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Iron Bioavailability to Piglets from Red and White Common Beans (*Phaseolus vulgaris*)

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Polyphenols in foods may chelate dietary Fe and lower its bioavailability. Concentrations of phenols are higher in red beans than in white beans. The aim of this study was to compare iron bioavailabilities from red and white beans in a piglet hemoglobin repletion model. Fe deficient cross bred piglets (Hampshire × Landrace × Yorkshire) were used. Nutritionally balanced diets (except for Fe) were formulated to contain 50% precooked, dehydrated beans (either small red or Great Northern white). At age 5 weeks, the piglets were assigned to two groups and fed diets containing either red or white beans for 4 weeks. Weight and hemoglobin (Hb) concentrations were monitored weekly. Feed intakes were measured daily. Hemoglobin repletion efficiency (HRE) was calculated as the gain in total body hemoglobin Fe (Hb-Fe) divided by Fe intake. Hb concentrations, Hb-Fe gains, and HRE were not different between the groups at any time point ($p > 0.05$). HRE values in the red bean group were 50% in the first week and 30% over the entire 4 week period. In the white bean group, they were 56 and 26%, respectively. Proline-rich protein mRNA concentrations in parotid glands were higher in the red bean group compared to the white bean group. These results show that iron bioavailabilities from red and white beans are similar and suggest that pigs adapt to the inhibitory effects of polyphenols on iron absorption by increasing the secretion of protective proline-rich proteins in the saliva.

KEYWORDS: Iron bioavailability; beans; polyphenols; pigs; hemoglobin repletion; proline-rich protein

INTRODUCTION

Pulses, including the common bean (*Phaseolus vulgaris*), are an economical source of protein, complex carbohydrates, minerals, and vitamins for millions of people. They are a dietary staple in many countries in Latin America where they have been part of the diet for centuries (*1*). They are also widely consumed in Africa and Asia (*2*).

Populations that rely on pulses and cereals as their major dietary staples tend to have high prevalences of iron deficiency. This may be caused, in part, by the low bioavailability of iron in these diets because of the presence of polyphenols, phytates, and other antinutrients (*3*). Beans contain significant amounts of a variety of polyphenolic compounds including flavanols, anthocyanins, and tannins (*4*). The concentrations and classes of these polyphenols vary substantially among different varieties of beans. Some polyphenols contribute to the color of beans, and colored beans tend to have higher concentrations of polyphenols compared to white beans (*5*). Tannins are a complex class of water soluble polyphenols that have the ability to precipitate proteins (*6*). They include galloyl-glucose esters (hydrolyzable tannins) and polymers of proanthocyandins (condensed tannins) (*7*). Tannins may inhibit digestibility of proteins and carbohydrates by binding to digestive enzymes. They may also interfere with the absorption of nutrients by binding to proteins on the surfaces of enterocytes (*8*). Polyphenols are recognized as potent inhibitors of iron absorption (*3, 9*).

Hu and colleagues compared iron bioavailabilities from white, red, pinto, and black beans using an in vitro digestion Caco-2 cell culture method (*5*). Iron concentrations in the different bean types were similar, but the bioavailable iron estimated from ferritin formation in the Caco-2 cells was extremely low in the three colored beans. It was 10-fold higher in the white beans, suggesting that color-imparting polyphenols inhibit iron absorption. Beiseigel and colleagues compared iron absorption from pinto (a colored bean) and Great Northern (a white bean) beans in nonanemic women by using an extrinsic radio iron tag method (*10*). They also showed low iron bioavailability, about 2%, but found no difference between the colored and white beans. Therefore, the aim of the present study was to compare iron

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bioavailabilities from white and red beans by using in vitro digestion/Caco-2 cell culture and piglet hemoglobin repletion and models.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from VWR (West Chester, PA), Sigma Chemicals (St. Louis, MO), or Fisher Scientific (Fair Lawn, NJ) unless stated otherwise. Water used in the preparation of reagents for cell culture and digesta and tissue analyses was double deionized. Glassware and utensils were soaked in 1.2 M HCl for no less than 4 h and rinsed with deionized water prior to use to remove contaminant iron.

Caco-2 Cell Trial. An in vitro digestion/Caco-2 cell culture model developed by Glahn et al. (*11*) was used in a preliminary assessment of iron bioavailability from the beans, and bean diets were selected for a piglet trial (see below). Briefly, samples (approximately 20 g) were ground to a fine powder by using a coffee grinder. Aliquots of the powders (approximately 1.0 g) were mixed on a rocking platform (~80 rpm) in 15 mL of buffer containing 140 mM NaCl and 5 mM KCl (pH 2). The pH of the mixture was then adjusted to 2 with 1.0 M HCl. Pepsin (0.5 mL of a 20 mg/mL, pH 2 pepsin solution; Sigma P6887, 3200−4500 units/mg protein) was added, and the mixture was incubated for 1 h at 37 °C on a rocking platform to simulate gastric digestion. The resulting pepsin digest was then adjusted to pH 7 with 0.1 M $NaHCO_3$, and a pancreatin−bile mixture (2.5 mL; 0.3 g pancreatin (Sigma P3292) and 1.8 g bile extract in 150 mL of 0.1 M $NaHCO_3$) was added. The mixture was incubated at 37 °C for two additional hours on a rocking platform to simulate intestinal digestion. This pancreatin−bile digestion took place in a small upper chamber positioned over a monolayer of cultured Caco-2 cells. Caco-2 cells (at passage 28−35) had been seeded at a density of 50 000 cell/cm^2 and were grown in Dulbecco's modified eagle medium (DMEM, Gibco) at 5% CO_2/95% air, relative humidity, and 37 °C. The experiment was conducted 14 days post seeding. Prior to the uptake assay, cultures were washed with tempered (37 °C) minimum essential medium (MEM) at pH 7, and 1 mL of MEM was placed in each well. The contents of the upper chamber were separated from the Caco-2 cell layer by a 15 000 molecular weight cutoff dialysis membrane (Spectra/Por 2.1, Spectrum Medical, Gardena, CA) that allowed iron released from the digested samples to diffuse into the medium bathing the cells in the lower chamber.

After the simulated intestinal digestion, the upper chamber was removed, and the cells were incubated at 37 °C for 23 h to allow ferritin to form. The growth medium was removed, and the cell monolayer was harvested from the bottom chamber by adding deionized water and placing it in a sonicator to disrupt the cells. Caco-2 cells synthesize ferritin in response to increases in intracellular iron concentration. Therefore, ferritin concentration in the cells, measured in an aliquot of the cell suspension by using an enzyme-linked immunoassay (RAMCO, Houston, TX), was used as an index of iron uptake. Cell ferritin was expressed as nanograms of ferritin per milligram of cell protein.

Piglet Feeding Trial. *Diets.* Diets were formulated to meet or exceed the National Research Council nutrient requirements (*12*), except for iron, of pigs weighing between 5 and 10 kg (**Table 1**). Whole dent corn, soybean meal, and red or white beans (ADM Edible Bean Specialties, Decatur, IL) were ground and mixed thoroughly with the other ingredients.

Piglets. Newborn crossbred piglets (Yorkshire × Hampshire × Landrace), farrowed at the Cornell University Swine Research Farm, were given an intramuscular injection of iron dextran (Sanofi Animal Health Inc., Overland Park, KS) containing 50 mg of Fe, half the recommended dose, so that they would develop iron deficiency by the time they were weaned. The piglets were weaned at age 21 days and fed an iron-deficient corn-soy transition diet (76.8 mg of Fe/kg feed) until they were switched to the treatment diets. When the piglets reached age 28 days, their weights and hemoglobin concentrations were measured, and barrows having similar weights and hemoglobin concentrations were selected and divided into two groups such that the mean weights and hemoglobin concentrations of each group were similar. Each piglet was kept in an individual metabolism cage to enable collection of orts. The barn was maintained at 22−25 °C, with a 12 h light/dark cycle. The groups were randomly assigned to either the red bean diet (RBD) or white bean diet (WBD). The treatment period began when the piglets were 35 days old. The piglets had free access to water at all times.

Table 1. Compositions of Transition and Experimental Diets

ingredient	diet (g/kg diet, calculated from formulation)		
	transition	red bean	white bean
ground dent corn	570	370	370
whey protein isolate, BiPro[a]	50	50	50
dehydrated whole small red bean[b]	0	500	0
dehydrated Great Northern white bean[c]	0	0	500
soybean meal	300	0	0
threonine, 100% pure	0	0.5	0.5
methionine, 50% pure	0	3	3
corn oil	20	20	20
tylan 10	5	5	5
vitamin premix[d]	10	10	10
mineral premix (no Fe)[d]	45	45	45
Fe and phytate concentrations in diets, determined by analysis			
Fe[e] in beans (mg/kg bean), mean ± SE, $n = 3$		47.9 ± 1.1	53.6 ± 0.2
Fe[e] in diet (mg/kg diet), mean ± SE, $n = 3$	76.8 ± 10.5	37.0 ± 1.0	42.3 ± 0.8
phytate[f] (mmol/kg diet), mean ± SE, $n = 3$		8.57 ± 0.47	9.00 ± 0.30

[a] BiPro is a Davisco Foods International, Inc. product. [b] Cooked, dehydrated small whole red beans (362001) were donated by ADM Edible Bean Specialties (Decatur, IL). [c] Great Northern White beans (562001) were donated by ADM Edible Bean Specialties (Decatur, IL). [d] Vitamin and mineral premixes were purchased from Dyets, Inc. (Bethlehem, PA). They were formulated to provide the following concentrations (per kg diet): thiamin HCl, 1 mg; riboflavin, 3.75 mg; pyridoxine HCl, 1 mg; niacin, 10 mg; Ca pantothenate, 12 mg; folic acid, 1.3 mg; biotin, 0.2 mg; vitamin B12, 15 μg; retinol palmitate, 8 mg; vitamin D3, 0.5 mg; α-tocopheryl acetate, 88 mg; menadione sodium bisulfite, 0.8 mg; Ca, 7.5 g; P (available), 3.5 g; Na, 1.5 g, Cl, 1.5 g; Mg, 0.4 g; K, 2.04 g; Cu, 5 mg; I, 0.13 mg; Fe, 0 mg; Se, 0.14 mg; Zn, 50 mg. [e] Iron concentrations in the diets were determined by an inductively coupled plasma/atomic emission spectrophotometer (ICAP 61E Thermal Jarrell Ash Trace Analyzer, Jarrell Ash Co. Franklin, MA) following wet ashing (*14*). [f] Phytate concentrations were determined by an HPLC method (*15*).

A wet mash was prepared before each meal by adding water to dry feed in a ratio of 1 part water to 4 parts feed and offered to the pigs twice daily. On experiment day, 0, 200 g of diet (dry weight) was given to each pig. Leftover and spilled food were collected and weighed daily to determine feed intake. The feed ration for each piglet was increased by 20 g/day if there was no leftover feed on the previous day. Body weights and hemoglobin concentrations were measured weekly.

Feed was withdrawn 8 h before slaughter. The animals were killed on experiment day 28 by electrical stunning and exsanguination. The gastrointestinal tracts were immediately removed, and digesta samples from various segments were collected and frozen for later analyses. Parotid gland tissue was excised immediately postslaughter, frozen in liquid nitrogen, and stored in a −80 °C freezer until analysis.

The protocol was approved by the Cornell University Institutional Animal Care and Use Committee.

Hemoglobin Repletion Efficiency Assay. Blood samples were drawn from the subclavian vein into Vacutainers tubes (BD Vacutainers sodium heparin, ref no. 367878, NJ). Hemoglobin concentrations were determined colorimetrically by the cyanmethemoglobin method (*13*). Total body hemoglobin Fe for each pig was calculated from hemoglobin concentration and estimated blood volume using the following formula:

$$\text{Hb Fe (mg)} = [\text{BW (kg)} \times 0.067 \text{ L of blood/kg of BW}] \times [\text{Hb (g/L of blood)}] \times [3.35 \text{ mg of Fe/g of Hb}]$$

where Hb Fe = the total body hemoglobin iron; Hb = hemoglobin; BW = body weight.

Hemoglobin repletion efficiency (HRE) was calculated as follows:

$$\mathrm{HRE} = \frac{\text{Hb Fe, mg (final)} - \text{Hb Fe, mg (initial)}}{\text{total Fe intake, mg}} \times 100\%$$

HRE values were calculated using data collected on days 7, 14, 21, and 28. At each time point, the value for initial Hb Fe was the value determined on day 0; that is, HRE values are cumulative, not weekly values.

HRE provides an estimate of the percentage of ingested iron that is absorbed. It is a slight underestimate because some absorbed iron (approximately 10%) is incorporated into myoglobin and other iron-containing proteins and a small amount is lost via urine and sloughed skin and intestinal epithelial cells.

Diet Analyses. Total iron concentration of the diets was measured by ICP-AES as described previously (*14*). Phytic acid was determined with a high pressure liquid chromatography method (*15*). Inulin and oligosaccharidies were measured using the method of Quemer et al. (*16*). Selected polyphenols were determined by HPLC analysis following extraction with methanol/1 mol/L HCl (*17*). Total phenols were quantified using the Folin−Ciocalteu total phenolics assay following extraction with 80% ethanol, drying the extract on a rotary evaporator, and reconstituting with 20% DMSO (*18*). Gallic acid was used as a standard, and concentrations of phenols are expressed as gallic acid equivalents per gram sample.

Digesta Analyses. Samples of colon digesta were collected from the proximal, mid, and distal sections of the colon immediately postslaughter and frozen at −20 °C until analysis. Soluble ferrous and total soluble iron concentrations were determined in the three sections of the colon digesta with a colorimetric ferrozine assay (*19*). Briefly, for the soluble iron measurement, 1 g of digesta sample was diluted in 10 mL of deionized water. The mixture was centrifuged at 15 000*g* for 15 min. Soluble ferrous iron: Aliquots (1 mL) of the supernatant were mixed with 0.1 mL of ferrozine solution (5 mg of ferrozine/mL, Sigma P9762, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazine-4′,4′′-disulfonic acid sodium salt), and the absorbance of the mixture was read at 562 nm after 15 min. Total soluble iron: 0.1 mL of reducing reagent (containing 1.2 M HCl, 50 g/L hydroxylamine monohydrochloride) was added to the mixture of supernatant and ferrozine; the absorbance of the sample was measured at 562 nm the next day. Standards: A standard curve was prepared using standards containing 0, 25, 50, 75, and 100 μM iron. The standard solutions were prepared by diluting an iron atomic absorption standard solution (Aldrich 30 595-2, Milwaukee, WI).

Total Iron in Digesta. The moisture content of the colon digesta was determined by drying a weighed sample in an oven. A portion of the dried sample, 0.25 g, was weighed and acid-digested as described previously for iron analysis with ICP-AES. Dry matter content in digesta: Fresh cecum and colon digesta samples were collected and weighed; the samples were oven-dried at 125 °C and reweighed. The dry weight was expressed as a percentage of the fresh weight.

Analysis of mRNA in Parotid Glands. Frozen parotid gland tissue (50−100 mg) was homogenized with 1.0 mL of TRI reagent (Molecular Research Center (MRC), Cincinnati, OH). The cells were lysed with 0.4 mL of chloroform to extract the total RNA. The samples were centrifuged at 16 000*g* for 17 min, and the clear supernatant was transferred into an empty centrifuge tube. Chilled isopropanol (1 mL) was added to the clear supernatant to purify the sample. The isopropanol/sample mixture was centrifuged at 16 000*g* for 11 min, and the supernatant was discarded. Chilled 70% ethanol (1 mL) was added to the sample pellet as additional purification, and the mixture was transferred into a 1.5 mL microcentrifuge tube. The ethanol/sample mixture was centrifuged at 16 000*g* for 5 min before the ethanol was discarded. The microcentrifuge tube was inverted to dry for 5 min, and then, 50 μL of nuclease-free water was added to the microcentrifuge tube. The microcentrifuge tube was capped and placed in the 37 °C water bath for 3 min to allow the RNA to dissolve. The sample was frozen at −80 °C until analysis. RNA was reverse-transcribed using oligo (dT) and Superscript II reverse transcriptase (MBI, fermentas). Primers were designed to correspond to the previously published sequence of human salivary proline-rich protein, PBII (7618 bp). Ribosomal 18S was used to normalize the results, with primers from the human ribosomal 18S mRNA (GI 124517659).

Table 2. Ferritin Concentrations in Caco-2 Cells Exposed to Bean and Bean Diet Digests

treatment	ferritin (ng/mg of protein)[a]
baseline[b]	5.56 ± 0.22[d]
red bean	2.13 ± 0.16[c]
white bean	4.90 ± 0.41[d]
red bean diet	1.16 ± 0.12[c]
white bean diet	5.35 ± 0.26[d]

[a] Cells were exposed to only MEM growth media without added food digests and Fe. [b] Means ± SE, $n = 6$. Means with no letters in common are significantly different, $p < 0.05$.

Table 3. Body Weights, Hemoglobin Concentrations, and Hemoglobin Repletion Efficiencies of Piglets Fed Red or White Bean Diets[a]

treatment	day 0	day 7	day 14	day 21	day 28
			body weight (kg)		
red bean	6.8 ± 0.2	7.1 ± 0.2	8.7 ± 0.3	10.9 ± 0.5	12.7 ± 0.7
white bean	6.9 ± 0.2	7.0 ± 0.3	8.5 ± 0.4	10.5 ± 0.6	12.8 ± 0.7
			hemoglobin concentration (g/L)		
red bean	71.8 ± 2.9	82.8 ± 3.9	77.4 ± 3.5	79.2 ± 4.4	73.8 ± 3.6
white bean	71.1 ± 3.6	83.6 ± 4.0	80.0 ± 3.4	78.4 ± 3.0	71.6 ± 3.7
			hemoglobin repletion efficiency[b] (%)		
red bean		49.9 ± 11.0	32.1 ± 4.4	35.2 ± 4.1	29.9 ± 2.9
white bean		55.6 ± 13.0	32.1 ± 4.0	31.0 ± 3.3	25.5 ± 3.4

[a] Means ± S.E., $n = 8$. Within a time period, there were no significant differences between the red and white bean groups for body weight, [Hb], or HRE, $p > 0.05$. [b] HRE values on days 7, 14, 21, and 28 are cumulative from day 0, e.g. the value on day 28 represents absorption over the entire 4 week feeding period.

Statistical Analyses. Data from the pig studies were analyzed by *t* test (MINITAB Release 14.20, State College, PA). Data from the Caco-2 cell experiments were analyzed by one-way analysis of variance. Tukey's pairwise comparison was applied when a significant difference was detected by one-way analysis of variance. Means were considered significantly different at $p < 0.05$.

RESULTS

Caco-2 Trial. Iron bioavailabilities of the beans and bean diets determined with in vitro digestion/Caco-2 cell culture are shown in **Table 2**. Ferritin concentrations were higher in cells exposed to the white beans and white bean diet compared to the red beans and red bean diet ($p < 0.05$). However, ferritin concentrations in all the treatments were at or below baseline (baseline refers to a reagent blank, i.e. cells were exposed to the enzyme and bile salt solutions used in treating the samples but contained no sample).

Pig Trial. Body weights, hemoglobin concentrations, and hemoglobin repletion efficiencies are shown in **Table 3**. There were no significant differences in hemoglobin concentration between the two groups during the study ($p > 0.05$). Weight gain in both groups averaged 6 kg during the four-week study ($p > 0.05$). Hemoglobin concentrations fluctuated during the study but did not differ between groups ($p > 0.05$), and concentrations at the end of the study were nearly identical to concentrations at the beginning. Due to growth and the resulting increase in blood volume, hemoglobin Fe (Hb Fe) increased continuously throughout the study. However, there were no differences between the groups in Hb Fe gain. The day 7 results for both hemoglobin concentration and HRE appear to be excessively high. A possible explanation for these high values may be that the pigs, in adapting to the very high bean concentrations in the diets, became slightly dehydrated due to diarrhea. Dehydration could explain both the low gains in body weight and the high hemoglobin concentrations on day 7.

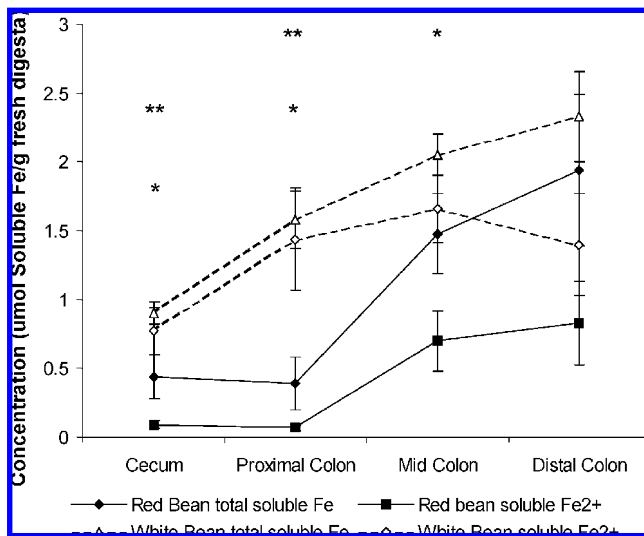

Figure 1. Soluble ferrous and total soluble Fe concentrations in fresh cecal and colon digesta (means ± SE, $n = 6$). Fresh digesta were collected postslaughter and were diluted 1:10 with deionized water and centrifuged. Ferrous and total Fe concentrations in the diluted supernatants were determined colorimetrically. Within a segment, * indicates a significant difference between RBD and WBD for soluble ferrous Fe, ($p < 0.05$); ** indicates a significant difference between RBD and WBD for total soluble Fe, ($p < 0.05$).

Dry matter content in cecum and colon digesta ranged between 12 and 27%; the percentage of dry matter increased as the digesta migrated from the cecum to the distal colon (data not shown). There was no significant difference in digesta dry matter content between the RBD and WBD groups ($p > 0.05$). The soluble Fe^{2+} concentration was significantly higher in the WBD group than in the RBD group for the cecum and proximal and mid colon contents, $p < 0.05$ (**Figure 1**). The cecum soluble Fe^{2+}concentration was approximately 7 times higher in the WBD group than in the RBD group. Both the soluble Fe^{2+} and total soluble Fe concentrations in the cecum digesta were significantly higher in the WBD group ($p < 0.05$). In the proximal colon, the soluble Fe^{2+} concentration was approximately 20 times greater in the WBD group compared to the RBD group ($p < 0.05$) while in the mid colon it was 2 times greater ($p < 0.05$). Total soluble Fe concentrations in the mid colon and both soluble Fe^{2+} and total soluble Fe concentrations in the distal colon did not differ between groups ($p > 0.05$). Iron concentrations in dried digesta from the proximal and mid colon were slightly higher in the WBD group (p < 0.05) (**Figure 3**).

The Folin−Ciocalteu assay provides an estimate of the total phenolics, expressed as gallic acid equivalents (μg/g). **Figure 2** shows that corn, which was added to the bean diets as a carbohydrate source, contains a high concentration of total phenolics. RB had a significantly higher concentration of total phenolics than WB ($p < 0.05$); however when incorporated into the diets, these differences were no longer significant (**Figure 2**).

The relative concentration of proline-rich protein mRNA in salivary gland tissue in the RBD group was approximately double that the WBD group, $p < 0.05$.

Phytate/Fe molar ratios were high in all samples and were higher in the diets than the beans due to the high phytate concentration in corn (**Table 4**). Red and white beans did not differ significantly from each other in the Fe/phytate molar ratio ($p > 0.05$). There was also no significant difference between the red and white bean diets ($p > 0.05$).

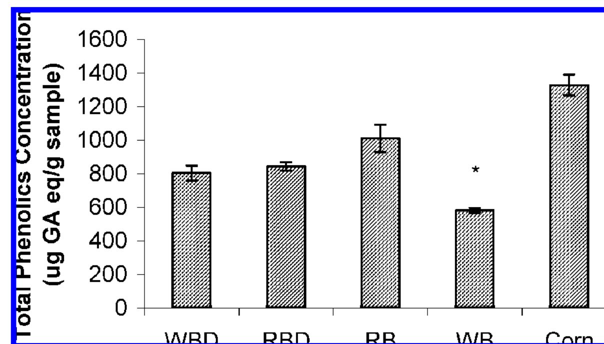

Figure 2. Concentrations of phenolic compounds in the diets and the beans and corn. Phenolic concentration is expressed as micrograms of gallic acid equivalent (GAE) per gram of sample. WBD = white bean diet; RBD = red bean diet; RB = red beans; WB = white beans. Means ± SE, $n = 3$. * indicates significantly different from RB, $p < 0.05$.

The oligosaccharide profiles were not significantly different between the red and white beans and between the red and white bean diets, $p > 0.05$. Concentrations of stachyose and raffinose were 1% or less (w/w) in the red and white beans and diets. No inulin was detected in the beans, corn, or soy (data not shown).

DISCUSSION

Iron concentrations in dry beans typically range from 50 to 80 μg/g (*20*), but there is considerable genetic variation (*21*). We have observed concentrations as high as 100 μg/g in lines bred for high iron content. Therefore, beans are promising candidates as vehicles for delivering iron to populations. However, the bioavailability of bean iron may be low. Nonanemic women with low iron stores absorbed less than 2% of iron from an isotopically labeled meal of common beans (*22*). Beiseigel and colleagues also observed low iron absorption by nonanemic women, reporting 2.2% from Great Northern beans and 1.6% from pinto beans (*10*). In the latter study, addition of ascorbic acid at molar ratios of 15−20 ascorbic acid/iron increased iron absorption by about 3-fold. Iron bioavailability from soybeans appears to be higher than from common beans. Murray-Kolb et al. (*23*) reported that women with low iron stores absorbed 27% of iron from intrinsically labeled soybeans.

Since phytate concentrations in the two varieties of beans used in the present study were very similar, it is unlikely that phytate is responsible for any differences in bioavailability between the beans. Polyphenol concentrations were higher in red beans compared to white beans, and therefore, we hypothesized that iron bioavailability would be higher in the white bean diets. Ferritin formation in Caco 2 cells was 4-fold higher in the white bean diet compared with the red bean diet, confirming our hypothesis. However, ferritin concentrations in all treatments were below baseline, suggesting that none of the treatments were providing available iron to the cells. Rather, they were all inhibiting iron uptake with the red beans and red bean diet being more inhibitory that the white beans and diet. In a previous in vitro digestion/Caco-2 study, a Great Northern bean from a different source produced ferritin concentrations well above baseline while a pinto bean (a colored bean) also fell below baseline (*10*). In numerous experiments, it has been our experience that white bean samples typically give ferritin formation values in the range 10−40 ng of ferritin/mg of cell protein, depending on the genotype (R. P. Glahn, personal observations). The results of the present study appear to be an aberration in that it is the first time we have seen such low values for white beans.

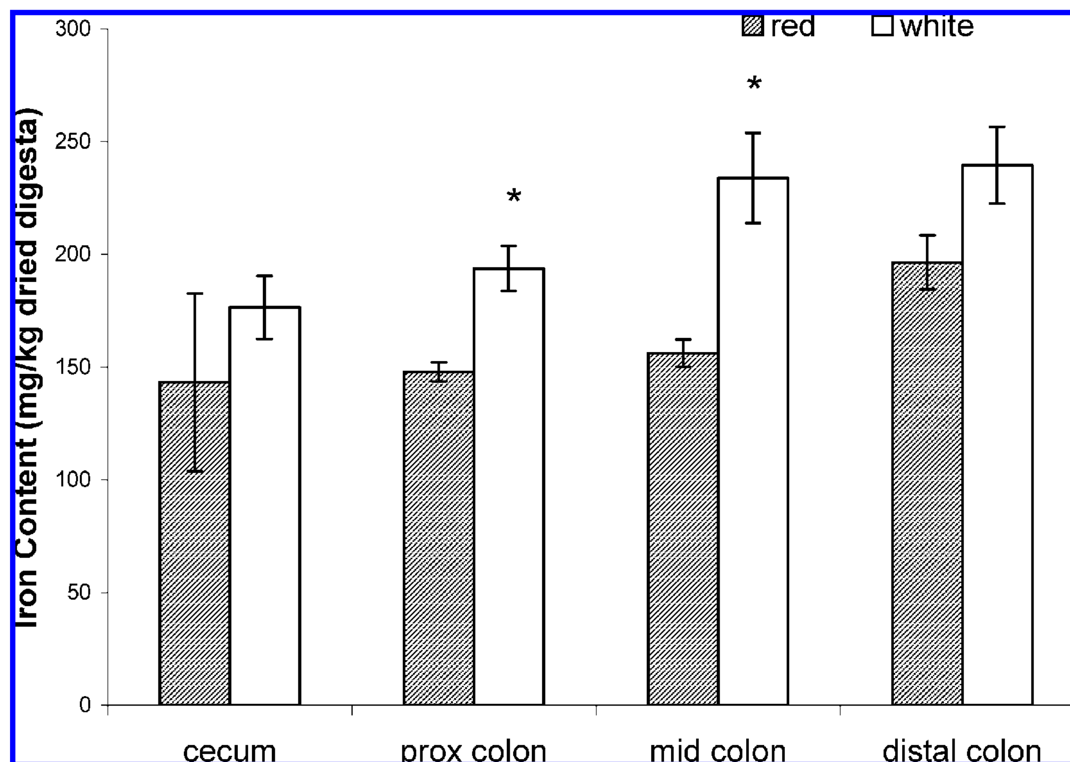

Figure 3. Total iron content of oven-dried digesta from cecum and colon. Fresh digesta that was collected from cecum and proximal, middle, and distal colon was oven-dried. Total Fe concentrations in the dried pig digesta from the two diet groups were expressed as mean ± SE. * indicates a statistically significant difference between RBD and WBD within an intestinal segment, $n = 8$ and $p < 0.05$.

Table 4. Phytate Concentrations and Phytate/Fe Molar Ratios in Beans and Bean Diets[a]

sample	phytate (μmol/g)	phytate/Fe molar ratio
red bean	8.33 ± 0.18	9.71 ± 0.41[b]
white bean	9.20 ± 0.26	9.59 ± 0.30[b]
red bean diet	8.57 ± 0.47	12.93 ± 0.39[c]
white bean diet	9.00 ± 0.30	11.89 ± 0.55[c]

[a] Means ± SE, $n = 3$. Within a column, means with no letters in common are significantly different, $p < 0.05$.

In contrast, results from our piglet hemoglobin repletion trial do not support the hypothesis. We saw no differences in hemoglobin concentration or hemoglobin repletion efficiency between the white and red bean groups. Beiseigel et al. (*10*) also observed no difference in absorption by women between white and red beans, but as mentioned above, absorption from both varieties of beans was very low in their study.

A possible explanation for the more than 10-fold higher iron absorption we observed in the pigs compared to absorption from women is that our pigs were severely anemic (hemoglobin concentrations between 70 and 80 g/dL) and therefore were highly upregulated for iron absorption. In both of the human studies mentioned above (*10, 22*), the women were not anemic, although one subject with very low serum ferritin absorbed about 8% of the iron in the beans (*22*). In both of these studies, iron absorption was inversely correlated to serum ferritin concentration, suggesting that iron deficient subjects are able to up-regulate iron absorption from beans. Therefore, a possible explanation for the high absorption rates in the present study (HRE values of 25−30% over the 4 week feeding period) may be that iron absorption was highly up-regulated in response to iron deficiency anemia.

Another possible explanation for the higher rates of absorption by our pigs is that the response indicator we used (change in hemoglobin iron over time) assessed accumulated iron absorption over an extended period, whereas Donangelo et al. (*22*) and Beiseigel et al. (*10*) fed a single meal of isotopically labeled beans following an overnight fast. Habitual exposure to polyphenols may illicit an adaptive response to the antinutritive properties of polyphenols. The adaptation may involve increased secretion of proline-rich proteins in the saliva. Fava bean hulls added to a casein-based diet increased the weight and proline-rich protein (PRP) content of parotid glands in rats (*24*). Rats exposed to purified hydrolyzable tannin for 10−18 days excreted significantly more tannin in the feces from an isotopically labeled single dose of tannin than rats given the same labeled dose but without previous exposure to tannins (*25*). This suggests that PRPs bind tannins and thereby prevent them from interacting with digestive enzymes and possibly with nonprotein dietary components. Recently, we showed that iron absorption from tea was 50% lower than that from an iron/water solution in rats not previously exposed to tea. In contrast, iron absorption from tea and water was identical in rats that had been fed diets containing tea for 5 days prior to the iron dose (*26*). This is clear evidence that chronic exposure to polyphenols reduces their inhibitory effect on iron absorption. In the present study, we measured concentrations of PRP-encoding mRNA in the parotid glands of our pigs to determine whether there might be differences between the red and white bean groups. We found significantly higher levels in the red bean group. This may explain the differences we saw between the Caco-2 trial and the piglet trial. It is unlikely that Caco-2 cells could have adapted to the higher levels of polyphenols in the red beans given the very short exposure time and the fact that we did not include parotid cells in the culture.

It is also possible, however, that some species of animals are more efficient at absorbing iron than others. Rats absorb nonheme iron from a variety of sources more efficiently than

humans do. One study with rats reported 53−75% (*27*) and another 48−65% (*28*) absorption of bean iron. High phytate concentrations in beans (**Table 1**) may explain the observed low iron bioavailabilities of bean iron to humans but not to rats. Differences in intestinal phytase activity between humans and rats could account for these species differences, since hydrolysis of phytate may reduce its inhibitory effect. Phytase activity in mucosal homogenates from human small intestine was 30 times lower than that in rat mucosal homogenates (*29*). Similar data on mucosal phytase activity for pigs has not been reported but supplemental phytase added to corn-soybean rations enhanced phytate phosphate utilization in weanling pigs and reduced fecal phosphate by 50% (*30*). This suggests that endogenous phytase activity in pigs is low and therefore is not an explanation for higher iron absorption in pigs compared to humans. An alternative explanation for the relatively high iron absorption observed in this study compared to the human studies is that the length of the pig small intestine is as much as 2 times the length of the human small intestine (*31, 32*).

The substantially higher levels of soluble ferrous and total iron in the colon in animals on the white bean diet were striking and might lead one to expect a higher percentage absorption in the white bean group (**Figures 1** and **2**). The fact that we did not see a difference in HRE between the two groups suggests that iron absorption from the colon is insignificant compared to iron absorption in the proximal small intestine.

Neither diet provided sufficient bioavailable iron to increase hemoglobin concentrations in the piglets, but there was enough iron to prevent a fall in hemoglobin. Therefore, since the pigs grew at a rapid rate, there were substantial increases in the total circulating hemoglobin iron over the 4 week period. More than 60% of the iron in the diets was from the beans, and this shows that, in spite of high concentrations of phytate and polyphenols, beans can provide substantial amounts of bioavailable iron to pigs. The fact that the pigs did not recover from their anemia is not surprising, since the iron concentration in both diets was only about 40% of the recommended concentration for piglets of this age (*12*). In previous studies where we formulated diets to contain >100 μg of Fe/kg of diet as ferrous sulfate, we observed significant increases in hemoglobin concentration in piglets in less than 2 weeks (*33, 34*).

In summary, our results suggest that corn-bean diets can provide sufficient iron to maintain iron status in rapidly growing piglets with high iron requirements but cannot replete low hemoglobin concentrations. The high percentage absorption we observed compared to absorption rates in human trials may indicate that pigs are more efficient at utilizing dietary iron than humans. Alternatively, differences in iron status between our pigs and the human subjects may be the explanation. We conclude that corn-bean diets have potential for delivering bioavailable iron to humans, especially when they suffer from iron deficiency anemia, if the diets are consumed over an extended period of time and beans bred for high iron concentration are consumed.

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