Stabilized–Solubilized Ferric Pyrophosphate as a New Iron Source for Food Fortification. Bioavailability Studies by Means of the Prophylactic–Preventive Method in Rats

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Abstract The purpose of the present work was to evaluate the iron bioavailability of a new ferric pyrophosphate salt stabilized and solubilized with glycine. The prophylactic–preventive test in rats, using ferrous sulfate as the reference standard, was applied as the evaluating methodology both using water and yogurt as vehicles. Fifty female Sprague–Dawley rats weaned were randomized into five different groups (group 1: $FeSO_4$; group 2: pyr; group 3: $FeSO_4$ + yogurt; group 4: pyr + yogurt and group 5: control). The iron bioavailability (BioFe) of each compound was calculated using the formula proposed by Dutra-de-Oliveira et al. where BioFe $\% = (HbFe_f - HbFe_i) \times 100/ToFeIn$. Finally, the iron bioavailability results of each iron source were also given as relative biological value (RBV) using ferrous sulfate as the reference standard. The results showed that both BioFe % and RBV % of the new iron source tested is similar to that of the reference standard independently of the vehicle employed for the fortification procedure ($FeSO_4$ 49.46±12.0% and 100%; Pyr 52.66±15.02% and 106%; $FeSO_4$ + yogurth 54.39±13.92% and 110%; Pyr + yogurt 61.97±13.54% and 125%; Control 25.30±6.60, $p < 0.05$). Therefore, the stabilized and soluble ferric pyrophosphate may be considered as an optimal iron source for food fortification.

Keywords Iron · Fortification · Rats · Bioavailability · Prophylactic-preventive method

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Introduction

Iron deficiency is still an unsolved nutritional problem all over the world. It affects developed as well as developing countries [1]. In developed countries, iron deficiency is associated with low ingest levels of absorbable iron, whereas in developing countries, it may respond to a poor availability of the dietary iron, resulting from the presence of iron absorption inhibitors in the diet. This circumstance is aggravated by the frequent incidence of intestinal worm infections, malaria, and vitamin A deficiency [1].

Food fortification has been shown to be an efficient strategy to prevent iron deficiency [2]. Many iron compounds are at our disposal to be used as potential sources for food fortification. However, only a few of them completely meet the requirements of high iron bioavailability, inertness in relation to the sensorial properties of the fortified food, absence of toxicity, resistance during storing or elaboration processes of the fortified food, and absorption mechanism following the same pattern as dietary iron. For these reasons, many efforts are still made to provide an adequate iron source for food fortification. With regard to fortification vehicles, different foods have been used such as milk and other dairy products, which are very attractive vehicles because they are consumed widely and have high nutritional properties [1].

For almost a decade, our group has been working in the area of food fortification with different micronutrients. In this case, the purpose of the present work was to evaluate the iron bioavailability of a new ferric pyrophosphate salt stabilized and solubilized with glycine. The prophylactic–preventive test in rats [3], using ferrous sulfate as the reference standard, was applied as the evaluating methodology both using water and yogurt as vehicles.

Materials and Methods

Animals

All animal experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996". Fifty female, inbred, Sprague–Dawley rats weaned at age 23 days were individually weighed (initial weight) and their initial hemoglobin concentrations (HbCi) were determined by the cyanomethahemoglobin method [4]. The animals were randomized into five different groups (group 1: $FeSO_4$; group 2: pyr; group 3: $FeSO_4$ + yogurt; group 4: pyr + yogurt, and group 5: control) as described below, and individually housed in stainless-steel cages in a temperature- and light-controlled environment.

Treatments

The AIN-93-G diet for rodents [5] modified without iron addition was used as the basal diet to evaluate the iron sources under study. In this sense, group $FeSO_4$ was fed with the basal diet fortified with 16.5 mg Fe/kg as $FeSO_4 \cdot 7H_2O$ (Fluka, Germany), group pyr with 16.25 mg Fe/kg as pyrophosphate salt stabilized and solubilized with glycine (Lipotech, Argentina), group $FeSO_4$ + yogurt with 14.7 mg Fe/kg as $FeSO_4 \cdot 7H_2O$ added in yogurt as vehicle and group pyr + yogurt with 15.4 mg Fe/kg as pyrophosphate salt stabilized and solubilized with glycine added in yogurt as vehicle. The control group received the basal diet without iron addition. The diets were freely administered to the rats as the only source

of solid nourishment and the amount of the consumed food was registered daily. The iron concentration of each diet was determined by atomic absorption spectroscopy in samples, which were previously mineralized. Free access to deionized water (Ametek, USA) was also allowed for the rats.

The diets were administered for 3 weeks. After this period, the rats were weighed, and final hemoglobin concentrations (HbCf) were determined as described for the initial ones. The iron bioavailability (BioFe) of each compound was calculated using the formula proposed by Dutra-de-Oliveira et al. [6]:

$$BioFe\% : (HbFef - HbFei) \times 100/ToFeIn$$

where HbFef is the final hemoglobin iron, HbFei is the initial hemoglobin iron, and ToFeIn is the total iron intake calculated as the product of the dietary iron concentration (DIC) and the food amount consumed by each animal during the experiment. Each HbFe (initial or final) was calculated considering a blood volume of 0.067 mL blood/g body weight, and a hemoglobin content of iron of 3.4 mg Fe/g Hb by means of:

$$HbFe = BW(0.067\,mL\,blood/g\,BW)(HbC)(3.4\,mgFe/g\,Hb)$$

were HbC is the hemoglobin content (HbCi or HbCf).

The bioavailability results of each iron source are also given as relative biological value (RBV), which was calculated as the percentage ratio between the BioFe of the studied source and the BioFe of the reference standard.

Statistics

The statistical analyses of the results were carried out by a one-way analysis of variance followed by the Student–Newman–Keuls test, fixing $p < 0.05$ as the limit for significance [7].
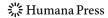
Results

Values of DIC of each diet and the ToFeIn per animal of each group are shown in Table 1. The ToFeIn of the control group is significantly lower than that of the others ($p < 0.05$) because of the lower DIC of this diet. Table 1 also shows weight variation, HbC variation,

Table 1 Dietary Iron Concentration; Total Iron Intake, Weight Variation, Hemoglobin Concentration Variation, and Hemoglobin Iron Variation in Animals Receiving the Diets Under Study

Group	DIC (mg/kg)	ToFeIn per animal (mg)	Weight variation (mg)	HbC variation (g/dl)	HbFe variation (mg)
FeSO₄	16.5	5.2±1.4	94.65±8.80	2.36±1.98	2.59±0.63
Pyr	16.3	4.0±1.6	88.60±7.86	1.73±2.31	2.56±0.73
FeSO₄ + yogurth	14.7	4.9±1.7	88.55±8.78	0.40±1.15	2.16±0.55
Pyr + yogurt	15.4	4.1±1.5	84.44±9.91	2.31±1.70	2.52±0.55
Control	3.8	0.6±1.3	75.01±6.90*	−3.80±1.80*	0.40±0.20*

DIC Dietary iron concentration, *ToFeIn* total iron intake, *HbC* hemoglobin concentration, *HbFe* hemoglobin iron

*Significantly different from the rest of the groups $p < 0.05$

and HbFe variation, and as it is evidenced from these results, the control group had the lowest values for each parameter in comparison with the other groups ($p<0.05$) which have no statistical differences among them.

The BioFe % and the RBV % of the studied iron sources are shown in Table 2. Although values of BioFe % pyr + yogurt were slightly higher than the others, statistically nonsignificant differences were found, except for the control group which showed a significantly low value of BioFe % in comparison with the others ($p<0.05$). RBVs obtained for each iron source under study are not significantly different among them, although pyr + yogurt values were also slightly higher than the others.

Discusion

Food fortification with iron can be an effective strategy to control iron deficiency anemia, but adding Iron to food still remains a challenge [8]. Water soluble iron compounds have high bioavailability but often cause adverse organoleptic changes in foods. Poorly soluble in water iron compounds, although more stable in foods, have low bioavailability. In order to overcome these problems, many strategies have been developed such as protect highly soluble iron compounds or reduce the particle size of insoluble iron compounds. Ferric pyrophosphate, which is a white-colored poorly soluble iron compound, does not change organoleptic properties of foods even in many difficult-to-fortify vehicles and has low bioavailability compared to ferrous sulfate (30–50%) [9]. Since particle size may be an important determinant of Iron absorption from poorly soluble iron compounds, many reports showed improvements of ferric pyrophosphate bioavailability when this parameter was modified from regular (21 μm) to 2.5 or 0.5 μm or nanoparticles and even protected by encapsulation [10, 11]. In this case, our study evaluated a new stabilized and soluble ferric pyrophosphate salt, a compound with high solubility which does not interact with the food matrix and does not modifies its sensorial properties. The study assayed the RBV of this new iron compound in comparison with the standard ferrous sulfate when both were added to diet in water or yogurt as food vehicles. In this way, the prophylactic–preventive test was used as the evaluating methodology because it is recognized to provide similar RBVs to the depletion–repletion assay (AOAC) but requiring a shorter experimental period [12]. Because nonsignificant differences of the ToFeIn or weight variations were found among the groups that received the diets added with ferrous sulfate or stabilized solubilized ferric pyrophosphate, it can be deduced that these sources have the same positive influence on the animals' growth. Therefore, we can conclude that this new stabilized and soluble ferric pyrophosphate may be consider as an optimal iron source for food fortification because it

Table 2 BioFe and RBV of the Studied Sources

Group	BioFe (%)	RBV (%)
$FeSO_4$	49.5±12.0	100
Pyr	52.7±15.0	106
$FeSO_4$ + yogurt	54.4±13.9	110
Pyr + yogurt	62.0±13.5	125
Control	25.3±6.6*	–

BioFe Iron bioavailability, *RBV* relative biological value

*Significantly different from the rest of the groups $p<0.05$

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has similar bioavailability to ferrous sulfate but it does not change sensorial characteristics of foods.

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