Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model

Amy K. Proulx
Iowa State University

Manju B. Reddy
Iowa State University, mbreddy@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/fshn_ag_pubs

Part of the Food Science Commons, Hematology Commons, Human and Clinical Nutrition Commons, Medical Pathology Commons, Plant Biology Commons, and the Plant Breeding and Genetics Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/fshn_ag_pubs/63. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.
Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model

Abstract
Heme iron has been identified in many plant sources—most commonly in the root nodules of leguminous plants, such as soy. Our objective was to test the effectiveness of soy root nodule (SRN) and purified soy hemoglobin (LHb) in improving iron bioavailability using an in vitro Caco-2 cell model, with ferritin response as the bioavailability index. We assessed bioavailability of iron from LHb (either partially purified (LHbA) or purified (LHbD)) with and without food matrix and compared it with that from bovine hemoglobin (BHb), ferrous sulfate (FeSO4), or SRN. Bioavailability of each treatment was normalized to 100% of the FeSO4 treatment. When iron sources were tested alone (100 ug iron/mL), ferritin synthesis by LHbD and BHb were 19% \( (P > 0.05) \) and 113% \( (P < 0.001) \) higher than FeSO4, respectively. However, when iron sources were used for fortification of maize tortillas (50 ppm), LHbA and BHb showed similar bioavailability, being 27% \( (P < 0.05) \) and 33% \( (P < 0.05) \) higher than FeSO4. Heat treatment had no effect on heme iron but had a significant reduction on FeSO4 bioavailability. Adding heme (LHbA) iron with nonheme (FeSO4) had no enhancement on nonheme iron absorption. Our data suggest that heme iron from plant sources may be a novel value-added product that can provide highly bioavailable iron as a food fortificant.

Keywords
Iron bioavailability; heme iron; soy root nodules; leghemoglobin

Disciplines
Food Science | Hematology | Human and Clinical Nutrition | Medical Pathology | Plant Biology | Plant Breeding and Genetics

Comments
Iron Bioavailability of Hemoglobin from Soy Root Nodules Using a Caco-2 Cell Culture Model

AMY K. PROULX AND MANJU B. REDDY*
Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011

Heme iron has been identified in many plant sources—most commonly in the root nodules of leguminous plants, such as soy. Our objective was to test the effectiveness of soy root nodule (SRN) and purified soy hemoglobin (LHb) in improving iron bioavailability using an in vitro Caco-2 cell model, with ferritin response as the bioavailability index. We assessed bioavailability of iron from LHb (either partially purified (LHbA) or purified (LHbD)) with and without food matrix and compared it with that from bovine hemoglobin (BHb), ferrous sulfate (FeSO₄), or SRN. Bioavailability of each treatment was normalized to 100% of the FeSO₄ treatment. When iron sources were tested alone (100 ug iron/mL), ferritin synthesis by LHbD and BHb were 19% (P > 0.05) and 113% (P < 0.001) higher than FeSO₄, respectively. However, when iron sources were used for fortification of maize tortillas (50 ppm), LHbA and BHb showed similar bioavailability, being 27% (P < 0.05) and 33% (P < 0.05) higher than FeSO₄. Heat treatment had no effect on heme iron but had a significant reduction on FeSO₄ bioavailability. Adding heme (LHbA) iron with nonheme (FeSO₄) had no enhancement on nonheme iron absorption. Our data suggest that heme iron from plant sources may be a novel value-added product that can provide highly bioavailable iron as a food fortificant.

KEYWORDS: Iron bioavailability; heme iron; soy root nodules; leghemoglobin

INTRODUCTION

Iron deficiency is a major nutritional problem, affecting over 2 billion people (1). Fortification of foods with iron has been a successful strategy for improving iron content of foods; however, the bioavailability of iron fortificants is often decreased due to the presence of inhibitors within the food matrix. Iron bioavailability is higher from heme iron sources because of lack of inhibition from chelating compounds including polyphenols and phytate and because of its intact absorption by pathways different than those of nonheme iron (2). Heme iron in the human diet is generally present in animal sources as a part of hemoglobin and myoglobin but is also found in many invertebrates, bacteria, fungi, and widely distributed in the plant kingdom (3). However, these heme proteins currently do not provide a significant amount of iron in human diets. Plant hemoglobins are most commonly found in nodulating legumes as part of the symbiotic nitrogen fixation pathway; however, nonsymbiotic hemoglobins also exist in many plants with diverse and yet undescribed roles in plant physiology (4).

Leghemoglobin (LHb), a symbiotic hemoglobin, is a monomeric heme protein originally identified in soybean root nodules and has been studied extensively (5, 6). Because of its high affinity for oxygen, LHb makes less oxygen available, enhancing the nitrogen fixation process. The nitrogenase enzyme produced by symbiotic bacteria within legume roots requires an anaerobic environment, and therefore, the plant produces a heme protein that is capable of scavenging oxygen within the cytosol of the root, resulting in a low-oxygen environment ideal for nitrogen fixation (7). Leghemoglobin accumulates iron in roots creating a large iron store. Iron levels of up to 2.5 mg total iron/g dry weight basis have been measured in soy nodules, with up to 26% of the total iron in heme form in the unpurified root (8). Most researchers are interested in the physiological role and structure of hemoglobin in the plant, but to our knowledge no studies have been reported on the use of plant hemoglobins for improving iron bioavailability of human diet.

Caco-2 cells are human intestinal adenocarcinoma cells exhibiting enterocyte-like biochemical and morphological characteristics and have been used widely for nonheme iron bioavailability studies (9, 10). Heme bioavailability in Caco-2 cells is not well studied, but recent work has shown that these cells synthesize enzymes involved in heme uptake and metabolism, in particular hemeoxygenase (11), and that the mechanisms of heme transport are similar between humans and Caco-2 cell models (12). This evidence makes the Caco-2 cell model appealing for its potential in evaluating heme iron bioavailability. The objective of this study was to determine the iron bioavailability of crude soy root nodule extract (SRN) and two purified soy leghemoglobins and to compare their bioavailability with that of bovine hemoglobin (BHb) using the Caco-2 model. The underlying objective of this study is to introduce the concept of using plant hemoglobin as a heme iron source in diets that are consumed by humans and to promote further research into this area.

* To whom correspondence should be addressed. Phone: (515) 294-2024. Fax: (515) 294-5390. E-mail: mbreddy@iastate.edu.
MATERIALS AND METHODS

LHb Preparation. Soybean plants (cultivar − OAC Bayfield) were field raised in sandy loam soil at Cambridge Research Station, University of Guelph, during the 2002 growing season on cropland used for potatoes in the prior two growing seasons. Seed was inoculated with Hi Stick Prep Rhizobium japonicum (Becker Underwood Canada, Saskatoon, Saskatchewan) at 1.8 g inoculant per 1 kg seed application rate. Fields were irrigated as needed. The root nodules were mechanically harvested at R7 maturity, removed from root structures, and lyophilized. Dried nodules were ground to pass a 30 mesh screen. Crude LHb preparation was prepared by reconstitution of lyophilized dried nodule powder with water:1:5 w/w, followed by centrifugation (5000g for 30 min). Supernatant was collected, lyophilized, and stored at −20 °C until use. The aqueous extract was also used to prepare partially purified LHb (referred to as LHbA) by using 50–80% ammonium sulfate ((NH4)2SO4) precipitation, followed by desalting by dialysis with water. The desalted protein extract was lyophilized and stored at −20 °C for further use. Ion exchange chromatography with DEAE Sepharose (GE Healthcare, Piscataway, NJ) was used to further purify the LHb obtained from (NH4)2SO4 precipitation. The column was equilibrated to pH 7.0 and run with a linear gradient elution starting with deionized (DI) water and ending with 1 mol/L NaCl at pH 7.0. Eluants that were in the 405 nm absorbance peak were pooled, desalted by dialysis with water. The desalted protein extract was lyophilized and stored at −20 °C for further use. The SDS–PAGE gel (12% acrylamide) was stained with Coomassie Brilliant Blue G250 (Biorad) and destaining with 10% (v/v) aqueous acetic acid. Protein purity was estimated by gel optical density using QuantOne software (BioRad).

Tortilla Preparation. Unfortified masa harina (Ultrawhite #1 tortilla flour, Cargill Foods, Paris, IL) was fortified with 50 ppm iron with one of the following: SRN, LHbA, BHb (98% pure, Sigma Aldrich, St. Louis, MO) or ferrous sulfate (FeSO4) (Sigma Aldrich). Masa harina (200 g) was mixed with fortificants, sealed in a plastic container, and thoroughly mixed by shaking 2 min. Masa harina was reconstituted with DI water (1:2 w/w), weighed to 25 g portions, flattened by tortilla press, and fried at 200 °C on a Teflon pan, 2 min on each side. Tortillas were lyophilized and ground to pass a 30 mesh screen, sealed, and stored in the dark at room temperature. Iron content of lyophilized tortilla was verified using the method described above for total iron, and the bioavailability was assessed using Caco-2 cells.

In Vitro Digestion. For experiments conducted without food matrix, SRN, LHbA, BHb, and FeSO4 were weighed to provide 1 mg iron in 10 mL final volume (100 μg/mL). Initially the iron sources were dissolved in 1 mL of a 140 mmol/L NaCl, 5 mmol/L KCl solution prior to digestion. Fortified tortilla samples were weighed to provide 200 μg total iron (−3.5 g to represent 50 ppb iron) and suspended in 5 mL of DI water. Pepsin was prepared by solubilizing 0.2 g of porcine pepsin A (1:60000) in 5 mL of 0.1 mol/L HCl. Pancreatin and bile solution were prepared by dissolving 0.05 g of porcine pancreatin (4 × USP) and 0.3 g of bile extract in 25 mL of 0.1 mol/L sodium bicarbonate (NaHCO3). Traces of minerals were removed from pepsin and pancreatin mixtures by treatment with Chelex-100 (BioRad) for 30 min, filtered through a separation column, and re-eluted with 5 mL of 0.1 mol/L HCl or 10 mL of 0.1 mol/L NaHCO3, respectively (15). The pH of all samples, either with or without food matrix, was adjusted to pH 2.0 with 0.5 mol/L HCl, pepsin was added (0.5 mL), and the digest was incubated at 37 °C for 1 h on an orbital shaker at 200 rpm. The pH was then adjusted to 6.5 with 1.0 mol/L NaHCO3 solution, and 2.5 mL of pancreatin solution was added. The samples were again incubated with shaking at 37 °C for 15 min and centrifuged, and the supernatant was then heat treated for 4 min at 100 °C in a boiling water bath to inactivate proteolytic activity as described by other researchers (16). Samples in aqueous matrix were adjusted to a final 10 mL volume with 140 mmol/L NaCl and 5 mmol/L KCl to provide a final concentration of 100 μg/mL. Samples with tortilla matrix were adjusted to final volume of 20 mL using DI H2O to provide a 10 μg/mL iron. All digests were centrifuged at 5000g for 5 min, and supernatants were used for cell bioavailability experiments.

Cell Culture. All reagents for cell culture work were from Sigma Aldrich or Gibco BRL (Grand Island, NY) unless otherwise mentioned. Caco-2 cells were purchased at passage 17 from American Type Culture Collection (Rockville, MD). The following experiments were conducted during passages 20–26. Cells were grown in a culture flask with Dulbecco’s Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 1% v/v nonessential amino acids, and 1% v/v antibiotic–antimycotic solution. Cells were maintained at 37 °C in an incubator with 5% CO2. Media was changed 3 times weekly. At 7 d, the cells were rinsed with Earle’s Balanced Salt Solution (EBSS), trypsinized with trypsin/EDTA (Gibco BRL) for 30 min, and seeded at a density of 5 × 104 cells/cm2 in a 75 cm2 culture flask for continued growth, or seeded on collagenized (Type 1 Rat tail collagen) 12-well cell culture plates (Corning Costar) at a density of 5 × 104 cells/cm2 for iron bioavailability experiments. The cell culture plates were maintained under incubator conditions similar to those of the cell culture flask. Iron bioavailability experiments were conducted 15 d post seeding after rinsing with EBSS.

Iron Bioavailability by Caco-2 Cells. Bioavailability of heme sources was determined using ferritin concentration as an index of bioavailable iron in response to iron uptake (15). Serum free media modified from a published study (14) (DMEM with 1% v/v nonessential amino acids, 1% v/v antibiotic–antimycotic solution, 10 mmol/L PIPES [piperazine-N,N′-bis(2-ethanesulfonic acid), hydrocortisone (4 mg/L), insulin (5 mg/L), selenium (5 μg/L), triiodothyronine (34 μg/L), and epidermal growth factor (20 g/L)] was applied to the cell culture (0.5 mL) before adding an equal volume (0.5 mL) of sample supernatants and was incubated for 2 h. An additional 0.5 mL of serum-free media was added to the initial 1 mL, followed by a further incubation for 22 h. After 24 h total incubation, the samples were removed by aspiration from the cell culture wells, and cells were rinsed with 1 mL of EBSS. The cells were then lysed by addition of 0.5 mL of deionized water to each well and sonicated with a probe-type sonic dismembrator at lowest setting (<1 W output) for 15 s. Total cellular protein was determined in the lysates by the Bradford Coomassie assay (Pierce Laboratories, Rockford, IL). Ferritin in the lysates was determined by radioimmunoassay (Fer-Iron II, Ramco Laboratories, Stafford, TX) and measured using Cobra-II Gamma Counter with SpectraWorks software (Packard BioSciences, Meriden, CT). After normalizing ferritin concentration to cell protein concentration, the values were normalized, defined as percentages compared to FeSO4, and expressed as relative biological values (RBV).

Effect of Heat on Fortificant Bioavailability. By adding the iron fortificant to the masa harina (subjected to heating) or to freeze-dried unfortified tortilla powder (subjected to no heating), the effect of heat treatment on iron bioavailability was assessed by using Caco-2 cells as described above.

Effect of Heme on Nonheme Iron Bioavailability. Masa tortilla was fortified using the largest band at ∼14 kDa, which is the reported molecular
FeSO₄ in aqueous solutions. Relative biological value (RBV) 
purified fraction of soy leghemoglobin), BHb (bovine hemoglobin), and
on ANOVA with Tukey’s multiple comparison test.

Fraction of the SRN extract (LHb A ). Lane 4: DEAE purified fraction following
(NH₄)₂SO₄ precipitation (LHb D ). Lane 5: standard 2 (6.5–26.6 kDa).

Iron bioavailability of SRN (soy root nodule), LHb D (DEAE 
extract, LHb A ((NH₄)₂SO₄ purified soy leghemoglobin), BHb (bovine hemoglobin), 
and FeSO₄ with 50 ppm iron. Relative biological value (RBV) = ng ferritin/
μg cellular protein relative to the mean FeSO₄ value. Bars (mean ± SE, n=5) with similar letters are not significantly different (P > 0.05) based
on ANOVA with Tukey’s multiple comparison test.

The results of the iron bioavailability study using aqueous solutions of SRN, LHb D, and BHb are shown in Figure 2. The relative biological values (RBV, compared to 100% with FeSO₄) were 28 ± 10%, 19 ± 17%, and 113 ± 13% higher than FeSO₄, respectively, (mean ± SEM) for SRN, LHb A, and BHb. The iron bioavailability of BHb was 2-fold higher than all other samples (P ≤ 0.001), but the iron bioavailability of SRN and LHb D was similar to that of FeSO₄. Since the bioavailability of SRN and LHb D was similar, LHb A, we found no advantage using the pure fraction. Hence, the partially purified LHb A fraction was used for tortilla fortification studies.

Unlike the previous results without food matrix, the RBV for 50 ppm fortified tortillas with SRN was 19% lower than that of FeSO₄, but was not significantly different (Figure 3). The LHb A and BHb tortillas exhibited 27 ± 6% and 33 ± 10% higher bioavailability than FeSO₄ (P < 0.05) and with no difference between them. Although based on dry weight, total iron content varied for SRN, LHb A, and BHb (1.42, 1.7, and 2.9 mg/g, respectively), and weight adjustment provided equal amounts of total iron in all the treatments prior to in vitro digestion. The heme iron content also varied based on dry weight basis, 1.0, 1.4, and 2.3 mg/g for SRN, LHb A, and BHb, respectively, but the 74–83% of added iron was in heme form in all the treatments.

Heat displayed no significant impact on bioavailability other than on FeSO₄ bioavailability (Figure 4). The bioavailability of FeSO₄ was 36 ± 6% lower in samples fortified before cooking (P < 0.001), indicating that iron bioavailability was decreased by heating the fortificant during cooking. Although not significant, SRN, LHb, and BHb showed increases in bioavailability with heating. Nonheme iron bioavailability was not affected by adding heme iron from the LHb A at any concentration ranging from 25 to 75% (data not shown).

Heme iron bioavailability has long been known to have higher bioavailability (18–29%) than nonheme iron (<10%) (17), because heme iron has a different uptake pathway than nonheme iron. Heme is released from the globin protein and iron is absorbed intact with porphyrin into the mucosal cells (18). High bioavailability of heme can be partly attributed to this different pathway and partly to the lack of inhibition from dietary factors such as phytate and polyphenols which strongly inhibit nonheme iron absorption (2, 17).

Currently heme iron in the human diet is almost exclusively from animal sources, and its intake has been shown to have a positive correlation with iron status (19, 20). Indeed, one major
bioavailability of Plant Hemoglobin from Soy

Bioavailability of Plant Hemoglobin from Soy J. Agric. Food Chem., Vol. 54, No. 4, 2006

recommendation for improving iron status in populations is to incorporate sources of heme iron in the diet because of its high iron bioavailability (21). However, the incorporation of animal-sourced heme iron is often unfeasible because of economic costs or because cultural and religious barriers forbid the consumption of meat in populations where iron deficiency is prevalent.

While it has long been known that plants produce heme proteins, they have not been extensively studied in human iron nutrition. A recent rat hemoglobin repletion study by one of the authors showed a bioavailability of 59% with SRN compared to FeSO4 which was similar to a 60% bioavailability with BHb (8). Unlike human studies, the lower bioavailability of BHb compared to FeSO4 raises some concern of this model for measuring heme iron absorption; however, the similarity in bioavailability between LHb and BHb promoted this current study. Since the rat is not shown to be the most reliable model to assess human bioavailability (22), we have used a cell culture model to further evaluate LHb iron bioavailability studies. The Caco-2 cell model is appealing because of its low cost, reliability, and wide use for nonheme iron bioavailability (9, 10). Interest in the use of this model for studying heme bioavailability and metabolism has been limited, but is increasing, as is described in recent studies (11, 12). Heme iron absorption in Caco-2 cells was shown to be affected by hemeoxygenase induction, more importantly by iron status similar to humans (12). Our results showing higher bioavailability of BHb compared to FeSO4 alone or with food further promote that the Caco-2 cell model may indeed be useful for evaluating heme iron bioavailability. Since iron within the epithelial cells has a similar metabolic fate regardless of its source, either heme or nonheme, ferritin may be a useful index of bioavailability, regardless of the source of the iron (15).

There are a number of reasons why a discrepancy exists in the bioavailability results in Figures 2 and 3. The first possible explanation is the difference in the iron concentrations in the uptake solutions, which was 10-fold higher in aqueous solution experiments (Figure 2), and the presence of solid milieu of food affecting uptake. Since we reported the values relative to FeSO4, it is also important to consider how FeSO4 bioavailability is affected by the food matrix when comparing the results from different experiments. Differences in FeSO4 bioavailability greatly influence the RBV of the treatments. Hence, the low RBV of BHb in Figure 3 should not be viewed as a reduction of bioavailability in the presence of food. Iron chemistry and solubility is highly dependent on the digestive milieu. Lower LHb bioavailability compared to that of BHb in aqueous solution (Figure 2) might also be attributed to the differences in globin fractions of those two proteins. Although structural homology between these two proteins is high (5), the amino acid sequence of bovine α or β compared to soy globin shows minimal sequence homology when compared with BLOSUM-62 (NCBI Protein-Protein BLAST). Globin protein has been shown to increase the bioavailability of heme iron (12), and the presence of hydrophobic peptides hydrolyzed from the globin protein during digestion is known to affect the absorption of heme iron (23). It is possible that globin degradation may be different between LHb and BHb and the solubility of iron may be better maintained in BHb with its globin degradation products. It is also possible that there may be a lipid enhancing effect in the BHb which is not present in the LHb. Since the BHb was derived from bovine reticulocytes, it may have trace amounts of lipids which in the aqueous environment may influence iron bioavailability (24). Our results suggest that LHb may not be anymore beneficial than FeSO4 if it is used as a supplement. However, our goal was to assess bioavailability with food, which is more applicable for determining the use of LHb for food fortification.

The low bioavailability of SRN with tortilla may be due to the presence of nonhemoglobin fractions in the extract compared to LHbA or BHb. Since we have to use a 30% higher amount of this fraction to get equal amounts of heme iron compared to LHbA, its use in food fortification may be limited due to organoleptic problems. Because of high purity, we can use a lesser amount of LHb to avoid acceptability problems, but it adds higher cost for preparation. However, LHb obtained from (NH4)2SO4 fractionation had a reasonable level of purity as well as a bioavailability with tortillas similar to that of BHb (Figure 3), suggesting the usefulness of this fraction in food fortification. The RBV of BHb decreased, from 113% to 27% of FeSO4, when heme iron was fortified into tortillas compared to without food matrix. Besides the lower concentration of fortificant in this experiment, the lower bioavailability of BHb with the food matrix might be due to the influence of calcium in tortillas due to nixtamalization treatment with calcium hydroxide. Calcium has been shown to decrease the bioavailability of heme iron bioavailability as well as nonheme iron (25, 26). Another explanation might be that FeSO4 availability is low in aqueous solution due to its low solubility at neutral pH without any chelating agents. Since the results are expressed as RBV to FeSO4, the decrease in bioavailability of BHb should be viewed as higher bioavailability of FeSO4 in the presence of tortillas compared to without food matrix. Unlike the results in Figure 2, BHb and LHb bioavailability is similar in Figure 3, suggesting that there might be differences in iron solubility or the effect of calcium.

Ferrous sulfate is considered an ideal positive control for bioavailability assessment because of its high bioavailability in most food products. However it is not practical for maize foods fortification because of the adverse organoleptic effects caused by FeSO4 catalyzed lipid peroxidation and changes in color of the food itself (27). As such, FeSO4 is not currently recommended for maize foods fortification (28), but our results with LHbA showing bioavailability higher than FeSO4 with food offer a promising new alternative iron fortification scheme.

The effect of heat on the bioavailability of heme iron sources is minimal, unlike FeSO4 which decreases in bioavailability during heating. Although not significant, the heat treatment tends to improve the bioavailability of heme iron, which may be due to denaturation of the globin proteins and enhancement of hydrophilic interactions. However, the decrease in FeSO4 bioavailability during heating might be due to interaction of iron with maillard browning products (29) or from oxidation of ferrous iron to the less bioavailable ferric form (30). It appears that the heme pyrrole provides a protective effect against the heat effect.

No beneficial effect of heme/nonheme iron combinations on bioavailability, compared to both iron alone at the same concentration, was shown in our study, suggesting that heme does not enhance the bioavailability of nonheme iron. Therefore, the positive effects of meat on nonheme iron absorption may be due to factors in meat other than the hemoglobin fraction. The enhancing effect of animal tissue on nonheme iron bioavailability was generally attributed to the “meat factors” rather than hemoglobin (31–32).

Our results showing the bioavailability of hemoglobin from soy root nodules similar to that of heme iron from animal sources when added with tortillas provide a unique alternative fortificant, hence to improve the iron status of the population.
However, no advantage of using LHb as a supplement was found because its bioavailability is similar to commonly used FeSO₄. Because of the known minimal inhibition of heme iron, unlike nonheme iron, by dietary factors and because of the high heat stability of this iron form, plant hemoglobins may have great potential to be used as a fortificant for improving nutrition. Soy root nodules have no current use in agriculture production and as such may provide a novel value-added product for soy producers. Given optimal nodulation conditions, 2–3 plants would provide sufficient LHb to meet 25% of the recommended daily requirement of iron, assuming 25% of heme iron absorption. The use of plant hemoglobins in iron nutrition warrants further study of sensory and safety issues related to fortification to make this a feasible reality.

LITERATURE CITED

(9) Au, A. P.; Reddy, M. B. Caco-2 cells can be used to assess human iron bioavailability from a semipurified meal. J. Nutr. 2000, 130, 1329–1334.

Received for review September 14, 2005, Revised manuscript received December 23, 2005. Accepted December 28, 2005. The authors acknowledge the financial support of the Nutrition Sciences Council W.S. Martin Grant, Iowa State University.