# Iron bioavailability in Wistar rats fed with fortified rice by Ultra Rice® technology with or without addition of yacon flour (*Smallanthus sonchifolius*)

*Ceres M. Della Lucia, Maria das Graças Vaz Tostes, Carlos Mário M. Silveira, Lívia A. Bordalo, Fabiana C. Rodrigues, Helena Maria Pinheiro-Sant'Ana, Hércia S. D. Martino, Neuza Maria B. Costa*

Department of Nutrition and Health, Federal University of Viçosa, Brazil.  Department of Husbandry, Federal University of Espírito Santo, Center for Agricultural Sciences, Alegre, Brazil.

**SUMMARY**. This study aimed to evaluate iron (Fe) bioavailability in Wistar rats fed with rice fortified with micronized ferric pyrophosphate (FP) by Ultra Rice® (UR) technology with or without addition of yacon flour as a source of 7.5% of fructooligosaccharides (FOS). Diets were supplied with 12 mg iron/kg from the following sources: ferrous sulfate (FS - control diet), fortified rice with micronized ferric pyrophosphate (Ultra Rice®) (UR diet), ferrous sulfate + yacon flour (FS + Y diet) or Ultra Rice® + yacon flour (UR + Y diet). Blood samples were collected at the end of depletion and repletion stages for determination of hemoglobin concentration and calculation of the relative biological value (RBV). Also, the content of short chain fatty acids (SCFA) (acetic, propionic and butyric acids) from animals' stools and caecum weight were determined. The UR diet showed high iron bioavailability (RBV = 84.7%). However, the addition of yacon flour in the diet containing fortified rice (UR + Y diet) decreased RBV (63.1%) significantly below the other three groups (p<0.05). Groups that received yacon flour showed higher acetic acid values compared to those who did not. In conclusion, fortified UR® with micronized ferric pyrophosphate showed high iron bioavailability but the addition of yacon flour at 7.5% FOS reduced iron bioavailability despite increased caecum weight and SCFA concentration.
**Key words:** Ultra Rice®, relative biological value; hemoglobin; iron deficiency; fructooligosaccharides.

**RESUMEN. Biodisponibilidad de hierro en ratas Wistar alimentadas con arroz fortificado por medio de la tecnología Ultra Rice® con o sin adición de harina de yacon (*Smallanthus sonchifolius*)**. Este estudio tuvo como objetivo evaluar la biodisponibilidad de hierro (Fe) en ratas Wistar alimentadas con arroz fortificado con pirofosfato férrico micronizado por medio de la tecnología Ultra Rice® (UR®), con o sin adición de harina de yacón. Las dietas contenían 12 mg de hierro/kg a partir de las siguientes fuentes: sulfato ferroso (SF - dieta de control), Ultra Rice® (dieta UR®), sulfato ferroso + harina de yacón (dieta SF + Y) o Ultra Rice® + harina de yacón (dieta UR® + Y). Al final del estudio, se recogieron muestras de sangre para la determinación de la concentración de hemoglobina y el cálculo del valor biológico relativo (RBV). También se determinó el contenido de ácidos grasos de cadena corta (AGCC) (ácidos acético, propiónico y butírico) en las heces de los animales. La dieta UR® mostró alta biodisponibilidad de hierro (RBV = 84,7%). Sin embargo, la adición de harina de yacón en la dieta que contenía arroz fortificado (dieta UR® + Y) disminuyó el RBV (63,1%) (p <0,05). Los grupos que recibieron harina de yacón mostraron los valores más altos de ácido acético en comparación con aquellos que no recibieron. En conclusión, el arroz fortificado con pirofosfato férrico micronizado por medio de la tecnología UR® mostró alta biodisponibilidad de hierro. La adición de harina de yacón, con el fin de proporcionar 7,5% de fructooligosacáridos (FOS) en la dieta, causó aumento del peso del ciego y de la concentración de AGCC, aunque disminuyó la biodisponibilidad de hierro.
**Palabras clave:** Ultra Rice®, valor biológico relativo, hemoglobina, deficiencia de hierro, fructooligosacáridos.

## INTRODUCTION

Iron deficiency is the most common and widespread nutritional disorder in the world, and is a public health problem in both industrialized and non-industrialized countries. Interestingly, although a marked decline of stunting and an increase in the obesity epidemic characteristic of the nutritional transition process have been observed, a high prevalence of anemia continues, with a modal frequency of 40-50% in children under five years and 30-40% in pregnant women. Anemia is, in terms magnitude, the main problem of deficiency in the world, apparently without major geographic differentiations (1).

Food fortification is a well recognized approach to overcome hidden hunger in many parts of the world, particularly in developing countries. Additionally, fortification is a method of controlling micronutrient deficiency as an intervention alternative mainly recommended for locations where high prevalence rates are found (2).

Several condiments and foods, including sugar, soybeans, milk, oil, wheat flour and rice have been explored as potential vehicles for fortification in different country contexts. Among these, rice is a potential candidate for fortification in countries where it is a staple food and specific deficiencies reach high prevalence rates among people with consistent rice consumption (3).

Previous attempts involving the fortification of rice flour were unsuccessful, due to the habit of washing and cooking rice with excess water, which results in the leaching of micronutrient used for enrichment (4). However, recently a new technology was created (Ultra Rice® - UR®) that overcame this barrier. Broken and cracked grains, which typically comprise 20% to 30% of the production and are generally destined for animal feed, can be transformed into rice flour, combined with a binder and other nutrients, and refurbished by extrusion as reconstituted rice grains with the same size, shape and texture of conventional rice (5).

Studies evaluating the efficacy and effectiveness of UR® in the improvement of the nutritional status of iron are still scarce. A study conducted in Indonesia demonstrated the viability of UR® in small rural mills and excellent market acceptance of the fortified product (6). In India, after seven months of using iron fortified-UR® in the meals of anemic schoolchildren, positive effects were observed with regards to serum ferritin (SF), but not hemoglobin (Hb) (7). In Brazil, it was found that micronized ferric pyrophosphate-UR® increased iron levels and reduced the incidence of anemia in children between 6 and 24 months old who were mildly anemic at baseline (8).

Iron bioavailability may be enhanced by dietary components, such as dietary inulin-type fructans (ITF) (inulin and fructooligosaccharides - FOS), as a result of their fermentation in the large intestine (9). Fermentation favors the production of short-chain fatty acids (SCFA), which affect luminal pH, in turn affecting mineral solubility. These effects are also accompanied by modifications in the mucosal architecture of the intestine as a result of increases in both the cellularity and number of crypts, mechanisms which may contribute to an increase in the mineral absorptive surface (9). However, in the study of Petry et al. (10) the authors found no effect of inulin on iron absorption. A possible explanation for this is that whereas it seems probable that iron is absorbed in the colon in human, colonic absorption is likely to be a minor component of total iron absorption compared with duodenal absorption

Yacon (*Smallanthus sonchifolius*) is an Andean tuberous root that accumulates large amounts of low degree of polymerization ITF (11). Its cultivation has expanded in various regions of the world (New Zealand, Japan, Czech Republic, South Korea, Thailand, Philippines, Russia, Estonia, Brazil etc..) because of its easy handling and processing and mainly because it is a source of bioactive components. Although it is considered a traditional food in South America, for the European Union yacon is a new food and therefore its safety must be evaluated. This has stimulated the interest of the scientific community in studies to characterize this root regarding to its chemical composition, technological and functional properties (12).

Yacon is regarded as a functional food given that it contains fructooligosaccharides (FOS), inulin and phenolic compounds. The consumption of FOS and inulin provides the growth of bifidobacteria in the colon, enhances mineral absorption and gastrointestinal metabolism (11). Therefore, the use of yacon, together with dietary sources of iron, may improve the mineral bioavailability and reduce the impact of dietary iron insufficiency.

In the study of Hunt JR (13), the bioavailability of some ferric pyrophosphate compounds in UR® was assessed. The authors found an relative biological value (RBV) varying from 75% to 94%. Therefore, although the ferric pyrophosphate bioavailability is good, the addition of yacon flour can improve it.

Thus, the present study aimed to determine whether the addition of yacon flour (*Smallanthus sonchifolius*) is able to increase iron bioavailability in Wistar rats fed with fortified rice with ferric pyrophosphate by UR® technology.

## MATERIALS AND METHODS

*Raw material*
Rice grains extruded from rice flour (Ultra Rice®

- UR®), produced by a pasta manufacturer (Adorella Foods Ltd.) located in Indaiatuba, Sao Paulo, Brazil, and kindly granted by *Program for Appropriate Technology in Health* (PATH) were used. The grains contained iron (in the form of micronized ferric pyrophosphate), zinc (as zinc oxide), thiamine (in the form of thiamine mononitrate) and folic acid.

### *Preparation of yacon flour*

Yacon roots (115.5 kg) were purchased from the local market (Viçosa, MG, Brazil), selected, weighted and subjected to the flour preparation process according to the methodology of Rodrigues, Castro, Martino & Ferreira (12). After washing in running tap water and sanitization in chlorinated water at 5 ppm during 5 minutes, peeling was made using a potato peeler and then roots were treated with a sodium citrate solution at 0.5% during 15 minutes. After grinding in multiprocessor (Walita, modelo RI7625), yacon pieces of 2 cm, whole peeled, was immersed in a sodium bissulfite solution at 0.5% during 15 minutes. After that, liquid was eliminated and drying was performed in airflow dryer (Polidryer-DP, Viçosa, Brazil) at 55ºC during 48 hours. At the end of the drying process, yacon was ground and the flour was weighted and stored in plastic bags at 10ºC. Chemical composition of yacon flour was determined as indicated by the AOAC method (14), resulting in the following values per 100 grams: 6.9 g of moisture, 2.7 g of proteins, 0.15 g of fat, 5.4 g of ash, 8.6 g of glucose, 21.1 g of fructose; 16.3 g of sucrose and 25.7 g of FOS.

### *Determination of iron in UR®, yacon flour and experimental diets*

Iron content in UR®, yacon flour and experimental diets was determined according to AOAC (14). One gram of each sample, in triplicate, was weighted in tubes and digested using 10 mL of concentrated $HNO_3$ at a temperature of 160 °C. After the first 8 hours of digestion, another 5 mL of $HNO_3$ were added. After completing digestion, the contents of the tube were transferred to 50 mL volumetric flasks. The samples were then mixed in a vortex and the volume was made up with deionized water. Iron was determined by plasma emission spectrophotometry (Perkin-Elmer Optima 3300 DV, Norwalk, USA). The glassware and utensils used for both the mineral and biological assays were demineralized, using a 10% $HNO_3$ solution, in which they remained for 24 h followed by rinsing in deionized water.

### *Evaluation of iron bioavailability in vivo*

The study was conducted according to Brazilian Standards of Animal Experimentation and was approved by the Ethics Committee for Animal Research (Project Identification Code: 33; date of approval: June 09th 2011).

Thirty-two 21-day old male *Wistar* rats (*Rattus novergicus*, albinus variety, Rodentia class), with initial body weight ranging from 60 - 90 g were used in the study. The animals were provided by the Central Biotery of the Universidade Federal de Viçosa, Minas Gerais, Brazil and were individually housed in stainless steel cages under controlled temperature (21°C ± 1°C) and 12 hour photoperiod for 14 days.

The depletion-repletion hemoglobin method was applied to determine iron bioavailability, according to AOAC (13), with a modification to the depletion phase which lasted three weeks, instead of four. This time was sufficient to cause iron deficiency anemia in the rats (6 mg/dL), based on the results of previous studies in our laboratory (15).

   1.Experimental diets

   Diets were prepared based on the nutritional needs of animals using AIN93-G composition, according to Reeves, Nielsen & Fahey (16). The composition of the diets is presented in Table 1. Ingredients were individually weighed and mixed using demineralized plastic tools, followed by mixing in a semi-industrial mixer (Lieme®, São Paulo, Brazil) at low speed for 15 minutes. The diets were stored in polyethylene bags at 10ºC. The amount of yacon flour was calculated to provide 7.5% of dietary fructo-oligossacharides (FOS).

   2. Depletion phase

   The animals were fed a modified AIN-93G diet (16) recommended for rats in the growing phase, utilizing an iron-free mineral mix and deionized water *ad libitum*, during 21 days to induce anemia. At the end of the depletion period, blood samples were collected by tail incision to determine hemoglobin (Hb) concentration. After making an incision at the terminal portion of the tail of each animal, blood was dripped on glass slide and immediately collected with a micropipette. A 10 µL blood aliquot was mixed with 2.5 mL of cyanide and potassium ferricyanide (Drabdkin so-

TABLE 1. Composition of experimental diets (g/100 g mixture)

| Components/ | Depletion Period | | | Repletion Period | |
|---|---|---|---|---|---|
| Concentration | AIN-93G | FS | UR® | FS + Y | UR + Y |
| FeSO$_4$ (mg) | - | 0.0059 | - | 0.0041 | - |
| Albumin* | 20.00 | 20.00 | 20.00 | 20.00 | 18.93 |
| Maltodextrin* | 13.20 | 13.20 | 13.20 | 9.77 | 9.77 |
| Sucrose* | 10.00 | 10.00 | 10.00 | - | - |
| Soybean Oil * | 7.00 | 7.00 | 7.00 | 6.95 | 6.95 |
| Fiber (microfine cellulose)* | 5.00 | 5.00 | 5.00 | 1.13 | 1.13 |
| Iron-free mineral mix * | 3.50 | 3.50 | 3.50 | 3.50 | 3.50 |
| Vitamin mix * | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| L-cystine* | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| Choline bitartrate * | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Corn starch* | 39.75 | 39.75 | 39.75 | 29.06 | 28.97 |
| Yacon flour [a] | - | - | - | 29.2 | 29.2 |
| Ultra Rice® [b] | 0.00 | - | 0.13 | - | 0.09 |

FS: Ferrous sulfate diet UR®: Ultra Rice diet FS + Y: Ferrous sulfate + Yacon flour diet  UR®+ Y : Ultra Rice + Yacon flour diet
* Rhoster, Industry and Commerce Ltd.  [a] 25.7% oligofrutans, 16.3% saccharose, 21.1% fructose, 0.0013% iron, soluble dietary fiber: 2,72 % insoluble dietary fiber: 10,53 % non-digestible carbohydrates: 38,95 % (Source: Rodrigues, Castro, Martino, Ferreira, 2011).
[b] 0.94% of iron

lution) and absorbance was measured at 540 nm in a spectrophotometer (Shimadzu UV-1601).

3. Repletion phase

Anemic rats were systematically assigned to four groups (n=8) according to their hemoglobin level to obtain groups with similar means. The groups were fed with diets containing 12 mg of iron/kg supplied from the following iron sources: ferrous sulfate (FS - control diet), fortified rice (Ultra Rice® - UR®) (UR® diet), ferrous sulfate + yacon flour (FS + Y diet) or Ultra Rice + yacon flour (UR® + Y diet). In diets FS + Y and UR® + Y, corn starch, sucrose and dietary fiber were quantitatively adjusted, taking into account the offer of 7.5% of FOS and the carbohydrate content of yacon flour (Table 1).

The rats were fed 17 to 18 g rations/day of repletion diet during 14 days and deionized water was provided *ad libitum*.

At the end of the repletion period, blood samples were collected by tail incision for further determination of Hb concentration.

For calculating Hb concentrations, absorbance for a standard Hb solution at a concentration corresponding to 11.4 g/dL was used as reference (Química Básica, Belo Horizonte, MG, Brazil).

Iron (Fe) consumption was calculated considering the total amount of diet consumed and the iron content of the specific diet, which was calculated for each animal according to the formula below:

(1) Fe consumption =
[total diet consumption during repletion period (g) x Iron in the diet (mg/1000 g)] / 1000

The results of the Hb concentrations and iron consumption were used to estimate the following indexes:

(2) Hb-Fe pool (mg), assuming the total blood volume was 6.7% the body weight and Fe content in

(3) Hb was 0.335%:
Hb-Fe pool (mg) = [Fe Hb (final) -
Fe Hb (initial)  x 100] / Fe intake (mg)
Where:
Hb Fe (initial) = [weight (g) (initial) x
Hb (g / dL) (initial) x 6.7 x 0.335] / 1000
Fe Hb (final)= [weight (g) x (final)
x Hb (g / dL) (final) x 6.7 x 0.335] / 1000
Hb Repletion Efficiency (HRE)
%HRE = (Hb Fe pool (final) −
Hb Fe pool (initial) x 100] / Fe intake (mg)

(4) Relative Biological Value (RBV):
RBV = 100 x (% HRE test group /

% HRE SF group)
(5) Biological Value Relative to Hb Gain (GHb RBV):
RBV GHb = gain of Hb by each animal Hb / Average gain of Hb in ferrous sulfate group

During the experimental period, body weight and food intake were monitored to determine the feed efficiency ratio (FER), calculated as the ratio between the body weight gain (g) and food intake (g).

*Reticulocyte count*

After the animals were sacrificed, a reticulocyte count was also obtained as an indicator of recent bone marrow activity. A 5 mL blood aliquot was collected in a tube containing EDTA, and then 0.5 mL of this solution was added to 0.5 mL of brilliant cresyl blue in a hemolysis tube, which was maintained in a water bath at 37 °C for 15 minutes. The smear was obtained from the homogenate which was focused using an objective lens. Counting was performed with at least 1.000 red blood cells, and the number of reticulocytes found in these fields was noted. Values were expressed in %.

*Determination of short chain fatty acids (SCFA) in animals stools*

For determination of SCFA (acetic, propionic and butyric acids) the method proposed by Smiricky-Tjarda, Grieshop, Flickinger, Bauer & Fahey (17) was used. SCFA concentrations were determined using high performance liquid chromatography system (HPLC) (Shimadzu, model SPD-10A VP), coupled to an Ultra Violet (UV) detector using a wavelength of 210 nm. After animals' euthanasia, stools from the caecum were mixed with 25% metaphosphoric acid in eppendorf tubes and then maintained at rest for 30 minutes at room temperature. After that, samples were centrifuged in a refrigerated microcentrifuge (Hitachi, CT15RE) at 16.100 g for 30 minutes. The supernatant was transferred to another eppendorf and was centrifuged again for 20 minutes under the same conditions. This supernatant was then used for determining the SCFA concentrations. The chromatographic conditions were: reversed phase column (C18), 30 cm x 4.5 mm, flow rate: 0.8 mL/min, column pressure: 181 kgf, mobile phase: 1% orthophosphoric acid in water, injection volume: 20 µl.

*Statistical Analysis*

Descriptive statistics were used and results are shown in terms of mean and standard deviation. Groups were compared using analysis of variance (ANOVA) and the Dunnett's post hoc test was applied to identify where significant differences occurred, considering ferrous sulfate as the control group and a significance level of 5% ($p < 0.05$). Data was analyzed using the software Statistical Package for the Social Sciences (SPSS), version 17.0.

## RESULTS

*Iron status of rats*

After 21 days of iron restriction (depletion period), it was observed that hemoglobin levels in the animals were low (Table 2), indicating the efficacy of iron depletion. No significant difference was observed in hemoglobin levels and hemoglobin iron among the experimental groups ($p > 0.05$). Also, there were no significant differences among groups regarding weight gain and food consumption during the depletion period ($p > 0.05$).

*Food intake and body weight in iron repletion period*

After analysis of diets, it was observed that the average ± SD iron content was 1.76 ± 0.23 mg iron/100 g in FS control diet, 2.11 ± 0.31 mg iron/100 g in FS + Y diet, 2.07 ± 0.29 mg iron/100 g in UR® diet and 2.53 ± 0.19 mg iron/100 g in UR® + Y diet.

There were no significant differences ($p > 0.05$) in food intake and FER among groups during the repletion period. However there was a difference in total iron intake ($p < 0.001$), where the FS group presented the lowest intake (4.58 ± 0.39 mg) and the UR® + Y group showed the highest (6.71 ± 0.51 mg). Body weight and weight gain at the end of repletion period were not different among groups (Table 3).

*Hematological indexes and iron bioavailability*

The hematological levels of animals consuming diets containing yacon flour and ferrous sulfate or UR® with ferric pyrophosphate as sources of iron at the beginning and end of the repletion period are described in Table 4 and Figure 1.

Iron intake was different among the groups since the iron concentration in diets were also different, al-

TABLE 2. Body weight, weight gain, feed intake, feed efficiency ratio and hematological indexes of animals after 21 days of iron-deficient diet (depletion period)

| Indexes | FS | FS + Y | UR® | UR® + Y |
|---|---|---|---|---|
| Weight gain (g) | 96.7 ± 7.4 | 97.0 ± 15.0 | 94.9 ± 14.8 | 92.5 ± 11.1 |
| Feed intake (g) | 295.7 ± 19.6 | 287.5 ± 37.2 | 302.4 ± 22.5 | 285.8 ± 22.6 |
| Feed efficiency ratio | 0.3 ± 0.03 | 0.3 ± 0.07 | 0.3 ± 0.05 | 0.3 ± 0.05 |
| Hemoglobin (g/dL) | 7.5 ± 1.2 | 7.5 ± 1.1 | 7.5 ± 1.1 | 7.4 ± 1.1 |
| Hemoglobin iron (mg) | 3.0 ± 0.6 | 3.1 ± 0.7 | 3.0 ± 0.5 | 2.9 ± 0.4 |

FS: Ferrous sulfate, FS + Y: Ferrous sulfate + Yacon flour, UR®: Ultra Rice, UR® + Y: Ultra Rice + Yacon flour.  Results expressed as mean ± SD from iron-deficient rats (n=8/group) after 21 day period fed with iron repletion diet.  SD = Standard deviation.  Means do not differ from each other according to ANOVA ($p > 0.05$)

TABLE 3. Data from food consumption, body weight and hematological parameters after 14 days of repletion diet

| Indexes | FS | FS + Y | UR® | UR® + Y |
|---|---|---|---|---|
| Total food intake (g) | 259.9 ± 22.3 | 255.9 ± 24.7 | 262.5 ± 25.8 | 264.9 ± 19.9 |
| Fe intake (mg) | 4.6 ± 0.4 [a] | 5.4 ± 0.5 [b] | 5.4 ± 0.5 [b] | 6.7 ± 0.5 [c] |
| Feed efficiency ratio | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.04 | 0.1 ± 0.04 |
| Weight gain (g) | 31.1 ± 13.9 | 31.5 ± 10.9 | 31.5 ± 8.9 | 25.7 ± 10.1 |

FS: Ferrous sulfate, FS + Y: Ferrous sulfate + Yacon flour, UR®: Ultra Rice, UR® + Y: Ultra Rice + Yacon flour. Results expressed as mean ± SD. Means followed by the same capital letters in columns do not differ from each other according to the Dunnett's test ($p > 0.05$), to compare each test group with ferrous sulfate group (control). SD = Standard deviation.

though they were prepared to reach 12 ppm Fe/ kg of diet. These differences can occur due to possible contamination in ingredients used during the preparation of diets, which is common. Since significant differences were detected in iron intake among the groups, specific ratios were calculated in an attempt to avoid misinterpretation of the results since groups with higher iron intake have proportionally higher hemoglobin concentration and higher hemoglobin gain. Thus, the ratios hemoglobin iron/iron intake and Hb gain/iron intake were calculated.

Hemoglobin levels at the end of the repletion period were higher than in the beginning (end of depletion), however Hb gains did not differ among groups ($p > 0.05$), even when this index was corrected by iron consumption.

Despite a tendency to increase in the groups that received yacon flour, the biological value relative to Hb gain did not differ among the groups receiving yacon compared with those that did not receive yacon ($p > 0.05$).

Reticulocyte values at the end of the repletion period were normal in all groups. It was observed that at the end of the experiment the animals had recovered from iron depletion, with reticulocyte values within normal limits (0.5 to 2.3%).

There was no significant difference ($p > 0.05$) in the levels of hemoglobin iron at the end of the repletion period; however, considering the iron intake, levels of hemoglobin iron were lower in the UR® + Y group compared to other groups.

Significant differences ($p < 0.05$) were observed among groups with respect to hemoglobin repletion efficiency (HRE) and the biological value relative to HRE (RBV), as shown in Figure 1.

*Intestinal analysis*

Yacon flour was associated with a significant increase in the absolute caecum weight of animals ($p < 0.001$), more pronounced in the diet containing ferrous sulphate and yacon flour. The relative caecum weight (caecal weight/body weight) was higher in ani-

TABLE 4. Hematological values of animals receiving diets containing yacon flour and ferrous sulfate or UR® with ferric pyrophosphate as sources of iron at the beginning and end of repletion

| Parameters | FS | FS + Yacon | UR® | UR® + Yacon |
|---|---|---|---|---|
| Initial hemoglobin | 7.5 ± 1.2 | 7.5 ± 1.1 | 7.5 ± 1.1 | 7.4 ± 1.1 |
| Final hemoglobin | 12.4 ± 1.5 | 12.7 ± 1.2 | 12.3 ± 2.7 | 12.4 ± 2.6 |
| Hemoglobin gain (g/dL) | 4.9 ± 1.4 | 5.2 ±1.1 | 4.7 ± 1.9 | 5,0 ± 2.7 |
| Hemoglobin gain /Iron intake | 1.1 ± 0.3 | 1,0 ± 0.2 | 0.9 ± 0.3 | 0.7 ± 0.4 |
| Relative biological value | 100.0 ± 27.9 | 106.8 ± 23.1 | 97.6 ± 39,0 | 102.6 ± 56.1 |
| Initial hemoglobin iron (mg) | 3.0 ± 0.6 | 3.1 ± 0.7 | 3.0 ± 0.5 | 2.9 ± 0.4 |
| Final hemoglobin iron (mg) | 5.8 ± 0.8 | 6.2 ± 0.8 | 5.8 ± 1.2 | 5.5 ± 0.9 |
| Final hemoglobin iron /Iron intake | 1.3 ± 0.3 [a] | 1.2 ± 0.2 [a] | 1.1 ± 0.2 [a] | 0.8 ± 0.1 [b] |
| Reticulocytes (%) | 1,0 ± 0.5 | 1.3 ± 0.8 | 1.1 ± 0.4 | 1.4 ± 0.6 |

FS: Ferrous sulfate, FS + Y: Ferrous sulfate + Yacon flour, UR®: Ultra Rice, UR® + Y: Ultra Rice + Yacon flour. Results expressed as mean ± SD. Means followed by the same capital letters in columns do not differ from each other according to the Dunnett's test (p > 0.05), to compare each test group with ferrous sulfate group (control).  SD = Standard deviation.
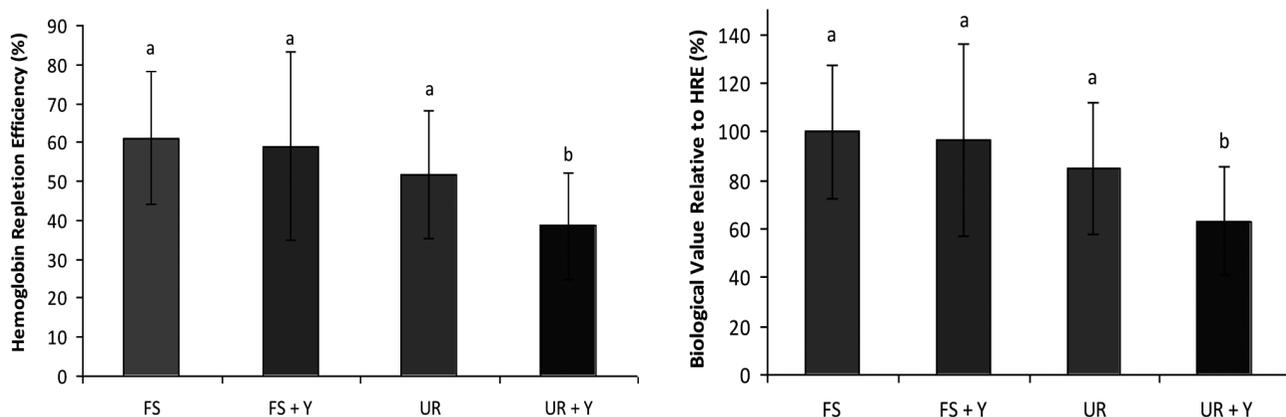


FIGURE 1. Hemoglobin Repletion Efficiency (HRE) and biological value relative to HRE in experimental diets. Mean values followed by the same superscript letter are not significantly different according to the Dunnett's test (p > 0.05) to compare each test group with ferrous sulfate (control).

TABLE 5. Caecum absolute and relative weights and production of short chain fatty acids

| Parameters | FS | FS + Yacon | UR® | UR® + Yacon |
|---|---|---|---|---|
| Caecum absolute weight (g) | 4.9 ± 1.53 [a] | 13.2 ± 3.1 [b] | 4.6 ± 1.2 [a] | 8.5 ± 1.8 [c] |
| Caecum relative weight (g) | 0.0 ± 0.008 [a] | 0.1 ± 0.02 [b] | 0.02 ± 0.006 [a] | 0.04 ± 0.01 [c] |
| SCFA (µmol/caecum) | | | | |
| Acid acetic | 88.0 ± 21.81 [a] | 184.8 ± 46.0 [b] | 72.5 ± 20.3 [a] | 152.9 ± 54.6 [b] |
| Acid propionic | 243.8 ± 48.41 [a] | 513.5 ± 154.7 [b] | 203.5 ± 62.5 [a] | 283.0 ± 105.2 [a] |
| Acid butyric | 7.1 ± 2.90 [a] | 17.6 ± 11.7 [b] | 7.3 ± 5.9 [a] | 10.5 ± 2.7 [a] |

FS: Ferrous sulfate, FS + Y: Ferrous sulfate + Yacon flour, UR®: Ultra Rice, UR® + Y: Ultra Rice + Yacon flour.  Results expressed as mean ± SD. Means followed by the same capital letters in columns do not differ from each other according to the Dunnett's test (p > 0.05), to compare each test group with ferrous sulfate group (control).  SD = Standard deviation.  SCFA: short chain fatty acids.

mals fed with yacon flour and more prominent in the group that received ferrous sulfate with yacon flour (p<0.001) (Table 5).

There was significant difference (p<0.001) in the concentration of SCFA, and with respect to acid acetic groups that received yacon flour showed higher values than those that did not. The FS + Y group produced higher butyrate and propionate concentrations, and no differences were observed in the other groups.

## DISCUSSION

Iron bioavailability can be affected by sources of FOS. Lobo et al. (18) found that iron bioavailability from ferric pyrophosphate was higher when offering yacon flour to animals with iron deficiency anemia, with an RBV of 97%. It is important to consider that ferric pyrophosphate is an insoluble salt of low iron bioavailability. In the rat model, the high FOS content could improve bioavailability of this mineral by acidification of the medium resulting from the activity of fermentative local microbiota or increased iron uptake due to hypertrophy of the caecum walls (18). However, in the present study, rice fortified with ferric pyrophosphate showed a high RBV (84.7%) unlike that found by Lobo et al. (40%) (17). However, , when offered in association with yacon flour , the iron in the fortified rice showed a reduction in bioavailability. This indicates that in the present study pyrophosphate in fortified rice had the highest bioavailability and the addition of yacon flour reduced the bioavailability of this mineral.

One of the feasible reasons for the high iron bioavailability obtained in the present study may be that the iron pyrophosphate in UR was in the micronized form, unlike that used by Lobo et al. (18). According to PATH (19) and Wegmüller et al (20), the micronization process produces very small salt particles (micrometers), thus increasing the surface area and improving iron bioavailability when compared to larger particle-size ferric FP and other insoluble salts. Whereas the prebiotic effect at the hind gut level did not seem to increase iron absorption, the flour may have decrease iron absorption where it matters most – the upper small intestinal mucosa.

HRE values for the FS control group (61%) and for the FP UR® group (51.8%) were higher than those found in the study by Lobo et al. (18), in which FS

showed an HRE around 25% and the FP group showed a HRE of approximately 10%. In the present study, despite having displayed the highest iron consumption among the groups (p<0.01), the group that received ferric pyrophosphate and yacon showed the lowest HRE and RBV (p<0.05).

One factor that may partially explain this result was the presence of a mild diarrhea observed in the group that received ferric pyrophosphate and yacon flour, which may have caused increased excretion of iron in the stool. However, the dose of FOS included in the diet was similar to that used in other studies, which did not report diarrhea in animals (9, 17).

Although negative effects of yacon flour on iron bioavailability in the present study were observed, there was a marked increase in caecal weight of the animals, being more pronounced in the group receiving yacon flour with ferrous sulfate. There was also an increased concentration of SCFA in these animals. Yacon flour contains a high proportion of carbohydrates in the form of oligofrutans such as inulin and FOS (21).

The effects of SCFA on both normal and neoplastic epithelial cells are known. While butyrate and, to a lesser extent, propionate, act by reducing the proliferation of tumor cells in vitro, all three major SCFA stimulate the proliferation of normal epithelial cells (22). It has also been reported that fermentation of FOS in the caecum of animals was accompanied by a hypertrophy in this portion of intestine or increase of the number and depth of caecal crypts (10), suggesting the cause/effect relationship between hyperplasia and hypertrophy and development of cell wall in the caecum.

Genta, Cabrera, Grau & Sanchez (23) presented results corroborating with those of the present study when feeding rats with diets containing yacon for a period of 4 months. Various levels of FOS (from 340 to 6800 mg/kg/day) were offered and the authors found significant increases in caecal weight only in the group supplemented with the highest level of FOS. These authors also found lack of toxicity and a certain beneficial metabolic activity in normal rats. In another study, Boyle et al. (24) fed rats with different oligofructose levels (0%, 0.55%, 1.65%, 4.96% and 9.91%), observing a significant increase in caecal weight for the supplemented diets. In these studies the increased caecum weight was dose dependent and was associated with the trophic effect of SCFA on colo-

nocytes. In the present study 7.5% of FOS added to the diet increased caecal weight, especially when yacon was combined with ferrous sulfate. Significant gut effects of yacon flour were therefore observed, but not reflected in increased iron absorption.

## CONCLUSION

Fortified rice with micronized ferric pyrophosphate by UR® technology showed high iron bioavailability. The addition of yacon flour, to provide 7.5% of fructo-oligosaccharides (FOS) in the diet, increased caecum weight and SCFA concentration, but reduced iron bioavailability, so its addition should not be encouraged.

Further studies are necessary regarding the effects of FOS, especially in diets with low bioavailability, aiming to reach further conclusions about its effects on iron absorption and contribution in the control of iron deficiency anemia.

## REFERENCES

1. Batista Filho M. Alimentação, nutrição & saúde. In: Rouquayrol ZM, Almeida Filho N, editors. Epidemiologia & Saúde, 5th ed., Rio de Janeiro: Medsi; 1999. p. 353-374.

2. Chakravarty I. Food-based strategies to control vitamin A deficiency. Food Nutr Bull. 2000; 21: 135-143.

3. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Review. 1997; 55: 210-222.

4. Hoffpauer DW. Rice enrichment for today. Cereal Foods World. 1992; 37: 757-759.

5. Lee J, Hamer ML, Eitenmiller RR. Stability of retinyl palmitate during cooking and storage in rice fortified with ultra rice fortification technology. J Food Sci. 2000; 65: 915-919.

6. PATH. Program for Appropriate Technology in Health. Seattle, Wa., Personal communication. 1999.

7. Moretti D, Zimmermann MB, Muthayya S, Thankachan P, Lee TC, Kurpad AV, Hurrell RF. Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in India schoolchildren: a double-blind randomized controlled trial. Am J Clin Nutr. 2006; 84: 822–829.

8. Pessoa MC. Eficácia do consumo de arroz fortificado com ferro no tratamento de crianças com anemia carencial [dissertation]. Belo Horizonte (MG): Universidade Federal de Minas Gerais; 2009.

9. Lobo AR, Colli C, Alvares EP, Filisetti TMCC. Effects of fructans-containing yacon (*Smallanthus sonchifolius* Poepp & Endl.) flour on caecum mucosal morphometry, calcium and magnesium balance, and bone calcium retention in growing rats. Brit J Nutr. 2007; 97: 776-785.

10. Petry N, Egli I, Chassard, Lacroix C, Hurrell R. Inulin modifies the bifidobacteria population, fecal lactate concentration, and fecal pH but does not influence iron absorption in women with low iron status. Am J Clin Nutr. 2012; 96: 325-331.

11. Delgado GTC, Tamashiro, WMSC, Maróstica Júnior MR, Pastore GM. Yacon (*Smalanthus sonchifoli*): A functional food. Plan Foods Hum Nutr. 2013. Available in: <http://link.springer.com/content/pdf/10.1007%2Fs11130-013-0362-0.pdf>. (accessed 10 Jun 2013).

12. Rodrigues FC, Castro ASB, Martino HSD, Ferreira CLLF. Farinha de yacon (*Smallanthus sonchifoli*): produção e caracterização química. Rev Inst Adolfo Lutz. 2011; 70. 290-295.

13 Hunt JR. Assessment of the bioavailability of ferric pyrophosphate in Ultra Rice using the AOAC rat hemoglobin repletion method. In: The research behind the Ultra Rice technology. Available in: <www.path.org>. (accessed 20 May 2013).

14. AOAC - Association of Official Analytical Chemists. Official methods of analysis of the Association of Official Analytical Chemists. 16.ed. Maryland. 1998.

15. Martino HSD, Carvalho AW, Silva CO, Dantas MIS, Natal DIG, Ribeiro SMR, Costa NMB. Heat-treated hull flour does not affect iron bioavailability in rats. Arch Latinoamer Nutr. 2012; 61: 135-142.

16. Reeves PG, Nielsen FH, Fahey GCJr. AIN-93G purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Comitee on the Reformulation of the AIN-76A rodent diet. J Nutr. 1993; 123: 1939-1951.

17. Smiricky-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey GC. Dietary galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial concentrations, and ileal fermentative characterisitics of growing pigs. J Animal Sci. 2003; 81: 2535-2545.

18. Lobo AR, Cocato ML, Borelli P, Gaievski EHS, Crisma AR, Nakajima K, Nakano EY, Colli C. Iron biovailability from ferric pyrophosphate in rats fed with eructan-containing yacon (*Smallanthus sonchifolius*) flour. Food Chem. 2011; 126: 885-891.

19. PATH. Introdução do Arroz Fortalecido Usando a Tecnologia Ultra Rice®. Available online: <www.path.org/files/MCHN_ultrarice_faq_port.pdf> (accessed 26 Jun 2011).

20. Wegmüller R, Zimmermann MB, Moretti D, Arnold M, Langhans W, Hurrell RF. Particle size reduction and encapsulation affect the bioavailability of ferric

pyrophosphate in rats. J Nutr. 2004; 134(12):3301-4

21. Cazetta ML, Martins PMM, Monti R, Contiero J. Yacon (*Polymnia sonchifolia*) extract as a substrate to produce inulinase by Kluyveromyces marxianus var. bulgaricus. J Food Eng. 2005; 66: 301–305.

22. Scheppach W, Bastram HP, Richter, F. Role of short-chain fatty acids in the prevention of colorectal cancer. Eur J Cancer. 1995; 31A: 1077-1080.

23. Genta SB, Cabrera WM, Grau A, Sanchez S.S. Subchronic 4-month oral toxicity study of dried *Smallanthus sonchifolius* (yacon) roots as a diet supplement in rats. Food Chem Toxicol. 2005; 43: 1657-1665.

24. Boyle FG, Wrenn JM, Marsh BB, Anderson WI, Angelosanto FA, Mccartney AL, Lien EL. Safety evaluation of oligofructose: 13 Week rat study and in vitro mutagenicity. Food Chem Toxicol. 2008; 46: 3132-3139.