Iron bioavailability from ferric pyrophosphate in rats fed with fructan-containing yacon (Smallanthus sonchifolius) flour

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A B S T R A C T
The effects of inulin-type fructans (ITF)-containing yacon flour (YF) on Fe bioavailability from ferric pyrophosphate (FP) were evaluated in Fe-deficient rats using the Hb repletion efficiency (HRE) assay. Weaning male Wistar rats were fed a low-Fe diet (12 mg/kg) for 15 days followed by 2 weeks of Fe repletion with diets providing 35 mg Fe/kg as either ferrous sulphate (FS) or FP, supplemented with 7.5% ITF as either YF or Raftilose (RAF), a purified ITF. ITF increased caecal fermentation, whereas YF was more butyrogenic than RAF. ITF improved HRE in FP-fed rats, and those fed YF had a higher relative biological value compared with those fed FP and RAF. Liver Fe was increased by ITF, but only YF led to values similar to those in the FS group. It is observed that ITF increased caecal fermentation and Fe bioavailability. These effects were more pronounced when YF was the ITF source.

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1. 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 (World Health Organization, 2006). Iron deficiency is the result of a long-term negative Fe balance: in its more severe stages, Fe deficiency causes anemia. About 40% of the world’s population (more than 2 billion individuals) is thought to suffer from anemia. According to World Health Organization, 39% of children younger than 5 years, 48% of children between 5 and 14 years, 42% of all women, and 52% of pregnant women in developing countries are anemic, with half having Fe deficiency anemia (WHO, 2006).

The main strategies for correcting Fe deficiency in populations are dietary modification or diversification to improve Fe intake and bioavailability; Fe supplementation and Fe fortification of foods; and biofortification by plant breeding which has been considered as a promising approach to improve dietary Fe nutritional quality (Zimmerman & Hurrel, 2007). The dietary habits of a population group strongly affect the bioavailability of both dietary Fe and added fortifying Fe. Although the efficiency of Fe absorption increases as Fe stores become depleted, the amount absorbed from foods, especially where diets are low in meat, fish, fruit and vegetables, is not enough to prevent Fe deficiency in many women and children, especially in the developing countries (Zimmerman & Hurrel, 2007). For instance, the main cause of increasing Fe deficiency in Brazil is that the consumption of food items considered Fe sources has continually decreased. Indeed, the search for new food standards, proposals for food distribution and knowledge about the diet composition must be the researcher’s target (Szarfarc, 2006).

In recent years, several studies have emphasised the positive effects of dietary inulin-type fructans (ITF; inulin and fructooligosaccharides [FOS]) on mineral bioavailability as a result of their fermentation in the large intestine (Lobo, Colli, Alvares, & Filisetti, 2007; Scholz-Ahrens & Schrezenmeir, 2007). The fermentation process favours the production of short-chain fatty acids (SCFA), which affect luminal pH, in turn affecting mineral solubility (Scholz-Ahrens & Schrezenmeir, 2007). 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 (Kleessen, Hartmann, & Blaut, 2003; Lobo et al., 2007).
Inulin-type fructans are commonly found in almost all species of the Asteraceae family, many of which are of economically important, such as *Chicorium intybus* and *Helianthus tuberosus* (Carvalho & Figueiredo-Ribeiro, 2001). *Yacon* (*Smallanthus sonchifolius* [Poepp & Endl.]) H. Robinson, Asteraceae) is an Andean tuberous root that accumulates large amounts of ITF with a low degree of polymerisation (DP < 10, FOS) (Itaya, Carvalho, & Figueiredo-Ribeiro, 2002). It has been grown in southeast Brazil since 1991, from August to September, yielding around 100 t/ha (Vilhena, Câmara, & Kakihara, 2000).

Our previous study demonstrated that the consumption of ITF-containing yacon flour (YF) enhanced the calcium (Ca) and magnesium (Mg) balance in healthy growing rats, contributing to a higher bone mineral retention and strength (Lobo et al., 2007). These effects were accompanied by an increase in caecum weight and in the number and depth of crypts, as well as in the number of bifurcated crypts, thus suggesting an increment in the absorptive surface. It seems likely that these effects contributed to a larger absorption and bioavailability of minerals in YF-fed animals (Lobo et al., 2007). In the present study, we evaluated the effects of supplementing a diet with ITF-rich YF on the bioavailability of iron (Fe) from ferric pyrophosphate (FP; Fe$_3$(P$_2$O$_7$)$_3$; a water-insoluble compound) in a rat model of Fe-deficiency anaemia. Intestinal parameters (caecal weight, caecal content pH and SCFA production) were assessed as a measurement of ITF fermentation in rats. Furthermore, Fe status alterations induced by YF consumption were compared with those obtained by consumption of a purified source of ITF (Raftilose P95; RAF; Orafti-Active Food International, Tienen, Belgium).

2. Materials and methods

The experimental protocol was approved by the Commission on Ethics in Animal Experiments of the Faculty of Pharmaceutical Sciences of the University of São Paulo (FCF/USP) (CEEA 88/2005 FCF-USP) according to the guidelines of the Brazilian College on Animal Experimentation.

### 2.1. Iron deficiency protocol

Female Wistar rats ($n = 12$) were obtained from the colonies for Animal Experimentation of FCF/USP, each of them breastfeeding six to eight male pups, were housed in plastic cages with ripcurl and fed a Fe-deficient powder diet (Association of Official Analytical Chemists, 2006) (12 mg Fe/kg; $n = 10$ female rats) or an AIN-93 M diet (Reeves, Nielsen, & Fahey, 1993) ($n = 2$ female rats) for 21 days.

On the weaning day, a total of 92 male rats, initially weighing 54–58 g, were transferred to individual metabolic cages under controlled temperatures (22 ± 2 °C) and relative humidities (55 ± 10%) with a 12-h dark-light cycle (lights on 08.00–20.00 h). They received demineralised water ad libitum and were fed Fe-deficient powder diet (ID group; $n = 80$) or an AIN-93G diet (Reeves et al., 1993) (CON group; $n = 12$) for 15 days (depletion period). During this period, 10 ID rats were selected to determine body weight and haemoglobin (Hb) concentration. When the Hb concentration of these animals reached the mean value of 68 ± 0.7 g/l, it was analysed in all animals. At this point, six ID rats and six CON rats were sacrificed for the determination of haematological and biochemical parameters as well as liver Fe concentrations to verify the effectiveness of the Fe depletion protocol.

### 2.2. Hemoglobin regeneration assay

Among the remaining deficient animals ($n = 74$), 44 were selected for the repletion period. These animals were distributed into four groups, based on the product of body weight (g) × Hb concentration (g/l), and fed modified AIN-93G diets containing 35 mg Fe/kg as microencapsulated ferrous sulphate (FeSO$_4$) (Cocoto, Rê, Trindade Neto, Chiebao, & Colli, 2007) (FeSO$_4$:7 H$_2$O: Fermavi Eletroquimica Ltda, São Paulo, Brazil) (FS group; $n = 8$) or FP (Fermavi Eletroquimica Ltda, São Paulo, Brazil) (FP group; $n = 12$) in the mineral mix, supplemented with YF (YF group; $n = 12$) or RAF (RAF group; $n = 12$) at 7.5% ITF (75 g/kg diet) (repletion period, Table 1). The animals consumed these diets for 14 days until the end of the experiment. The remaining six healthy animals continued with the AIN-93G diet during the repletion period, and at the end of this period, the Hb mean concentration in this group was 132 ± 17 g/l.

At the end of the repletion period, the rats were anaesthetised through an intraperitoneal route with a 1:1:0.4:1.6 (v/v/v/v) mixture of ketamine (10 mg/kg body weight; Vetasert, Fort Dodge, Iowa, USA), xylazine (25 mg/kg body weight; Virbaxil 2%, Virbac, São Paulo, Brazil), acepromazine (2 mg/ml; Aceprom 0.2%, Univet S/A Indústria Veterinária, São Paulo, Brazil) and demeralised H$_2$O. After anaesthesia, blood was collected from the abdominal aorta for analysis and the liver was perfused through the subhepatic vein with a NaCl solution (9 g/l) to drain blood out of the organ. The liver was then removed, rinsed with saline, weighed and stored at −20 °C until analysis. The caecum (and its contents) was removed, weighed and put in a Petri dish with ice (Lu, Gibson, Muir, Fielding & O’Dea, 2000) and cut open along the small curvature. The caecal content pH was measured in situ by inserting an electrode (UP-25; Denver Instrument, Denver, Colorado, USA) through the ileocaecal junction. Aliquots of the contents were adequately stored at −80 °C for SCFA concentration analysis. The faeces were quantitatively collected during the last 5 days of the repletion period, pooled and stored at −20 °C.

### 2.3. Chemical composition of the experimental diets

The diet offered to the CON group was formulated according the AIN-93G diet (Reeves et al., 1993). In the YF and RAF diets (Table 1), cornstarch, sucrose and dietary fibre were quantitatively substituted, taking into consideration the carbohydrate content in the

<table>
<thead>
<tr>
<th>Ingredient (%)</th>
<th>FS</th>
<th>FP</th>
<th>YF</th>
<th>RAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein$^a$</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Fibre</td>
<td>5.0</td>
<td>5.0</td>
<td>0.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Soybean oil$^b$</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>i-Cystine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate (41.1% choline)</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Vitamin mixture$^e$</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture$^d$</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Succrose</td>
<td>10.0</td>
<td>10.0</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Corn starch</td>
<td>52.95</td>
<td>52.95</td>
<td>25.45</td>
<td>52.39</td>
</tr>
<tr>
<td>Yacon flour$^f$</td>
<td>–</td>
<td>–</td>
<td>41.7</td>
<td>–</td>
</tr>
<tr>
<td>Raftilose P95$^g$</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8.1</td>
</tr>
</tbody>
</table>

$^a$ Casein = 85% protein ($N \times 6.25$).

$^b$ Carril Agrícola S/A.

$^c$ AIN-93-XX vitamin mixture (Reeves et al., 1993).

$^d$ Modified from a mineral mixture (AIN-93G-MX; Reeves et al., 1993): Fe$_3$(P$_2$O$_7$)$_3$ at 4.12 g Fe/kg or FeSO$_4$.7 H$_2$O at 2.71 g Fe/kg mix was utilized in the mineral mix of FP, YF and RAF diets.

$^e$ São Sebastião Farm (Ibiúna, São Paulo, Brazil); 18% ITF, 28.3% sucrose, 15.7% fructose, 6% IDF, 4% SDF, 4 mg Fe/g, 19 mg Cu/g and 16 mg Zn/g.

$^f$ Orafti Active Food International (Clariant, São Paulo, Brazil), 93% ITF.
ITF sources. The yacon tuberous roots, donated by São Sebastião Farm (Ibiúna, São Paulo, Brazil) were properly processed as described in a previous study (Lobo et al., 2007). They were autoclaved (121 °C, 20 min), freeze-dried (Liotécnica Ind. Com. Ltda, Embu, São Paulo, Brazil) and ground for obtaining the flour. Raffi- lose used in this study was donated by the company Clariant S/A (São Paulo, Brazil). According to the analysis certificate, this product contains around 93% ITF containing molecules (GFm) with an average DP of 4. Inulin-type fructans in RAF were obtained by partial hydrolysis of inulin, which was extracted from chicory roots (Chicorium intybus).

Total fructans, glucose, fructose and sucrose in the YF were analysed by spectrophotometry (Steegmans, Ilians, & Hoebregs, 2004). Insoluble (IDF) and soluble (SDF) dietary fibres were determined by the enzymatic–gravimetric method (Prosky, Asp, Schweizer, Devries, & Furda, 1988). The Fe concentrations in the diets and in the YF were analysed by atomic absorption spectrophotometry (AAS; Analyst 100, Perkin Elmer, Norwalk, CT, USA) using a hollow cathode lamp at 283.4 nm and a slit of 0.2 nm after wet digestion (HNO3:H2O2, 5:1, v/v). The working standard solution was prepared with ferric chloride (FeCl3) (Tritisol, Merck, Darmstadt, Germany). YF analysis showed 18% total fructans, 28.3% sucrose, 15.7% fructose, 6% IDF, 4% SDF and 4 μg Fe/g.

2.4. Iron bioavailability, haematological parameters and liver mineral contents

The diet consumption in the repletion period was determined every 2 days, and the Hb concentration in the blood was obtained through tail puncture every 7 days (days 0, 7 and 14) via the cyanide Hb method (Drabkin & Austin, 1935). The Hb concentrations and Fe consumption results were used to estimate the following parameters (Mahoney, Van Orden, & Hendricks, 1974):

1. Hb Fe pool (mg), assuming the total blood volume was 6.7% body weight and Fe content in Hb was 0.335%:

\[
\text{Hb Fe pool} = \frac{\text{(body wt (g) \times Hb (g/l) \times 6.7 \times 0.335)}}{10,000}
\]

2. Hb regeneration efficiency (HRE):

\[
\%\text{HRE} = \frac{\text{[Hb Fe pool (final) – Hb Fe pool (initial)]}}{\text{Fe intake (mg)}} \times 100
\]

3. Relative biological value (RBV):

\[
\text{RBV} = 100 \times \frac{\%\text{HRE test group}}{\%\text{HRE SF group}}
\]

At the time of euthanasia, blood was obtained from the abdominal aorta. Blood samples obtained without anticoagulant were collected, and serum Fe concentrations and the unsaturated Fe-binding capacity (UIBC) were determined (Labtest Diagnóstica S/A, Lagoa Santa, Minas Gerais, Brazil). The total Fe-binding capacity (TIBC) and transferrin saturation were calculated from the values of serum Fe and UIBC. Blood samples with anticoagulant (EDTA; 1 mg/ml; Sigma Chemical Co., St. Louis, MO, USA) were obtained for complete and differential cell counts (Dacies & Lewis, 1949). The femoral cavity was flushed with McCoy's 5A medium (Sigma Chemical Co., St. Louis, MO, USA) to collect bone marrow cells. Spleen cells were obtained after disrupting the splenic capsule and dissociating the tissue in McCoy's 5A medium. The total number of cells was quantified in a standard haemocytometer (Neubauer chamber; Herka, Berlin, Germany), and reticulocyte counts were carried out according to Brecher's method (Brecher, 1949). The liver Fe concentrations were analysed by AAS, as described for dietary Fe analysis.

2.5. Apparent mineral absorption

The faecal moisture content was determined through sample weight loss in an oven at 105 °C. The dried faeces were ground and the samples were used for mineral analysis. The apparent intestinal absorption of Fe was calculated according to the equation:

Apparent absorption(%) = 100 × (ingestion-faecal excretion)/ingestion

2.6. Determination of short-chain fatty acids in the caecal contents

Caecal SCFA (acetic, propionic, isobutyric, butyric, isovaleric, valeric, caproic and heptanoic acids) analyses were carried out according to the method described by Zhao, Nyman, and Jönsson (2006) with modifications. The SCFA concentrations were determined using a GC 2010 gas chromatograph (Shimadzu Scientific Instruments Inc., Kyoto, Japan) equipped with a flame ionisation detector (FID). One gram of caecal content was thawed and suspended in 5 ml H2O and homogenised for about 3 min. After that, the pH was adjusted to 2–3 by adding 5 M HCl and the solution was kept at room temperature for 10 min with occasional shaking. The suspension was centrifuged (20 min; 3000 rpm) and 1 μl of the supernatant was injected onto a Nukol-fused silica capillary column (30 m × 0.25-mm i.d.; 0.25-μm thickness film; Supelco, Bellefonte, Palo Alto, CA, USA). The column temperature was held at 100 °C for 0.5 min, increased from 20 °C/min to 200 °C and finally held for 5 min. Hydrogen was used as the carrier gas at a flow rate of 1.8 ml/min, and the split ratio was 1:2. Injector and FID temperatures were 200 °C and 240 °C, respectively. Individual fatty acids were identified by comparison with the retention times of standards (Volatile Free Acid Mix, code 46975; Sigma Chemical Co., St. Louis, MO, USA) and quantified using GC Real Time Analysis 1 software (GC Solution version 2.30.00, LabSolutions, Shimadzu Scientific Instruments Inc., Kyoto, Japan).

2.7. Statistical analysis

The data analysis was carried out with SPSS (SPSS Inc., Chicago, Illinois, USA) for Windows (version 11.5, 2002). All tests were performed assuming bilateral hypotheses and a 5% significance level. Initially, descriptive statistics were used to evaluate the mean and standard deviation (SD) of the studied variable. Data are shown as mean ± SD. In the depletion period, comparison of mean values between CON and ID groups was performed by using an unpaired t-test. In the repletion period, the variable means of the groups were compared by using analysis of variance (ANOVA). A Tukey’s post hoc test was applied to identify where significant differences occurred. A non-parametric Kolmogorov–Smirnov test was applied to verify the normality of the observations and, when the normality hypothesis was rejected, an unpaired t-test and ANOVA were substituted with non-parametric Mann–Whitney and Kruskal–Wallis tests, respectively. The observed power was 85–95% for most tests.

3. Results

3.1. Iron deficiency protocol

A discrete to moderate microcytosis and marked hypochromia was observed in the ID rats when compared to those in the CON group (mean corpuscular volume of 64 ± 9.7 and 40.3 ± 6.3 fl.; mean corpuscular Hb of 19.2 ± 3.2 and 11.8 ± 0.7 pg for CON and ID groups, respectively; P < 0.001) with significant reductions in
Hb concentration and in the Hb Fe pool ($P < 0.001$; Table 2). These changes were the result of a marked reduction in the serum Fe levels and in transferrin saturation ($P < 0.001$) as well as in liver Fe stores ($P < 0.001$). Moreover, the consumption of an ID diet resulted in a significant increase in the reticulocyte count ($P < 0.001$), although an associated discrete decrease in the number of bone marrow cells ($P = 0.076$) and a significant reduction in the total number of spleen cells ($P = 0.017$) compared to the CON group (Table 2) were also observed, indicating that the bone marrow maintained its capacity to restore the erythroid pool.

### 3.2. Analysis of chemical composition of repletion diets

There are no differences in the chemical component (moisture, protein, ether extract and total ash) levels among the diets. However, there are differences in dietary Fe concentrations, with FS diet showing the lowest value (53.0, 60.4, 64.7 and 61.7 for FS, FP, YF and RAF diets, respectively).

### 3.3. Body weight gain and food consumption

No significant differences were observed in body weight among the groups at days 0, 7 and 14 of the repletion period. Moreover, total food intake did not vary among the groups after 7 (105.2 g for FS group) and 14 days (222.6 g for FS group). However, on day 7, the FP rats (FP, YF and RAF groups) showed a higher Fe intake than those in the FS group ($P = 0.03$), whereas on day 14 the Fe intake was similar between the groups.

### 3.4. Haematological and iron bioavailability parameters

In general, no statistically significant differences were observed in the haematological parameters among the experimental groups at the end of the repletion period, except for the higher reticulocyte count observed in the FP group when compared to the other groups (3.6% in FP group; $P = 0.010$). Haemoglobin values, Hb Fe pool, HRE and RBV during the 14-day repletion period are shown in Fig. 1. Considering that the groups presented similar Hb concentrations on day 0 ($P = 0.347$), the mean Hb concentration in YF group increased by 18% ($P < 0.001$) and 7% in comparison to the RAF group and 40% and 24% ($P < 0.001$) in comparison to the FP group on days 7 and 14 of the repletion period, respectively (Fig. 1-A). These data reinforce the reticulocyte count results in the FP group which, after the repletion period, still remained above the values of the control group.

The efficiency of Hb recovery reflects the ratio of dietary Fe conversion into Hb to the amount of ingested Fe over the course of the repletion period. In the present study, only YF animals showed higher HRE values compared to those in the FP group and similar HRE values compared to FS group, on day 7 of the repletion period ($P < 0.001$; Fig. 1C). The Hb concentration observed at the end of the repletion period in the FS animals was considered the reference. Hence, the FS group was taken as a reference for expressing the bioavailability of Fe from FP (FS is assigned the value 100%).

### Table 2

Body and liver weights, biochemical iron parameters, liver Fe concentrations and haematological parameters of rats fed control and iron-deficient diets for 14 days.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>CON ($n = 6$)</td>
<td>ID ($n = 6$)</td>
</tr>
<tr>
<td>Liver weight (g wet wt/100 g body wt)</td>
<td>125.0 ± 8.8</td>
<td>116.3 ± 3.3</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>135.6 ± 6.7</td>
<td>68.1 ± 6.5</td>
</tr>
<tr>
<td>Hb Fe pool (mg)</td>
<td>3.3 ± 0.4</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>Serum Fe (µg/dl)</td>
<td>249.3 ± 34.8</td>
<td>16.9 ± 3.5</td>
</tr>
<tr>
<td>TIBC (µg/dl)</td>
<td>299.2 ± 44.8</td>
<td>469.9 ± 113.4</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>83.5 ± 1.0</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>3.2 ± 1.8</td>
<td>23.6 ± 1.8</td>
</tr>
<tr>
<td>Liver Fe (µmol/g dry wt)</td>
<td>57.0 ± 13.4</td>
<td>10.3 ± 1.7</td>
</tr>
<tr>
<td>Bone marrow total cells (×10⁷/ml)</td>
<td>2.7 ± 1.0</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Spleen total cells (×10⁷/ml)</td>
<td>14.8 ± 2.4</td>
<td>10.1 ± 3.3</td>
</tr>
</tbody>
</table>

CON, control group; ID, Fe-deficient group; TIBC, total Fe-binding capacity. Results expressed as mean ± SD. Mean values followed by the same superscript letter are not significantly different ($P < 0.05$).

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Fig. 1. Iron bioavailability in iron-deficient rats fed diets containing ferrous sulphate or ferric pyrophosphate supplemented with 7.5% inulin-type fructans as yacon flour or Raftilose P95. Abbreviations: FS, ferrous sulphate; FP, ferric pyrophosphate; YF, yacon flour; RAF, Raftilose P95; RBV, relative biological value; HRE, haemoglobin recovery efficiency. Results are expressed as mean ± SD ($n = 8$ for FS group, and $n = 12$ for the other groups). Mean values followed by the same superscript letter are not significantly different ($P < 0.05$).
(RBV). The RBVs of the FP in the YF-supplemented group were 84% and 97% on days 7 and 14 of the repletion period, respectively. These values were significantly higher than those of FP and RAF groups on day 7 ($P < 0.001$; Fig. 1D). Moreover, at this time, no significant difference was observed between the RAF and FP groups in terms of RBV. On day 14, however, there was no difference in the RBV between both ITF groups, but their mean RBV was higher than that of FP group ($P < 0.001$).

### 3.5. Intestinal parameters and liver iron concentrations

ITF supplementation led to an increased caecum (wall and contents) weight and decreased the caecal pH values, whereas these effects were more pronounced in YF-fed rats ($P < 0.001$; Table 3). The total caecal pool of SCFA was significantly increased after ITF consumption (despite the lack of significant effects on total SCFA concentration), and the YF group showed higher values than did the RAF-fed group ($P < 0.001$). Moreover, the butyrate concentrations were increased only when YF was the ITF source ($P < 0.001$; Table 3). As expected, the FP group presented a lower apparent Fe absorption when compared to the FS group, assessed in the last 5 days of the repletion period (days 10–14; $P < 0.001$). However, ITF consumption did not significantly affect the apparent Fe absorption. The liver Fe concentrations were lower in FP than FS rats, whereas YF consumption recovered to levels comparable to those seen in the FS group. Moreover, RAF consumption resulted in increased hepatic Fe levels compared to the levels in the FP group, although the values remained lower than those of the FS group ($P < 0.001$; Fig. 2).

### 4. Discussion

Several factors in the diet can influence the mineral bioavailability, the magnitude of which depends on inhibitors and promoters in a meal, and hence on the food matrix (Gibson, 2007). Over the past years, the positive effects of ITF on macromineral (Ca, Mg) absorption and bioavailability have been frequently observed in animal (rats, pigs) (Lobo et al., 2007; Scholz-Ahrens & Schrezenmeir, 2007) and human studies (Van Der Heuvel, Muys, Van Dokkum, & Schaafsma, 1999). However, data concerning their effects on micromineral bioavailability are relatively scarce and so far have presented contradictory results (Scholz-Ahrens & Schrezenmeir, 2007). In particular, although there is some evidence that Fe bioavailability is positively affected (Tako et al., 2008; Yasuda, Roneker, Miller, Welch, & Lei, 2006), to our knowledge, there are no studies using a non-purified source of ITF on Fe bioavailability in a rat model. In the present study, our results showed that the consumption of diets supplemented with YF (7.5% ITF) improved the bioavailability of Fe from FP (around 30–50% the bioavailability of Fe from FS; Hurrel, 2002), as evaluated by Hb repletion assay in anaemic rats. Moreover, such effects were more pronounced than those observed after dietary supplementation with 7.5% ITF from RAF, a purified source of ITF from chicory roots.

The consumption of ITF led to a higher HRE compared to values observed in the FP group, and this effect was similar to that observed in FS group. Moreover, the RBV of FP in ITF-fed animals was equivalent to that of FS group (considered the reference in Fe bioavailability studies; Hurrel, 2002; Mahoney et al., 1974; Poltronieri, Arèas, & Colli, 2000), and this effect was even more significant in the YF group on day 7 of the repletion period. Moreover, although statistically significant values were not observed, Fe apparent absorption increased by 80% and 34% after ITF consump-

### Table 3

Intestinal parameters (caecal weight, caecal content pH and SCFA production, and apparent mineral absorption) in iron-deficient rats fed diets containing ferrous sulphate or ferric pyrophosphate supplemented with 7.5% inulin-type fructans as yacon flour or Raftilose P95.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dietary treatment</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FS ($n = 8$)</td>
<td>FP ($n = 12$)</td>
</tr>
<tr>
<td>Caecal wall weight (g wet wt/100 g body wt)</td>
<td>0.26 ± 0.02$^a$</td>
<td>0.33 ± 0.03$^a$</td>
</tr>
<tr>
<td>Caecal content weight (g wet wt/100 g body wt)</td>
<td>1.0 ± 0.2$^a$</td>
<td>1.5 ± 0.2$^a$</td>
</tr>
<tr>
<td>Caecal content pH</td>
<td>7.0 ± 0.2$^a$</td>
<td>7.1 ± 0.2$^a$</td>
</tr>
<tr>
<td>SCFA concentration (μmol/g wet wt)</td>
<td>30.5 ± 4.6$^a$</td>
<td>29.1 ± 6.3$^b$</td>
</tr>
<tr>
<td>Acetate</td>
<td>7.0 ± 1.2$^a$</td>
<td>6.4 ± 1.8$^a$</td>
</tr>
<tr>
<td>Propionate</td>
<td>4.8 ± 0.8$^a$</td>
<td>4.2 ± 0.8$^a$</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>47.3 ± 6.3$^a$</td>
<td>44.1 ± 8.6$^a$</td>
</tr>
<tr>
<td>Total SCFA pool (μmol/caecum)</td>
<td>79.7 ± 23.3$^a$</td>
<td>94.8 ± 13.6$^a$</td>
</tr>
<tr>
<td>Acetate</td>
<td>18.4 ± 6.1$^a$</td>
<td>21.1 ± 5.9$^a$</td>
</tr>
<tr>
<td>Propionate</td>
<td>12.5 ± 3.3$^a$</td>
<td>13.0 ± 2.2$^a$</td>
</tr>
<tr>
<td>Butyrate</td>
<td>123.5 ± 35.2$^a$</td>
<td>144.1 ± 18.9$^a$</td>
</tr>
<tr>
<td>Apparent Fe absorption (%)</td>
<td>64.4 ± 21.3$^a$</td>
<td>23.7 ± 6.4$^a$</td>
</tr>
</tbody>
</table>

FS, ferrous sulphate; FP, ferric pyrophosphate; YF, yacon flour; RAF, Raftilose P95. Total SCFA is the sum of acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, caproic, isocaproic and heptanoic acids. Results expressed as mean ± SD. Mean values followed by the same superscript letter are not significantly different ($P < 0.05$).
tion (for YF and RAF groups, respectively), compared with the FP group.

Only 10% of dietary Fe is absorbed in the small intestine (mainly in the duodenum), which indicates that significant amounts of Fe are recovered in the luminal content of the large intestine (Lund, Wharf, Fairweather-Tait, & Johnson, 1998). Recent evidence suggests that, under some circumstances, the proximal colon may significantly contribute to Fe absorption (Frazer, Wilkins, & Anderson, 2007; Takeuchi et al., 2005). In this context, bacterial fermentation of non-digestible carbohydrates in the large intestine results in SCFA production, which reduces the luminal pH and improves mineral solubility (Scholz-Ahrens & Schrezenmeir, 2007). Low pH and high SCFA (mainly butyrate) concentrations both result in intestinal tissue hypertrophy, leading to increased surface area in the large intestine and thus enhanced mineral absorption (Lobo et al., 2007; Scholz-Ahrens & Schrezenmeir, 2007). Hence, the lower luminal pH and the larger caecum observed in the ITF-fed rats (mainly in the YF group) could have contributed to increased Fe bioavailability compared to FP rats.

In this study, the ITF consumption increased caecal SCFA production, and this effect was more pronounced in the YF-supplemented group than in the RAF group (~70%, YF vs. RAF). Moreover, butyrate content (μmol/caecum) increased by 108% in the YF-supplemented group when compared to the RAF group. In rats, the trophic effects in the caecum caused by bacterial fermentation of non-digestible carbohydrates are attributed to the increase in cell proliferation as a consequence of changes in the intestinal tissue architecture (Kleessen et al., 2003; Lobo et al. 2007). In this respect, a prior study demonstrated an increase in crypt bifurcation in rats as a response to the consumption of YF containing 7.5% ITF after 27 days (Lobo et al. 2007), an effect which may have contributed to the increase in the mineral absorption surface area. In addition, in this study, the presence of YF in the large intestine may have resulted in more non-digestible substrates being fermented given the DF content of YF (6% IDF and 4% SDF). In this context, other physico-chemical properties of DF may affect the increase in cell proliferation as a consequence of changes in the mucosal architecture (Kleessen et al., 2003; Lobo et al. 2007). In this manner, other factors should be taken into account, such as the degree of mineral deficiency, as well as the composition of the food matrix in which Fe is found, which may influence the physico-chemical properties of the bolus in the intestinal lumen. These effects, if confirmed in humans, might contribute to the formulation of specific diets for individuals with Fe deficiency.

Conflicts of interest

There are no financial, professional, or personal conflicts of interests for any of the authors.

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