Biofortified Black Beans in a Maize and Bean Diet Provide More Bioavailable Iron to Piglets Than Standard Black Beans

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Abstract

Our objective was to compare the capacities of biofortified and standard black beans (Phaseolus vulgaris L.) to deliver iron (Fe) for hemoglobin (Hb) synthesis. Two lines of black beans, one standard and the other biofortified (high) in Fe (71 and 106 µg Fe/g, respectively), were used. Maize-based diets containing the beans were formulated to meet the nutrient requirements for swine except for Fe (Fe concentrations in the 2 diets were 42.9 ± 1.2 and 54.6 ± 0.9 mg/kg). At birth, pigs were injected with 50 mg of Fe as Fe dextran. At age 28 d, pigs were allocated to the experimental diets (n = 10). They were fed 2 times per day for 5 wk and given free access to water at all times. Body weights and Hb concentrations were measured weekly. Hb repletion efficiencies (means ± SEM) did not differ between groups and, after 5 wk, were 20.8 ± 2.1% for the standard Fe group and 20.9 ± 2.1% for the high Fe group. Final total body Hb Fe contents did not differ between the standard [539 ± 39 mg (9.7 ± 0.7 µmol)] and high Fe [592 ± 28 mg (10.6 ± 0.5 µmol)] bean groups (P = 0.15). The increase in total body Hb Fe over the 5-wk feeding period was greater in the high Fe bean group [429 ± 24 mg (7.7 ± 0.4 µmol)] than in the standard Fe bean group [361 ± 23 mg (6.4 ± 0.4 µmol)] (P = 0.034). We conclude that the biofortified beans are a promising vehicle for increasing intakes of bioavailable Fe in human populations that consume beans as a dietary staple. J. Nutr. 139: 305-309, 2009.

Introduction

Iron (Fe) deficiency is the most prevalent nutrient deficiency worldwide (1). Strategies for reducing its prevalence include distribution of Fe supplements to at-risk groups, food fortification, and diversification of diets. Whereas these strategies have been effective in industrialized countries, they have met with limited success in resource-poor countries because of cost, lack of access to health care, limited availability of centralized food processing facilities required for postharvest fortification, and other factors (2-4). Recently, HarvestPlus, an alliance of research institutions, launched a global effort to reduce micronutrient malnutrition by breeding staple food crops for enhanced mineral and vitamin content (5). HarvestPlus affiliated plant breeders screen germplasm banks for crop varieties that are high in Fe, Zn, and/or β-carotene. Promising germplasm accessions are crossed with commercial varieties that are high yielding and agronomically suitable for a particular area. Ultimately, the seeds of these "biofortified" crops will be distributed to farmers who will plant them and produce foods with enhanced nutritional value for their families and local markets.

The common bean (Phaseolus vulgaris L.), one of the staple food crops targeted for nutritional enhancement by HarvestPlus, is a grain legume (6). It provides significant quantities of protein and energy and is a source of vitamins and minerals including Fe (7). The common bean is an attractive candidate for biofortification, because there is genetic variability of Fe concentration and therefore it is possible to breed for significant increases in Fe concentrations in beans (8). Also, Fe concentrations in beans are high relative to other crops and therefore beans can deliver substantial amounts of Fe. Bean genotypes with high Fe concentrations delivered more absorbed Fe to rats than genotypes with lower concentrations of Fe (8).

Breeders at Centro Internacional de Agricultura Tropical, Cali, Colombia, developed a biofortified bean that contains >100 µg Fe/g, a substantial increase over standard beans (S. Beebe, unpublished observations). However, an increase in Fe concentration in beans or other staple food crops may not necessarily
translate into a proportional increase in absorbed Fe, because genotypes with high Fe concentrations may also have increased (or decreased) concentrations of Fe absorption inhibitors or enhancers. Therefore, it is necessary to measure the amount of bioavailable Fe as well as the concentration of Fe in these new Fe-enhanced crops. Beans are usually consumed with foods that are high in starch such as maize, rice, wheat, sweet potatoes, or cassava. For example, black beans are consumed with maize in Guatemala, southern Mexico, and Haiti. The objective of this study was to compare the capacities of biofortified and standard common beans to deliver Fe for hemoglobin (Hb) synthesis in Fe-deficient pigs.

Materials and Methods

Diets, study design, and blood sample collection. Two lines of black beans (Phaseolus vulgaris L.), one standard and the other biofortified in Fe, were provided by Centro Internacional de Agricultura Tropical. The Fe concentrations in the beans were 71 ± 6.5 (standard Fe) and 106 ± 8.6 (high Fe) μg Fe/g beans. Upon arrival, beans were mixed with water (~1 kg beans: ~1.5 L water) and cooked in an autoclave at 121°C for 30 min. Following cooking, they were freeze-dried and ground to a coarse powder for incorporation into diets. Maize-based diets (Table 1) containing the beans were formulated to meet current recommendations for nutrient requirements of swine (9). Diets had no supplemental Fe but were adequate in protein, essential fatty acids, vitamins, and minerals for pigs of this age. Pigs were fed the experimental diets for 5 wk. Blood samples (~100 μL) were collected weekly from the ear vein using micro-hematocrit heparinized capillary tubes (Fisherbrand).

Animals and protocols. Weanling crossbred pigs (Yorkshire × Hampshire × Landrace) from the Cornell University Swine Farm were used. All animal protocols were approved by the Cornell University Institutional Animal Care and Use Committee. Pigs were selected from litters that were injected with 50 mg of Fe as Fe-dextran at birth (one-half the normal Fe dose). Thus, the pigs developed Fe deficiency by the time they were weaned. Piglets were weaned at 3 wk of age and fed an Fe-deficient diet for 1 wk. At age 4 wk, body weight and Hb concentration were (mean ± SEM) 8.0 ± 0.13 kg and 106.1 ± 0.17 g/L, respectively. The piglets were allocated to 2 treatment groups (high Fe and standard Fe bean diets, n = 10) so that mean body weight and Hb concentration in the groups were similar. The groups were also balanced for gender and litter. Animals were housed individually in stainless steel cages in a temperature-controlled barn (22 to 25°C) with a light/dark cycle of 12:12 h, pigs were fed 2 times per day, had free access to water, and were checked daily. The groups were randomly assigned to the treatment diets at age 4 wk and maintained on these diets for 5 wk. Feed intakes were measured daily. Fe intakes were calculated from feed intakes and Fe concentration in the diets. Body weights and Hb concentrations were measured weekly.

Fe bioavailability was calculated as Hb repletion efficiency (HRE) (10):

\[
HRE = \frac{Hb\ Fe\ mg\ (final) - Hb\ Fe\ mg\ (initial)}{Total\ Fe\ Intake,\ mg} \times 100
\]

where Hb Fe = total body Hb Fe. Hb Fe was calculated from Hb concentrations and estimates of blood volume based on body weight (a blood volume of 67 mL/kg body weight was assumed) (11): Hb Fe (mg) = body weight (kg) × 0.067 L blood/kg × Hb (g/L blood) × 3.35 mg Fe/g Hb.

Fe intake was calculated from feed intake data and Fe concentrations in the feed.

Hb measurements. Blood Hb concentrations were determined spectrophotometrically using the cyanmethemoglobin method (Pointe Scientific) following the kit manufacturer’s instructions.

Phytate analyses in beans and diets. Phytate was extracted from 250 mg of a lyophilized diet sample by suspending in 10 mL of 1.25% H2SO4 for 2 h. Supernatants of the extracts were diluted 1:10 with deionized water and 10 μL aliquots were analyzed by HPLC. A Dionex Liquid Chromatograph System (AS50 Autosampler) equipped with a conductivity detector (model ED30) and a gradient pump (GS50) was used. An IonPac AG11 guard column and an IonPac AS11 column (4 × 250 mm) were used. The mobile phases were 200 mmol/L NaOH (carbonate-free) and deionized water, running at a flow rate of 1 mL/min. PeakNet 6.40 software was used to process the chromatographic data (Dionex).

Polyphenol concentrations in the beans and diets. Samples (1.5 g) of the cooked beans and the diets were extracted in 4 mL of acidified methanol (methanol and 1.0 mol/L HCl, 85:15, v/v). The samples were shaken for 2 h, vortexed to mix, and centrifuged at 12,800 g; 10 min. Total phenol content in the supernatants was measured as previously described (12). Briefly, to 0.125 mL of the supernatant, 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteau reagent were added. The mixture was allowed to stand at room temperature for 5 min and then 0.125 mL of aqueous Na2CO3 (7% wt/v) was added. The final volume of the mixture was adjusted to 3 mL with deionized water and was allowed to stand for 60 min at room temperature. The absorbance was measured at 760 nm against a reagent blank. The amount of total phenolics was expressed as gallic acid equivalents (μg/g diet).

Statistical analysis. One-tailed Student's t tests were performed to compare differences between means using the JMP software (SAS Institute). Values were considered significantly different at P < 0.05. Values in the text are means ± SEM.

Results

Phytate:Fe molar ratios in the bean-maize diet samples. The concentrations of phytate and Fe in the diets were used to

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Standard Fe bean diet</th>
<th>High Fe bean diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, μg/g</td>
<td>42.9 ± 1.2</td>
<td>54.6 ± 0.9</td>
</tr>
<tr>
<td>Total phenols (gallic acid), μg/g</td>
<td>113.8 ± 9.5</td>
<td>112.78 ± 8.4</td>
</tr>
<tr>
<td>PhytaTe:Fe molar ratio</td>
<td>8.87 ± 1.02</td>
<td>9.75 ± 1.65</td>
</tr>
</tbody>
</table>

1 GRANDE, Ultra 8000-Grade A.
2 Vitamin and mineral premix provided (per kg diet): retinyl palmitate, 1208 μg; menadione, 0.05 mg; d-biotin, 0.05 mg; choline chloride, 0.5 g; folic acid, 0.3 mg; nicin, 15 mg; Ca-panthothenate, 10 mg; riboflavin, 3.5 mg; thiamin, 1 mg; pyridoxine, 1.5 mg; cyanocobalamin, 17.5 μg; CuSO4·5H2O, 6 mg; Na2SO3·5H2O, 370 μg; Tylan3, 5 μg; ZnO, 100 mg.
3 Tylan is a macrolide antibiotic used as growth promoter in commercial swine operations (El Lilly).
4 Values are means ± SEM, n = 3 (by analysis).

Hb measurements. Blood Hb concentrations were determined spectrophotometrically using the cyanmethemoglobin method (Pointe Scientific) following the kit manufacturer’s instructions.
calculate the phytate to Fe molar ratios. The ratios of phytate:Fe did not differ between diets and were 8.67 ± 1.02 for the standard Fe diet and 9.75 ± 1.65 for the high Fe diet (n = 3; Table 1).

**Total phenolic concentration in the beans and bean-maize-based diet samples.** The total phenolic concentrations in the bean samples [gallic acid equivalents (μg/g of bean; n = 3)] were 299.0 ± 11.7 μg/g in the high Fe beans and 366.9 ± 6.8 μg/g in the standard Fe beans. Total phenolic concentrations did not differ between the standard (113.9 ± 9.5 μg/g) and high Fe (112.7 ± 8.4 μg/g) bean diets (Table 1).

**Growth rates, Hb, and HRE.** The pigs grew well over the 5-wk study. Feed intakes and body weights did not differ at any time throughout the study. Fe intakes were consistently higher in the high Fe group than in the standard Fe group after wk 1 of the 5-wk feeding period was only 5 wk. In a similar study, Schaffer et al. (13) compared the effects of high Fe (13.4 μg/g) and low Fe (2.2 μg/g) rice on Fe status indices in early weaned piglets. At the end of the 33-d feeding trial, none of the indices differed. A likely reason for the lack of effect in this study is that both diets were Fe deficient but not anemic Filipino women in a 9-mo efficacy trial, that of standard beans. We used HRE values as an estimate of Fe bioavailability, which we defined as the proportion of ingested Fe that is absorbed and utilized in metabolic processes or sequestered in storage depots. HRE is an underestimate of Fe bioavailability, because it does not include absorbed Fe used in the synthesis of myoglobin and other proteins, nor does it account for any Fe entering stores. However, in Fe-deficient pigs, between 80 and 90% of absorbed Fe is utilized for Hb synthesis (E. Tako, unpublished observations). HRE values were identical for the 2 groups and exceeded 20%. This shows that even though the biofortified beans were higher in Fe concentration, Fe bioavailability did not differ. This is not surprising given that the phytate:Fe ratios and total phenol concentrations were similar in the 2 diets.

The main objective of the present study was to compare the capacities of biofortified and standard beans to deliver Fe for Hb synthesis in Fe-deficient pigs. The pigs receiving the high Fe bean diet gained significantly more Hb Fe than the piglets on the diet containing standard beans. This result clearly shows that Fe-biofortified beans can enhance Fe status in Fe deficient pigs even when fed as part of a complete diet, where the difference in Fe concentration between the diets was only 12 μg/g and the feeding period was only 5 wk. HRE values did not differ between treatments (Table 2). However, the increase in total body Hb Fe from the beginning of the study to the end of wk 5 was greater in the high Fe group [429 ± 24 mg (7.7 ± 0.4 μmol)] than the standard Fe group [361 ± 33 mg (6.4 ± 0.4 μmol)] (P = 0.034; Table 2).

**Discussion**

One of our goals for the study was to address the question of whether Fe bioavailability of the high Fe beans differed from

**TABLE 2** Body weights, feed and Fe intakes, HRE, blood Hb concentrations, and total body Hb Fe content in piglets fed diets containing standard or high Fe beans from d 0 to d 35.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 0</th>
<th>d 7</th>
<th>d 14</th>
<th>d 21</th>
<th>d 28</th>
<th>d 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>8.3 ± 0.2</td>
<td>10.6 ± 0.4</td>
<td>14.1 ± 0.9</td>
<td>17.4 ± 1.2</td>
<td>21.6 ± 1.3</td>
<td>25.6 ± 0.7</td>
</tr>
<tr>
<td>High Fe</td>
<td>7.7 ± 0.2</td>
<td>9.7 ± 0.3</td>
<td>13.2 ± 0.7</td>
<td>16.3 ± 1.1</td>
<td>21.2 ± 1.2</td>
<td>27.3 ± 0.9</td>
</tr>
<tr>
<td>Feed intake, kg/d</td>
<td>-</td>
<td>0.48 ± 0.02</td>
<td>0.70 ± 0.04</td>
<td>1.07 ± 0.04</td>
<td>1.25 ± 0.04</td>
<td>2.14 ± 0.06</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>0.45 ± 0.02</td>
<td>0.65 ± 0.04</td>
<td>1.06 ± 0.05</td>
<td>1.20 ± 0.05</td>
<td>2.11 ± 0.06</td>
</tr>
<tr>
<td>Hb concentration, g/L</td>
<td>107.4 ± 4.1</td>
<td>327.6 ± 13.4</td>
<td>664.2 ± 9.8</td>
<td>1059.9 ± 11.6</td>
<td>1727.2 ± 18.6</td>
<td></td>
</tr>
<tr>
<td>High Fe</td>
<td>121.9 ± 4.7</td>
<td>370.8 ± 15.8</td>
<td>779.2 ± 13.9</td>
<td>1241.1 ± 21.1</td>
<td>2049.5 ± 16.0</td>
<td></td>
</tr>
<tr>
<td>Hb Fe content, mg</td>
<td>106.6 ± 2.6</td>
<td>102.4 ± 2.9</td>
<td>97.4 ± 4.2</td>
<td>96.8 ± 4.6</td>
<td>98.8 ± 3.6</td>
<td>100.0 ± 5.1</td>
</tr>
<tr>
<td>Standard</td>
<td>105.7 ± 2.8</td>
<td>103.2 ± 4.9</td>
<td>102.1 ± 3.9</td>
<td>105.2 ± 4.1</td>
<td>105.6 ± 1.9</td>
<td>108.1 ± 3.5</td>
</tr>
<tr>
<td>HRE, %</td>
<td>100.0 ± 3.5</td>
<td>100.0 ± 5.1</td>
<td>100.0 ± 3.5</td>
<td>100.0 ± 3.5</td>
<td>100.0 ± 3.5</td>
<td>100.0 ± 3.5</td>
</tr>
<tr>
<td>Changes in total body Hb Fe (cumulative increases from d 0), mg</td>
<td>41.8 ± 9.3</td>
<td>104.1 ± 24.3</td>
<td>162.4 ± 24.0</td>
<td>258.4 ± 36.0</td>
<td>360.5 ± 23.0</td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>36.1 ± 9.3</td>
<td>105.1 ± 12.6</td>
<td>173.8 ± 14.5</td>
<td>286.5 ± 24.7</td>
<td>426.5 ± 24.2</td>
</tr>
</tbody>
</table>

* Values are means ± SEM, n = 10. * Different from standard Fe at that time, P < 0.05.
* Values are mean daily feed intakes for the 7 d preceding the day designated in the column heading.
* Atomic mass for Fe is 55.8 g/mol.
* HRE values for each week were calculated using initial Hb Fe (start) values and values at the end of each respective week, i.e. HRE values are cumulative.
* Cumulative increases in total body Hb Fe. Values were calculated as the difference in total body Hb Fe at the end of each week and the total body Hb Fe at the start of the feeding study (d 0).
even though Fe concentrations in the polished rice they used were extremely low (3.2 µg/g for the high Fe rice and 0.57 µg/g for the control rice). The longer feeding duration in the Haas study (14) and lower Fe requirements in adult women compared with early weaned pigs may explain why they found an effect whereas Schaffer et al. (13) did not.

We chose the piglet as a model for Fe bioavailability studies because of similarities in gastrointestinal anatomy and physiology between pigs and humans. Pigs, like humans, are truly omnivorous and digestive and metabolic processes in pigs are similar to those in humans (15). Moreover, pigs readily consume monotonous diets that can be formulated to simulate human diets that are common in resource-poor regions of the world. Also, Fe deficiency develops rapidly in young pigs unless they are given i.m. Fe injections shortly after birth (16).

We chose to use the increase in body Fe in the pigs as an index of Fe absorption rather than use isotope tracers because of concerns that have been raised about the validity of single meal isotope tracer studies for estimating Fe bioavailability (17,18). To do this, we needed to accurately measure the accumulation of Fe in the animal over an extended feeding period. Unfortunately, we did not have access to an antibody against pig ferritin and therefore were unable to assess Fe stores in our pigs. We reasoned that if we could keep our pigs moderately anemic, then their Fe stores should be minimal and we could use Hb Fe as a reasonable index of absorbed Fe. This assumption is based on the concept that storage Fe is almost completely depleted before Fe deficiency anemia develops (19). We attempted to “titrate” the Fe status of the pigs so that they would be Fe-deficient anemic, but not severely so, at the start of the study. This was accomplished by reducing the usual amount of Fe given by i.m. injection to the newborn pigs. Piglets of this age grow extremely rapidly and therefore have very high Fe requirements that cannot be met by diet alone. Mean Hb concentrations in both groups at the start of the feeding period were ~105 g/L. Hb concentrations were maintained at this level throughout the study in the group receiving biofortified beans but fell slightly in the standard bean group (Table 2).

Baker and DeMaeyer (20) define nutritional anemia as “... a condition in which the Hb concentration is below the level that is normal, for a given individual, due to deficiency of one or more of the nutrients required for hemopoiesis, and conversely, as a condition in which the Hb concentration can be raised by increasing the amount of nutrient(s) absorbed.” The WHO (1) defines anemia as a Hb concentration <110 g/L for children 6–59 mo and <115 g/L for children aged 5–11 y. Cut-off points for anemia for pigs have not been clearly established, but some estimates have been suggested. In a 1980 review, Zimmerman (16) wrote that, according to the NRC, Hb concentrations ≥ 100 g/L are normal for pigs, 90 g/L is the minimum for optimal performance, 80 g/L is borderline anemic, and 60 g/L will result in retarded growth. However, these values are based on growth performance and not on normal levels as defined by Baker and DeMaeyer (20). Zimmerman (16) went on to state that absorption of Fe begins to increase at a Hb concentration < 110 g/L, suggesting that pigs with Hb concentrations <110 are Fe deficient. More recently, Rincker et al. (21) reported on a 35-d feeding trial where weaning piglets had initial Hb concentrations of ~110 g/L. Basal diets were supplemented with either 0.25, 50, 100, or 150 mg Fe/kg diet as ferric sulfate. The concentration of supplemental Fe was related to the Hb concentration (r = 0.001), suggesting that Hb concentrations of 107 g/L were not normal, i.e. they could be raised by increasing the amount of absorbed Fe. Therefore, we are confident that our pigs, with Hb concentrations of ~105 g/L, were anemic and that most of the absorbed Fe went to Hb synthesis and not to stores.

The pig model used in this study has its limitations for predicting Fe bioavailability to humans. Growing pigs must retain ~20 mg Fe/kg weight gain (16). Our pigs gained ~0.5 kg/d, so they needed to absorb 10 mg/d Fe to meet requirements. The requirement for absorbed Fe for children under 5 y is only ~0.6 mg/d or 3% of the pig’s daily requirement. Thus, one might argue that pigs are not a good model for the human, because they grow so much faster and their Fe requirements are so much higher. On the other hand, an advantage of the pig is that the length of the feeding period required for obtaining meaningful results is proportionally shorter than with humans because of their rapid growth. Another difference between pigs and humans is that the small intestine in pigs is longer than the small intestine in humans (15). This may give the pig a comparative advantage in Fe absorption because of the greater intestinal surface area. However, when expressed on a per-kilogram body weight basis, intestinal lengths are similar at ~0.1 m intestinal length per kg body weight (22).

We conclude that the biofortified beans are a promising vehicle for increasing intakes of bioavailable Fe in human populations where beans are a dietary staple and that black beans contain relatively high levels of bioavailable Fe. An efficacy trial comparing biofortified and standard beans in a human population is needed to confirm the findings reported here.

Acknowledgments
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Literature Cited