

# Bioavailability of Stabilized Iron (II) Sulfate in an Industrialized Fortified Infant Dessert. Studies in Rats by Means of the Prophylactic-Preventive Method

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**Abstract:** The purpose of the present work is to evaluate the iron bioavailability of stabilized iron (II) sulphate in industrialized fortified infant dessert. The prophylactic-preventive test in rats, using ferrous sulphate as the reference standard, was applied as the evaluating methodology. Thirty female Sprague-Dawley rats weaned, were randomized into three different groups (group 1: FeSO<sub>4</sub> + IDF; group 2: FeSO<sub>4</sub> stabilized + IDF and group 3: control). The iron bioavailability (BioFe) of each compound was calculated using the formula proposed by Dutra-de-Oliveira *et al* where BioFe = (HbFef-HbFei) / 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 % were not different among the groups assayed (FeSO<sub>4</sub> + D 0.38±0.01 and 100%; FeSO<sub>4</sub> stabilized + D 0.39±0.02 and 102%).

**Key Words:** Iron, fortification, rats, bioavailability, prophylactic-preventive method.

## INTRODUCTION

Iron deficiency is still an unsolved nutritional problem all over the world. It affects developing as well as industrialized countries [1], producing physical and psychological diseases, reducing productivity, and affecting the health budget [2]. In industrialized countries, iron deficiency is associated with low intake of absorbable iron, while in developing countries, it may respond to a poor availability of the dietary iron because of 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 [3]. Food fortification has demonstrated to be an efficient strategy to prevent iron deficiency [4]. However, any iron source used for food fortification must comply with some important requirements [5, 6]. These are high iron bioavailability (BioFe), inertness in relation to the sensorial properties of the fortified food, absence of toxicity, stability during storing, or elaboration processes of the fortified food and absorption mechanism of the iron in the fortified food should follow the same pattern as dietary iron. Many iron compounds are now at our disposal to be used as potential sources for food fortification; however, only a few of them completely meet the requirements mentioned above because BioFe and reactivity with the food matrix depend on the solubility of the iron source [3]. In fact, only soluble iron is available for absorption, but also soluble iron catalyzes oxidation processes that affect fatty acids, vitamins, and amino

acids, promoting the development of off-flavors and off-odors in the fortified products and reducing the food nutritional value [7]. For these reasons, many efforts are still done to provide an adequate iron source for food fortification.

Stabilized iron (II) sulfate has a natural composition (ferrous sulfate stabilized with glycine and malic acid) [8]; therefore, undesirable effects or interactions are not expected at nutritional levels. We have previously determined the bioavailability of stabilized iron (II) sulfate for its potential use in food fortification [8]. The iron bioavailability (BioFe) and the relative biological value (RBV) of stabilized iron (II) sulfate were closely similar to the reference standard [8]. However, that conclusion was reached when adding the iron source directly to the mineral mix of the diet, and no vehicle was used. On the present work, the iron source was added in an infant dessert and since it contains other minerals such as calcium and zinc, the BioFe could be affected due to interactions between them. Thus, more studies should be performed to evaluate if there are any changes in other minerals bioavailability other than iron. In this work, our aim is to determine the bioavailability of stabilized iron (II) sulfate when it is used as an iron source for industrialized fortification of an infant dessert with a composition similar to petit suisse cheese (Danonino, Danone Argentina). For this purpose, we used the prophylactic-preventive method in rats [9].

## MATERIALS AND METHODOLOGY

We used 30 inbred Sprague-Dawley male rats aged 25 days, which were weaned and weighed at the beginning of the experiment. The animals were randomized into three different groups and individually housed in stainless steel cages in a temperature- and light-controlled environment.

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Blood samples were taken on day zero of the treatment by capilar retroorbital sinus puncture. The initial hemoglobin concentration (HbC) of each rat was determined by the cyanomethemoglobin method [10].

The AIN-93-G diet for rodents [11] modified without iron addition was used as the basal diet to evaluate the iron sources under study. In this sense, group 1 received the basal diet added with the infant dessert fortified (FeSO<sub>4</sub> + IDF) in our laboratory with FeSO<sub>4</sub> 7H<sub>2</sub>O (Fluka, Switzerland); group 2 received basal diet added with industrialized fortified infant dessert (FeSO<sub>4</sub> stabilized + IDF; ferrous sulfate salt stabilized with glycine and malic acid; Lipotech, Argentina) and group 3 (control) received the basal.

The iron content of each diet was determined by atomic absorption spectroscopy after the samples were mineralized as previously described elsewhere [12]. The animals began to receive these diets after being weaned; no other type of nourishment was offered meanwhile. Free access to deionized water (Ametek) was allowed to the rats, food intake was also provided *ad libitum* but daily registered. The diets were administered during 3 weeks. After this period, the rats were weighed again, treated with 1,500 IU of heparin per kilogram body weight (BW), anesthetized with ethyl ether, and finally bled by means of retroorbital sinus puncture, collecting between 8 and 10 mL of blood from each animal. Final HbC was determined as the initial ones.

The BioFe% of each compound was calculated using the formula proposed by Dutra-de- Oliveira [13]: BioFe% : [(HbFef-HbFei) / ToFeIn] x 100 where HbFef is the final haemoglobin iron, HbFei is the initial haemoglobin 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 (BW), and a haemoglobin content of iron of 3.4 mg Fe/g Hb by means of: HbFe = BW(0.067 mL blood/g BW) (HbC) (3.4 mg Fe/g Hb) were HbC is the haemoglobin content (HbCi or HbCf).

The bioavailability results of each iron source were also given as RBV %, which was calculated as the percentage ratio between the BioFe% of the studied source and the BioFe% of the reference standard.

The statistical analysis of the results were carried out by a one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test, fixing  $p < 0.05$  as the limit for significance [14].

**Table 1. Dietary Iron Concentration (DIC); Total Iron Intake (ToFeIn), Weight Variation, Haemoglobin Concentration (HbC) Variation and Haemoglobin Iron (HbFe) Variation in Animals Receiving the Diets Under Study**

Group	DIC (mg/kg)	ToFeIn per Animal (mg)	Weight Change (mg)	HbC Change (g/dl)	HbFe Change (mg)
FeSO <sub>4</sub> + IDF	12.5 ± 0.2	4.5 ± 0.5	77.65 ± 10.94*	0.69 ± 0.75	1.75 ± 0.27
FeSO <sub>4</sub> stabilized + IDF	13.4 ± 0.3	4.9 ± 0.6	73.80 ± 13.39	1.12 ± 1.51	1.94 ± 0.37
Control	1.7 ± 0.2	0.5 ± 0.1	63.3 ± 12.7*	-0.49 ± 1.01*	1.09 ± 0.38*

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

## RESULTS

Table 1 shows the following parameters related to the BioFe% and RBV calculation: DIC, ToFeIn, weight variation, HbC variation, and HbFe variation. The DIC of each diet were, respectively, 1.7, 12.5, and 13.4 mg/kg for control, FeSO<sub>4</sub>, and stabilized iron (II) sulfate.

The ToFeIn of the control group was lower and significantly different ( $p < 0.05$ ) than those of the remaining groups. The results of the HbC variation of the basal diet group were different from ferrous sulfate and stabilized iron (II) sulfate groups. The control group showed lower and significantly different ( $p < 0.05$ ) HbFe values than the other groups.

The BioFe% and RBV % of the studied iron sources are shown in Table 2. Non significant difference was found in the BioFe% values.

**Table 2. Iron Bioavailability (BioFe%) and Relative Biological Value (RBV %) of the Studied Sources**

Group	BioFe%	RBV (%)
FeSO <sub>4</sub> + IDF	38 ± 1	100
FeSO <sub>4</sub> stabilized + IDF	39 ± 2	102

## DISCUSSION AND CONCLUSION

As we mentioned above, the purpose of this work was to determine the bioavailability of stabilized iron (II) sulfate when it is used in food fortification and particularly in the industrialized fortification of an infant dessert. The prophylactic-preventive test used as the evaluating methodology is recognized to provide similar RBVs than the depletion-repletion assay (AOAC) but requiring a shorter experimental period of time [15]. Because non significant differences of the ToFeIn or weight variations were found among the groups that received the diets added with infant dessert fortified with ferrous sulfate or stabilized iron (II) sulphate (Table 1), it can be deduced that these sources have the same positive influence on the animals' growth. The HbC variation of the basal diet group was different from ferrous sulfate and stabilized iron (II) sulfate groups (Table 1). It should be pointed out that while the HbC of the control group greatly decreases, the other groups showed positive changes. Therefore, it can be deduced that the iron provided by these fortified diets is efficiently incorporated into hemoglobin.

The BioFe% and RBV % of infant dessert fortified with stabilized iron (II) sulfate were closely similar to the reference standard. Stabilized iron (II) sulfate has a natural composition (ferrous sulfate stabilized with glycine and malic acid); therefore, undesirable effects or interactions are not expected at nutritional levels. In preliminary studies performed in our laboratory as well as industrial trials, stabilized iron (II) sulfate was used as the iron source to fortify fluid milk and petit suisse cheese with doses of 15 mg per liter and 20 mg of iron per kilogram, respectively. Metallic taste and rancidity, which usually happen when ferrous sulfate is used, were not detected in the case of this stabilized form.

Therefore, our results showed that fortification with this iron source represents a strategy to provide iron of high bioavailability and minimal food interaction. This conclusion is of great interest since a diary intake of fortified food with high iron bioavailability could help prevent the deficiency of this nutrient during infancy mostly after the exclusively breast feeding six months when complementary alimentation is needed.

#### REFERENCES

- [1] De Maeyer E, Tegman E, Raystone M. The prevalence of anemia in the world. *World Health Stat Q* 1985; 38: 302-316.
- [2] Boccio J, Iyengar V. Iron deficiency and anemia: causes, consequences and strategies to overcome this nutritional problem. *Biol Trace Elem Res* 2003; 94: 1-32.
- [3] Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro R, Weill R, Boccio J. Strategies to combat zinc and iron deficiency. *Nutr Rev* 2002; 60: 52-58.
- [4] Bothwell TH, McPhail AP. Prevention of iron deficiency by food fortification. In: Fomon SJ, Zlotkin S, Eds. *Nutritional Anaemias*. Nestlé Nutrition Workshop series 30. Raven, New York, 1992: 183-192.
- [5] Boccio JR, Zubillaga MB, Caro RA, Gotelli CA, Gotelli MJ, Weill R. Bioavailability and stability of microencapsulated ferrous sulfate in fluid milk: studies in mice. *J Nutr Sci Vitaminol* 1996; 42: 233-239.
- [6] Cook JD, Reusser ME. Iron fortification: an update. *Am J Clin Nutr* 1983; 38(4): 648-659.
- [7] Beltz HD, Grosch W. *Food chemistry*. Berlin, Heidelberg, New York: Springer, 1987.
- [8] Salgueiro J, Torti H, Meseri E. Bioavailability studies of stabilized iron (II) sulfate by means of the prophylactic-preventive method. *Biol Trace Element Res* 2007; 120: 110-113.
- [9] Motzok I, Pennell MD, Davies MI, Ross HU. Effect of particle size on the biological availability of reduced iron. *J Assoc Off Anal Chem* 1975; 58: 99-103.
- [10] Bernard HJ. Hematology and coagulation. In: Todd JC, Ed. *Todd-Sanford-Davidsohn Clinical diagnosis and management by laboratory methods*, 17th ed. Saunders, Philadelphia, USA, 1988: 721-773.
- [11] Reeves PG, Nielsen FH and Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc Writing Committee on the Reformulation of the AIN-76 Rodent Diet. *J Nutr* 1993; 123: 1939-1951.
- [12] Lysionek AE, Zubillaga MB, Calmanovici GP, et al. Modification of the ferrozine technique to analyze iron contents in different foods. *Biol Trace Elem Res* 1998; 65: 87-94.
- [13] Dutra-de-Oliveira JE, Freitas MLS, Ferreira JF, Gonçalves AL, Marchini JS. Iron from complex salts and its bioavailability to rats. *Int J Vitam Nutr Res* 1995; 65: 272-275.
- [14] Sokal RR, Rohlf FJ. *Biometry*. San Francisco: Freeman, 1981.
- [15] Fritz JC, Pla GW, Harrison BN, Clark GA, Smith EA. Measurement of the bioavailability of iron, using the rat hemoglobin repletion test. *J Assoc Off Anal Chem* 1978; 61: 709-714.

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