vehicles.

DISCUSSION

Iron deficiency is prevalent in developing countries where the diet is low in animal protein and heme iron. Dietary staples are cereal and vegetable products. Inhibitory factors commonly present in such a diet contribute significantly to a high incidence of iron deficiency anemia. Fortification of a universally available and inexpensive food staple is a practical intervention strategy for achieving long-term enhancement of iron status. For this reason, grain amaranth is a logical, nutritionally promising choice as a cereal food vehicle. It grows well under poor agricultural conditions and is inexpensive to prepare as a food. Other dietary intervention strategies have utilized local bread varieties, although the high bran and phytate contents in bread as well as high baking temperatures tend to inhibit iron absorption (El Guindi et al 1988).

In the present study, the RBV for the group fed a diet fortified with ferrous fumarate was significantly higher than that of the NaFeEDTA-fortified group. Several investigators (Layrisse and Martinez-Torres 1977, Viteri et al 1978, MacPhail et al 1981) have shown that NaFeEDTA is absorbed about twice as well as $FeSO_4$ from the same vegetable source. In a study of ethnic Indian females of South Africa, Ballot et al (1989) reported that NaFeEDTA-fortified curry powder, which was used as an intervention strategy, decreased the incidence of iron deficiency anemia from 22 to 5%. Lower tannin concentration, intrinsic ascorbic acid, and the RBV of innate iron in grain amaranth, however, seem to affect the optimum absorption of the iron fortification

compounds. In a recent report, which was based on both human and animal studies, Hurrell et al (1989) proposed that ferrous fumarate or ferrous succinate be the iron fortifier in infant cereals. The same study also demonstrated the relative stability and high RBV of these compounds.

In summary, cooked amaranth cereal maintained a favorable protein efficiency ratio for a vegetable product. The relatively high protein and oil contents in grain amaranth make it a desirable weaning food (Imeri et al 1987). An additional advantage is that the leaves of grain amaranth are also nutritious (Marx 1977). Cooking the grain further enhances its nutritional qualities. In an animal study, Bressani et al (1987b) showed that although raw grain amaranth does not promote optimal growth, grain processed by wet cooking produces excellent growth. In the present study, normal body weight gain and high RBV indicated good iron absorption from the cereal alone, although animal groups receiving iron fortification showed a significant increase in final hemoglobin or hemoglobin gain in comparison with the group fed unfortified cereal. Thus, a cereal composed of iron-fortified grain amaranth would be useful in an intervention strategy to combat protein-calorie malnutrition and iron deficiency anemia in infants and young children.

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