# Iron and Zinc Bioavailability in Rats Fed Intrinsically Labeled Bean and Bean−Rice Infant Weaning Food Products

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Beans are the core of the Latin American diet and contain iron and zinc. However, the bioavailability of these trace minerals from beans is low. The objective of this study was to determine if the bioavailability of iron and zinc could be improved with the use of fermentation and germination processing technologies. Black beans native to Costa Rica were grown hydroponically with either radioactive iron or zinc. The influence of fermentation and germination on iron and zinc bioavailability from intrinsically labeled infant weaning food products based on black beans and beans−rice was determined in rats. Mineral bioavailability was determined using whole-body $^{59}$Fe retention for iron, and whole-body $^{65}$Zn retention and incorporation of radiolabel into bone for zinc. Percent absorption of $^{59}$Fe from fermented products ranged between 48.0 and 58.0. Percent absorption of $^{65}$Zn ranged from 57.0 to 64.0. Fermentation did not increase iron bioavailability in rats fed fermented beans without rice. Fermentation of cooked beans significantly increased zinc retention. Germination significantly enhanced iron retention from cooked beans from 46 to 55% and from cooked beans−cooked rice from 34 to 48%. Germination significantly improved zinc absorption and retention from cooked beans without added rice.

**Keywords:** *Iron; zinc; bioavailability; intrinsic labeling; beans; rice; fermentation; germination*

## INTRODUCTION

Globally, ~2.15 billion people suffer from iron deficiency anemia (*1*). Iron deficiency affects >80% of the Latin American population (*2*). About 25% of Latin American school children suffer from iron deficiency anemia (*3*). Ten percent of infants in developed countries and 30−80% in developing countries are iron deficient by 1 year of age (*4*). In the United States, iron deficiency affects children aged 1−2 years, and iron deficiency anemia affects many poor or minority children (*5*). Infants are particularly vulnerable to iron and zinc deficiency during the weaning process. Iron deficiency anemia can decrease mental and psychomotor development in infants (*6*, *7*). Marginal zinc nutrition may limit skeletal growth (*8*), suppress immunity, lead to poor healing and dermatitis, and impair neuropsychological functions in infants (*9*).

About two-thirds of the global population derives 70% of calories from cereals and legumes (*10*). In Costa Rica, and in much of South and Central America, beans and rice are the core of infant diets; beans provide approximately 8 and 24% of calories and proteins, respectively, in weaning diets (*11*). Beans contain iron and zinc. However, the iron and zinc in beans are not well absorbed (*12*), partially due to antinutritional factors such as phytate and tannins (*13*, *14*).

Cereal grains such as rice provide calories and protein. Cereal grains, however, provide only trace amounts of iron and zinc. Small amounts of these micronutrients are found mostly in the aleurone layer cells associated with the bran and germ, which are removed during milling. Therefore, polished rice (rice after milling) tends to be low in micronutrients (*15*, *16*). Furthermore, rice contains relatively high levels of antinutrients, which reduce the bioavailability of iron and zinc, and lower levels of substances that promote the bioavailability of these micronutrients, further reducing the nutritional value of rice with respect to iron and zinc (*16*). Deficient levels of iron and zinc in rice can be overcome by combining rice with legumes such as beans, which are richer in micronutrients (*16*).

Legumes such as black beans are limiting in sulfur amino acids, cysteine, and methionine. Cereal grains including rice are limiting in lysine (*17*). Combining cooked black beans and cooked rice is likely to result in enhanced protein quality due to the resulting benefit of protein complementation.

In Costa Rica and in many Latin American countries, household food processing methods such as fermentation and germination are used in formulating infant foods (*18*−*21*). Besides improving organoleptic properties and enhancing food safety (*22*), these processes activate phytases (*23*, *24*). Activated phytases, in turn, hydrolyze phytate into lower inositol phosphates, rendering iron and zinc more bioavailable (*25*). Micronutrient availability has been evaluated for the fermented or germinated foods of tempeh and for rice−mungbean, rice−cowpea, and corn−mungbean mixtures (*26*). This potential, however, has not been assessed for black bean- and bean−rice-based foods.

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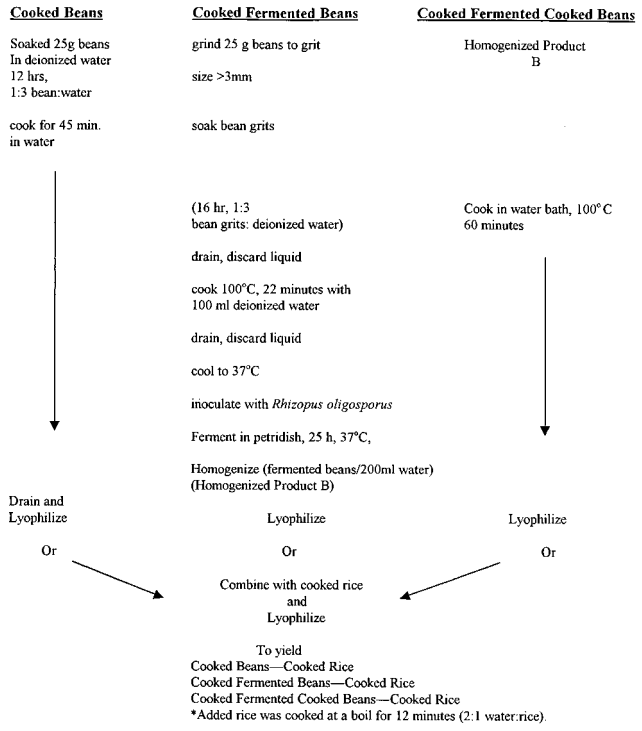


**Figure 1.** Processing of cooked bean and fermented bean products for experimental diets.

The current study was designed to determine iron and zinc bioavailability from processed black bean- and bean−rice-based products. To determine the relative influence of processing, black beans were intrinsically labeled with $^{59}Fe$ or $^{65}Zn$ and were then subjected to fermentation or germination and fed alone or combined with cooked rice. The influence of processing on iron and zinc bioavailability then was evaluated in rats.

## MATERIALS AND METHODS

**Plant Growth.** *Hydroponic Production of Beans.* Black bean seeds (*Phaseolus vulgaris* var. Talamanca) were provided by George Hosfield, Department of Crop and Soil Science, Michigan State University, East Lansing, MI. Long-grain rice was purchased locally (West Lafayette, IN). Beans were germinated and grown to seedlings in a misting booth for 6 days in a pearlite−vermiculite mixture. Seedlings then were transplanted to troughs in a circulating hydroponic growth system containing nutrient solution as described by Hoagland and Arnon (*27*) and as modified by Weaver (*28*). The pH of this solution was maintained between 5.8 and 6.0 with 0.5% $HNO_3$. The hydroponicum consisted of two fiberglass-lined troughs in which electrical pumps circulated the nutrient solution (46 L per trough, each of which contained ~40 plants; 1:128, nutrient solutionn/deionized water). Inside the greenhouse, daylight temperatures were ~27 °C. Supplemental lighting provided 146 $\mu$einstein/$m^2$ with a 14 h light period.

*Dosing of Plants.* $^{59}FeCl_3$ and $^{65}ZnCl_2$ (37 MBq each) were obtained from New England Nuclear (Boston, MA). From the stock $^{59}Fe$ and $^{65}Zn$ solutions, dilutions were made to provide 3.404 MBq per dose of $^{59}Fe$ or $^{65}Zn$. The first dose was given at initial flowering and for 4 weeks after flowering. Upon maturity, beans were harvested, and bean seed radioactivity was determined in a gamma counter (Packard Cobra II Auto-Gamma, Meridien, CT).

**Processing Methods.** *Fermentation.* Beans were fermented following the protocol of Rodriguez-Burger et al. (*29*). Fermented beans were lyophilized and ground into a fine powder or combined with cooked rice, lyophilized, and ground into a fine powder for experimental diets (Figure 1).

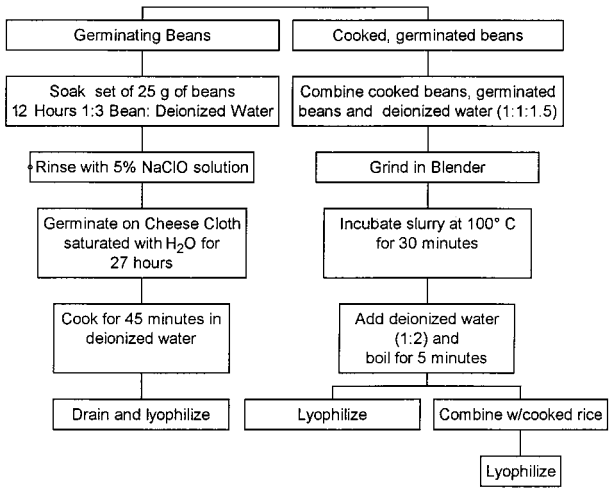


**Figure 2.** Processing of germinated bean products for experimental diets.

*Germination.* Figure 2 shows the protocol for germinating beans. Beans (25 g) were cleaned, soaked in water (35 °C; 1:3 bean/water for 12 h), and rinsed one time with 5 mL of sodium hypochlorite solution (200 ppm as chlorine) to prevent mold growth. Beans were germinated for 72 h at 30 °C and at 100% relative humidity. On day 4, when the coleoptiles were ~5 cm in length, beans were boiled in a water bath (100 °C) for 5 min, drained, cooled to room temperature (25 °C), and ground into a fine paste, after the addition of 1:1.5 ratio of beans to water, using a blender for 25 s at the lowest setting (Waring blender model 1002, Waring Products, Co., Winsted, CT). The germinated bean paste was mixed with cooked beans, then mixed with water, freeze-dried [freeze-dryer model PAC-TC-44 (FTS Systems, Inc., Stone Ridge, NY)], and ground to a fine powder using a mortar and pestle.

**Analytical Methods.** All products were analyzed in triplicate for moisture and fat using AOAC Methods 925.09 and 920.39C, respectively (*30*). Protein content of beans and rice was determined measuring the nitrogen content of duplicate samples with a conversion factor of 6.25, using Kjeldahl Method 960.52 (*30*). Amino acids (including sulfur-containing amino acids) were analyzed by CN Laboratories (Courtland, MN) using the AOAC Official Procedure 994.12. Duplicate samples were analyzed using performic acid oxidation to protect cystine and methionine followed by 6 N HCl hydrolysis. As this oxidation step is often detrimental to the recovery of tyrosine and phenylalanine and may cause interferences with the histidine peak, analysis is done with only a 6 N HCl hydrolysis for recovery of tyrosine, phenylalanine,and histidine.

Following hydrolysis, excess performic acid is destroyed using sodium metabisulfate. The sample pH is adjusted with sodium hydroxide to 3.2. Amino acids are separated on an Interaction amino acid column (ion exchange) using a three-buffer system. Following elution, amino acids are derivatized to form a fluorescence product, which is detected by a fluorometer. Samples are compared to standard curves of amino acid of known concentration. A control canola meal sample is analyzed with every 10 sample injections.

Tryptophan is extracted with base hydrolysis (6 N NaOH). Maltodextrin is added to samples, which are purged with nitrogen prior to being sealed in a hydrolysis tube. Samples are autoclaved for 18 h. Samples are then neutralized with 6 N HCl and diluted with acetate buffer. Tryptophan is separated from other components on a 5 $\mu$m C18 column (Waters Sperisorb) using paired-ion chromatography. The native fluorescence of tryptophan is used for detection. Tryptophan is determined by comparison to a standard curve prepared from reference tryptophan.

The Soxhlet extraction Method 920.39C (*30*) was used to estimate fat content. Total iron and zinc contents in harvested beans, in formulated bean products, and in test meals were

**Table 1. Iron Radiolabeled Test Diet Composition[a]**

| diet ($n$ = 10 rats per group) | CB[b,c] | CFB[b,c] | CFCB[b,c] | CGB[b,c] | CB-CR[b,c] | CFB-CR[b,c] | CFCB-CR[b,c] | CGB-CR[b,c] | CTRL[d] |
|---|---|---|---|---|---|---|---|---|---|
| product (g) | 0.9 | 0.9 | 0.9 | 1.0 | 1.6 | 1.5 | 1.3 | 1.0 | 0.9 |
| corn oil (g) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Alphacel (g) | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| sugar (g) | 1.8 | 1.7 | 1.8 | 1.6 | 1.4 | 1.1 | 1.1 | 1.6 | 1.9 |
| protein (%) | 7.0 | 7.0 | 7.0 | 7.0 | 7.2 | 7.3 | 7.0 | 7.0 | 7.0 |
| Fe (ppm) | 24.0 | 25.0 | 25.0 | 27.0 | 20.0 | 19.0 | 22.0 | 24.0 | 24.0 |
| phytate ($\mu$g/g) | 17.0 | 13.0 | 13.0 | 10.0 | 11.0 | 7.0 | 6.0 | 9.0 | 15.3 |
| phytate/iron molar ratio | 7.1 | 5.4 | 5.2 | 3.6 | 1.8 | 3.4 | 2.7 | 3.9 | 6.8 |
| cpm | 2010 | 2210 | 2028 | 1564 | 2734 | 2600 | 1939 | 1622 | 1895 |

[a] CB, cooked beans; CFB, cooked fermented beans; CFCB, cooked fermented cooked beans; CGB, cooked germinated beans; CB-CR, cooked beans cooked rice; CFB-CR, cooked fermented beans cooked rice; CFCB-CR, cooked fermented cooked beans cooked rice; CGB-CR, cooked germinated beans cooked rice. [b] Values reported for iron, zinc, and phytate contents and for cpm are the average of duplicate assays. [c] Beans intrinsically labeled with $^{59}FeCl_3$; rice not intrinsically labeled. [d] Cooked beans extrinsically labeled with $^{59}FeSO_4$.

**Table 2. Zinc Radiolabeled Test Diet Composition[a]**

| diet ($n$ = 10 rats per group) | CB[b,c] | CFB[b,c] | CFCB[b,c] | CGB[b,c] | CB-CR[b,c] | CFB-CR[b,c] | CFCB-CR[b,c] | CGB-CR[b,c] | CTRL[d] |
|---|---|---|---|---|---|---|---|---|---|
| product (g) | 0.9 | 0.9 | 0.9 | 1.0 | 1.3 | 1.3 | 1.2 | 1.0 | 1.0 |
| corn oil (g) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Alphacel (g) | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| sugar (g) | 1.7 | 1.7 | 1.7 | 1.7 | 1.3 | 1.3 | 1.6 | 1.6 | 1.7 |
| protein (%) | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| phytate ($\mu$g/g) | 14.9 | 14.3 | 12.7 | 15.0 | 5.7 | 5.5 | 4.8 | 5.8 | 14.1 |
| phytate/zinc molar ratio | 6.5 | 6.0 | 5.2 | 5.2 | 2.8 | 2.6 | 2.4 | 2.6 | 6.9 |
| Zn (ppm) | 22.8 | 23.8 | 24.6 | 28.9 | 20.2 | 21.1 | 20.1 | 22.2 | 20.3 |
| cpm | 14681 | 14079 | 14457 | 13681 | 9100 | 8583 | 10326 | 6320 | 9786 |

[a] CB, cooked beans; CFB, cooked fermented beans; CFCB, cooked fermented cooked beans; CGB, cooked germinated beans; CB-CR, cooked beans cooked rice; CFB-CR, cooked fermented beans cooked rice; CFCB-CR, cooked fermented cooked beans cooked rice; CGB-CR, cooked germinated beans cooked rice. [b] Values reported for iron, zinc, and phytate content and for cpm are the average of duplicate assays. [c] Beans intrinsically labeled with $^{65}ZnCl_2$; rice not intrinsically labeled. [d] Cooked beans extrinsically labeled with $^{65}ZnCl_2$.

determined by atomic absorption spectroscopy after sample digestion with $HNO_3$ (model 5100 PC, Perkin-Elmer, Norwalk, CT). Phytate in test diets was analyzed colorimetrically following separation by ion-exchange chromatography (*31*). Tibia zinc was analyzed by atomic absorption spectroscopy as described above.

**Formulation of Bean and Bean—Rice Products.** In the development of the fermented or germinated bean−rice products, the proportion of bean to rice was determined on the basis of the amino acid composition of processed beans and rice and the FAO/WHO/UN amino acid requirement for infants (requirements for lysine and sulfur-containing amino acids, including methionine and cysteine). Products contained 53% bean protein and 47% rice protein and provided equal ratios of sulfur-containing amino acids and lysine. Iron loads in the test meals ranged between 19 and 27 $\mu$g; zinc loads ranged between 20 and 29 $\mu$g in the 3 g portion. The intrinsically labeled products developed were cooked beans (CB), cooked fermented beans (CFB), cooked fermented cooked beans (CFCB), cooked germinated beans (CGB), cooked beans−cooked rice (CB-CR), cooked fermented beans−cooked rice (CFB-CR), cooked fermented cooked beans−cooked rice (CFCB-CR), and cooked germinated beans−cooked rice (CGB-CR), as shown in Table 1 (iron-labeled products) and Table 2 (zinc-labeled products). A control product consisting of field-grown cooked black beans extrinsically labeled with $^{59}Fe$ or $^{65}Zn$ was included to provide data for comparison with intrinsically labeled experimental beans that were grown hydroponically.

**Formulation of Experimental Diets.** Composition of the experimental diets is shown in Tables 1 and 2. Experimental diets were formulated following the protocol developed by Mason and Weaver (*32*).

Test and control diets contained the same components and were isonitrogenous (7% protein). Protein and iron or zinc in test diets was supplied by $^{59}Fe$ or $^{65}Zn$ intrinsically labeled beans or bean−rice products. Cooked beans extrinsically labeled with $^{59}FeSO_4$ or $^{65}ZnCl_2$ provided protein and iron or zinc for the control diet. The iron and zinc levels in the extrinsically labeled control diet were Fe = 23.76 ppm and Zn = 20.30 ppm per 3 g portion of the diet. The radioactivity and mineral content of control diets were equivalent to the intrinsically labeled test diets.

**Table 3. Diet Composition**

| component | diet: basal[a] (g/kg) | diet: iron deficient[b] (g/kg) | diet: marginal zinc[c] (g/kg) |
|---|---|---|---|
| sucrose[d] | 500 | 500 | 499.5 |
| casein (vitamin-free) | 200 | 200 | |
| egg white (spray-dried) | | | 200 |
| corn starch | 150 | 150 | 150 |
| fiber (Alphacel)[f] | 50 | 50 | 50 |
| corn oil[e] | 50 | 50 | 50 |
| AIN-93 mineral mix[f] | 35 | 9.62 | |
| AIN-93 mineral mix[f,g] (iron-free) | | 25.38 | |
| AIN-93 mineral mix[f,g] (zinc-free) | | | 35 |
| AIN-93 vitamin mix[f] | 10 | 10 | 10 |
| DL-methionine | 3 | 3 | 3 |
| choline bitartrate | 2 | 2 | 2 |
| *d*-biotin[h] | | | 3.5[i] |
| zinc mix[j] | | | 0.5 |

[a] Contained 24.3 $\mu$g/g iron, 33.05 $\mu$g/g zinc, 20% protein, 4.6% fat, and 0.6% moisture. [b] Contained 9.89 $\mu$g/g iron. [c] Contained 8.87 $\mu$g/g zinc. [d] Purchased locally. [e] Purchased locally. [f] Dyets Inc., Bethlehem, PA. [g] Difference made up with sucrose. [h] ICN Biochemicals, Cleveland, OH. [i] Units are mg/kg. [j] Zinc mix 0.249 g of ZnO and 0.9751 g of sucrose/g of mix provides 20 $\mu$g of zinc/g of diet when added as 1.0 g/kg.

**Rat Feeding and Handling Protocol.** *Fe Bioavailability.* Male Sprague−Dawley rats ($n$ = 70; 21 days old; Harlan Industries, Indianapolis, IN) were individually housed in stainless steel cages on a controlled 12 h light−dark cycle. Care and use of rats was in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23). Rats were provided free access to iron-deficient (Fe = 9.89 ppm) basal diet and deionized water for 24 days (Table 3). During this period, body weights of rats and food intake were monitored. Rats were tail-bled weekly, and iron status was determined by measuring hemoglobin (Hb) and hematocrit (PCV). Hb levels were determined according to the cya-

nomethemoglobin method (*33*), and PCV levels were obtained using heparinized microhematocrit capillary tubes.

*(a) Rat Feeding with Fermented Test Products*. On day 24, 70 rats were randomized for body weights, blood hemoglobin, and PCV (body weights = 148 ± 7.3 g; Hb = 6.7 ± 1.3 g/dL; PCV = 24 ± 3.4%). Rats were assigned to seven groups of 10 to consume fermented test diets (CB, CFB, CFCB, CBCR, CFB-CR, CFCB-CR, or extrinsic control, Table 1). Animals were fasted for 12 h and were fed a 3 g portion of the $^{59}$Fe radiolabeled fermented test diets.

*(b) Rat Feeding with Germinated Test Products*. On day 24, 50 rats were randomized for body weights, blood hemoglobin, and PCV (body weights = 153 ± 6.5 g; Hb = 7.0 ± 1.5 g/dL; PCV = 23 ± 3.0%). Rats were assigned to five groups of 10 to consume germinated test diets (CB, CGB, CB-CR, CGB-CR, or extrinsic control, Table 2). Animals were fasted for 12 h and were fed a 3 g portion of the $^{59}$Fe radiolabeled germinated test diets.

*Whole-Body Radioactivity Counting of $^{59}$Fe.* Animals consumed the entire portion of the respective radioactive test diets and control diet in 3−4 h. On day 0, and on subsequent counting days, radioassays of an $^{59}$Fe standard and a cesium ($^{137}$Cs) standard were conducted to ensure counter stability. The iron and cesium standards used had the following amount of radioactivity: $^{59}$Fe, day 0 = 2565 cpm; $^{137}$Cs, day 0 = 435746 cpm. Rats then were assayed by introduction into a large-well crystal whole-body gamma counter that consisted of a thallium-activated sodium iodide crystal scintillation detector (Harshaw Chemical Co., Cleveland, OH). This counter was equipped with a Canberra series 30 multichannel analyzer (Canberra Industries Inc). Coincidence loss of this system was <3% at 25 × $10^4$ cpm. Activity of $^{59}$Fe was measured using a window setting of 965−1490 keV, which included the two $^{59}$Fe photopeaks (1099 and 1291 keV). Animals were returned to cages and were fed iron-adequate basal diet ad libitum for the remainder of the experimental period (Table 3). Whole-body iron retention was measured on day 0 and again on days 1, 2, 4, 6, 8, and 10. On day 10, all animals were sacrificed by overexposure to $CO_2$.

*Zinc Bioavailability*. Rats were fed a marginal zinc diet for 9 days (Zn = 8.87 ppm) (Table 3). On day 10, rats were randomly assigned to 12 groups ($n$ = 6), fasted for 12 h, and fed a 3 g portion of the $^{65}$Zn radiolabeled test diets. Whole-body $^{65}$Zn was determined as described above for $^{59}$Fe except that the counting window was 700−1644 keV. Radioactivity was measured on days 0, 1, 2, 4, 6, 8, and 10. After overexposure to $CO_2$ on day 10, tibias were removed from animals to determine percent zinc retention.

**Data Analysis.** Whole-body radioactivity data were corrected for background, radionuclide decay ($^{59}$Fe = 45 days, $^{65}$Zn = 245.1 days), and daily whole-body gamma counter fluctuations and checked for normality (Wilk-Shapiro W test). Normality plots were used to detect outlier data. Percent retention of administered radionuclide was plotted against time and was determined for day 0, 1, 2, 4, 6, 8, and 10 using the following formula:

$$\% \text{ retention} = \frac{\text{whole-body count at time } t}{\text{whole-body count at time 0}} \times 100$$

Radioactivity measured on day 0 (4 h after rats were given radioactive test meals) represents absorbed radionuclide plus unabsorbed radioactivity in the gastrointestinal tract before excretion of the nuclide. Because not all of the administered radionuclide was absorbed on day 0, percent apparent absorption of radionuclide was calculated by extrapolating the linear portion of the plot of percent retention versus time for the period extending from day 2 to day 10 after the isotope administration (*34*). The SAS Regression Procedure provided $y$-intercepts for each test group (true absorption on day 0). Calculated $y$-intercepts of the linear regression analysis were the percent absorption of $^{59}$Fe and $^{65}$Zn. To test whether differences among test groups for true percent absorption and percent nuclide retention were significant, one-way analysis of variance was employed [PROC GLM, $P$ < 0.05, Student−Newman−Keuls (SNK) test]. Relative zinc bioavailability from each of the products was evaluated by comparing significant differences in tibia zinc retention 10 days post product feeding.

**Table 4. Absorption and Retention of $^{59}$Fe from Fermented Products[a]**

| diet group | calcd % absorption[b] | % retention on day 10[b] |
|---|---|---|
| CB | 57.9 ± 3.9[a] | 47.9 ± 5.6[a] |
| CFB | 53.1 ± 1.6[a] | 44.0 ± 4.9[a,b] |
| CFCB | 57.2 ± 2.3[a] | 47.0 ± 3.8[a] |
| CB-CR | 48.8 ± 2.2[b] | 39.4 ± 3.5[b,c] |
| CFB-CR | 55.2 ± 3.6[a,b] | 44.3 ± 5.2[a,b] |
| CFCB-CR | 48.4 ± 3.1[b] | 38.3 ± 5.2[c] |
| CTRL | 53.4 ± 4.2[a,b] | 38.3 ± 4.4[c] |

[a] CB, cooked beans; CFB, cooked fermented beans; CFCB, cooked fermented cooked beans; CB-CR, cooked beans cooked rice; CFB-CR, cooked fermented beans cooked rice; CFCB-CR, cooked fermented cooked beans cooked rice; CTRL, cooked beans extrinsically labeled with $^{59}FeSO_4$. [b] Mean ± SD, $n$ = 10 per group. Different superscripts within columns denote significant differences at $P$ < 0.05 SNK.

**Table 5. Absorption and Retention of $^{59}$Fe from Germinated Products[a]**

| diet group | calcd % absorption[b] | % retention on day 10[b] |
|---|---|---|
| CB | 56.9 ± 4.7[b] | 45.6 ± 6.6[b] |
| CGB | 59.5 ± 4.4[a,b] | 54.5 ± 4.7[a] |
| CB-CR | 58.2 ± 4.7[a,b] | 34.2 ± 6.8[c] |
| CGB-CR | 56.7 ± 6.8[b] | 48.0 ± 3.4[b] |
| CTRL | 64.4 ± 5.4[a] | 35.5 ± 5.7[c] |

[a] CB, cooked beans; CGB, cooked germinated beans; CB-CR, cooked beans cooked rice; CGB-CR, cooked germinated beans cooked rice; CTRL, cooked beans extrinsically labeled with $^{59}Fe$-$SO_4$. [b] Mean ± SD, $n$ = 10 per group. Different superscripts within columns denote significant differences at $P$ < 0.05 SNK.

## RESULTS

Absorption of $^{59}$Fe in rats fed fermented bean products was 48−58%, and retention of $^{59}$Fe was 38−48% (Table 4). The highest percent iron retention was recorded for CB and the lowest for CFCB-CR and extrinsically labeled CB (control). There was no difference ($P$ < 0.05) in percent absorption or retention of $^{59}$Fe between rats fed fermented and nonfermented products.

Absorption and retention data ($^{59}$Fe) for germinated beans are presented in Table 5. Germination increased retention of $^{59}$Fe from CB from 46 to 55% and that from CB-CR from 34 to 48% ($P$ < 0.05). The highest retention of $^{59}$Fe was from CGB (55%) and the lowest from the extrinsically labeled CB control (36%). Comparison of the absorption of $^{59}$Fe intrinsically and extrinsically labeled cooked beans showed a significant difference. There was also a significant difference in retention of the label, with the intrinsic label being retained the most.

The influence of fermentation on zinc absorption and retention is presented in Table 6. The $^{65}$Zn absorption from bean products was 79−87%, and retention of $^{65}$Zn from fermented products was 59−69%. Retention of zinc was highest in rats fed CFB (69%). Fermentation increased zinc retention but did not improve absorption of $^{65}$Zn. Retention of $^{65}$Zn was significantly higher in rats fed CFB (69%) compared to CB (60%).

As can be seen from Table 7, zinc absorption for germinated bean products was 75−87% and retention was 61−72%. Absorption and retention of $^{65}$Zn significantly increased upon germination. There was no difference in zinc absorption or retention in rats fed the germinated bean products that were combined with rice, as compared to germinated beans alone.

**Table 6. Absorption and Retention of $^{65}$Zn from Fermented Products[a]**

| diet group | calcd % absorption[b] | % retention on day 10[b] |
|---|---|---|
| CB | 78.9 ± 9.3[a,b] | 59.94 ± 5.7[b] |
| CFB | 85.8 ± 4.5[a] | 69.22 ± 7.2[a] |
| CFCB | 86.6 ± 6.5[a] | 62.81 ± 4.9[a,b] |
| CB-CR | 80.3 ± 3.4[b] | 60.40 ± 6.9[b] |
| CFB-CR | 82.0 ± 8.9[a,b] | 58.78 ± 5.7[b] |
| CFCB-CR | 80.9 ± 4.3[a,b] | 62.25 ± 5.5[a,b] |
| CTRL | 82.0 ± 3.6[a,b] | 64.68 ± 4.7[a,b] |

[a] CB, cooked beans; CFB, cooked fermented beans; CFCB, cooked fermented cooked beans; CB-CR, cooked beans cooked rice; CFB-CR, cooked fermented beans cooked rice; CFCB-CR, cooked fermented cooked beans cooked rice; CTRL, cooked beans extrinsically labeled with $^{65}ZnCl_2$. [b] Mean ± SD, $n$= 6 per group. Different superscripts within columns denote significant differences at $P < 0.05$ SNK.

**Table 7. Absorption and Retention of $^{65}$Zn from Germinated Products[a]**

| diet | calcd % absorption[b] | % retention on day 10[b] |
|---|---|---|
| CB | 75.1 ± 8.7[c] | 60.7 ± 9.1[b] |
| CGB | 87.2 ± 2.3[a] | 72.4 ± 4.4[a] |
| CB-CR | 85.4 ± 5.0[a,b] | 65.4 ± 3.8[a,b] |
| CGB-CR | 84.9 ± 5.6[a,b] | 66.0 ± 5.7[a,b] |
| CTRL | 79.6 ± 3.9[b,c] | 64.7 ± 9.4[b] |

[a] CB, cooked beans; CGB, cooked germinated beans; CB-CR, cooked beans cooked rice; CGB-CR, cooked germinated beans cooked rice; CTRL, cooked beans extrinsically labeled with $^{65}ZnCl_2$. [b] Mean ± SD, $n$ = 6 per group. Different superscripts within columns denote significant differences at $P < 0.05$ SNK.

Average incorporation of zinc into tibias was 94.5 μg/g. There was no difference between diet groups in percent $^{65}$Zn retention in rat tibia bone ($P < 0.05$). Erdman et al. (*35*) reported that there was no difference in tibia zinc content in rats that were fed soybean products of varying pH values. Similar to the lack of influence of processing on tibia zinc retention observed in the present study, Lihono and Serfass (*36*) reported that mean total femur zinc was not significantly different between hydrothermally cooked and conventionally processed soy milks.

The phytate content of iron-radiolabeled test diets ranged from 6 to 17 μg/g (Table 1). For zinc-radiolabeled test diets, phytate content was 5−15 μg/g (Table 2). In $^{59}$Fe-labeled diets, CB had the highest phytate content. In $^{65}$Zn-labeled diets, CB and CGB had the highest phytate levels, and similar to the iron diets, the lowest level of phytate was measured in CFCB-CR. Phytate levels are lower in the presence of rice (Table 1), but despite this decrease in the level of phytate, a decrease in iron absorption and retention was observed when the cooked bean and the cooked fermented cooked bean products were combined with rice (Table 4).

## DISCUSSION

This paper is the first to document iron and zinc bioavailability from fermented or germinated black bean−rice products. Results showed that both fermentation and germination improved zinc retention and that only germination positively influenced iron retention. This is in contrast to published findings from in vitro studies conducted with soybean-based products by Mugula (*26*), in which a 2.5-fold increase in in vitro iron availability from fermented maize−soybean tempeh was shown. Svanberg et al. (*25*) have reported that lactic acid fermentation of non-tannin cereals with added flour from germinated sorghum seeds increased iron availability by 5%.

The finding in the present study that fermentation increased zinc retention (by 15%) from the bean products is similar to findings documented in the literature. Modjapawiro et al. (*37*) showed that *Rhizopus* fermentation increased the relative biological value of zinc from 73% in nonfermented soybeans to 85% in the fermented product. Larsson et al. (*38*) reported that retention of zinc was higher from germinated versus nongerminated oats.

The varying influences of fermentation and germination on iron versus zinc availability observed in this study warrant discussion. Phytate is a major inhibitor of iron and zinc availability in cereals and legumes (*39*). Sandberg and Svanberg (*40*) have shown that even trace amounts of phytate in wheat bran have strong inhibitory effects on iron absorption. Similarly, Beard et al. (*41*) showed that a 2-fold variation in phytate content of soybeans did not lead to an increase in iron availability. Our results show that a decrease in phytate levels did not result in improved iron absorption and retention. These results indicate that other factors such as tannins, amount and source of protein, selected amino acids, chemical form of iron, and duration of feeding may also play a role in iron absorption and retention from these products (*42*). Consistent with our results, Welch and Graham (*16*) have shown that there was no apparent relationship between the amount of iron absorption and the concentration of phytate in the bean products.

It has been reported that the higher the fermentation time of cereal grains, the greater the reduction in phytic acid (*43*). Fermentation and germination activate phytases in cereal grains or flours, degrade phytic acid, and improve iron absorption (*40*). Bean grits in the current study were fermented for 24 h compared to other studies that have used higher fermentation times, for example, 48 or 72 h. Under the current fermenting conditions, phytate levels decreased from 17 to 13 μg/g in iron-labeled diets and the phytate/iron ratio decreased from 7 to 5 μg/g (Table 1). With increased fermentation time, it is expected that a further reduction in phytate content would occur, thus leading to a potential increase in availability of iron. Bean surface area and particle size of the bean grits can affect fermentation. Grits are larger particles than bean flour. This may have affected the extent of fermentation achieved in the products.

Germinating beans decreased the phytate level, in the iron study, by 50% (from 17 for CB to 10 for CGB) and the phytate/iron ratio from 7.1 to 3.6 (Table 1). This decrease in phytate content with germination probably explains the increase in the availability of iron from germinated beans, 46% (CB) versus 55% (CGB) (Table 5).

Fermentation and germination did not alter the phytate levels in test diets in the zinc study (Table 2). Differences in absorption and retention after fermentation and germination were observed without phytate level modifications. The ratio of phytate/zinc in food has been used as a predictor of zinc bioavailability (*44*, *45*). The phytate/$Zn^{2+}$ molar ratios of the test diets were 2.4 and 6.5 (Table 2). A minimum molar ratio of 15:1 (phytate/zinc) has been shown to decrease zinc absorption from cows milk and infant formulas (*46*).

The extent to which the intrinsic versus extrinsic form of iron is retained remains controversial. In the present

study, retention of the intrinsic form of the iron label was higher than that from the extrinsic label. Retention was similar for intrinsic and extrinsic forms of zinc. Boza et al. (*47*) showed that the intrinsic iron label had a lower retention than the extrinsic label. They proposed that due to a relatively slower release of intrinsic iron, this form of iron may be absorbed at a slower rate than the more freely available extrinsic form of iron. However, Bjorn-Rasmussen et al. (*48*) showed that extrinsic tags typically equilibrate with the non-heme iron in the diet, thus reflecting an absorption pattern similar to that of intrinsic iron. Other researchers have shown that the extrinsic label frequently overestimates absorption by 10−20%. The method in which the extrinsic label is incorporated merits consideration. In the present study, the extrinsic isotope was added after the test diet was formulated. Weaver et al. (*49*) showed that when $^{65}ZnCl_2$ was mixed with soy flour prior to incorporation in a diet, the absorption ratio was 0.99 compared to the ratio of 1.1 when the label was added to the premixed diet.

The findings of this research indicate that the incorporation of fermented and germinated bean-based food products into animal diets was beneficial. The 40% retention of $^{59}Fe$ and 60% retention of $^{65}Zn$, if applicable to humans, could help meet the infant's dietary requirements for iron and zinc, respectively.

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