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Stabilized Ferrous Gluconate as Iron Source for Food Fortification

Bioavailability and Toxicity Studies in Rats

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ABSTRACT

The iron bioavailability and acute oral toxicity in rats of a ferrous gluconate compound stabilized with glycine (SFG), designed for food fortification, was studied in this work by means of the *prophylactic* method and the Wilcoxon method, respectively. For the former studies, SFG was homogenously added to a basal diet of low iron content, reaching a final iron concentration of 20.1 ± 2.4 mg Fe/kg diet. A reference standard diet using ferrous sulfate as an iron-fortifying source (19.0 ± 2.1 mg Fe/kg diet) and a control diet without iron additions (9.3 ± 1.4 mg Fe/kg diet) were prepared in the laboratory in a similar way. These diets were administered to three different groups of weaning rats during 23 d as the only type of solid nourishment. The iron bioavailability of SFG was calculated as the relationship between the mass of iron incorporated into hemoglobin during the treatment and the total iron intake per animal. This parameter resulted in 36.6 ± 6.2% for SFG, whereas a value of 35.4 ± 8.0% was obtained for ferrous sulfate. The acute toxicological studies were performed in 2 groups of 70 female and 70 male Sprague–Dawley rats that were administered increasing doses of iron from SFG. The LD_{50} values of 1775 and 1831 mg SFG/kg body wt were obtained for female and male rats, respectively, evidencing that SFG can be considered as a safe compound from a toxicological point of view.

Index Entries: Iron; bioavailability; fortification; toxicity.

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INTRODUCTION

For almost for a decade, our group has been working in the area of food fortification with different micronutrients. Much of our work was dedicated to study the nutritional properties of SFE-171 *(1–7),* an iron fortifying source designed for milk and dairy products, which is successfully used in Argentina and other countries. More recently, we have focused our efforts on producing and studying new iron sources to fortify solid foods for massive consumption, such as flour or cereals. Ferric and a ferrous gluconate compounds, both stabilized with glycine, resulted from this research. The iron bioavailability of the former was studied by the *prophylactic–preventive* method in rats and it was found to be closely similar to that of ferrous sulfate *(8).* In the case of the latter (SFG), this property was studied by the same methodology and the results are presented in this work, together with those of its acute oral toxicity also in rats.

AU: Pls spell out SFE at first use.

MATERIAL AND METHODS

Bioavailability Studies in Rats

Thirty female inbred Sprague–Dawley rats weaned at 25 d old were individually weighed while their hemoglobin concentrations were determined by the cyanomethahaemoglobin method *(9).* The animals were separated into 3 different groups of 10 rats each and housed in stainless-steel cages in a temperature- and light-controlled environment (25°C with a half-day light/dark cycle, light up, 9:00–21:00).

The AIN-93-G diet for rodents *(10)* modified without iron addition was used as the basal diet to evaluate the iron sources under study. In this sense, one of the referred lots of rats (group 1) was fed with the basal diet fortified with 10 mg Fe/kg as SFG (Lipotech, Argentina). A second lot (group 2) received the basal diet fortified with 10 mg Fe/kg as $FeSO_4 \cdot 7H_2O$ (Fluka, Germany), which was used as reference standard. Finally, the third lot of rats (group 3) was used as a control, receiving the basal diet without iron additions. 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 the Ferrozine technique modified for foods *(11).* Free access to deionized water (Ametek, USA) was also allowed for the rats.

After 23 d, each animal was weighed again and then treated with 1500 IU heparin/kg body wt, anesthetized with diethyl ether, and sacrificed by bleeding through retro-orbital sinus puncture, collecting a few milliliters of blood per animal. The hemoglobin concentration in the collected blood of every rat was once again determined by the cyanomethahaemoglobin method *(9).* Each animal liver was entirely removed, washed with

Table 1
Dietary Iron Concentration (DIC), Total Iron Intake per Animal (ToFeIn), and Weight Variation (ΔW) for Each Group

Group	Number of animals	DIC (mg/kg)*	ToFeIn (mg)*	ΔW (g)*
1. SFG	10	20.1 ± 2.4^a	4.1 ± 1.1^a	77 ± 9^a
2. Ferrous sulphate	10	19.0 ± 2.1^a	3.7 ± 0.9^a	76 ± 11^a
3. Control	10	9.3 ± 1.4^b	1.9 ± 0.6^b	55 ± 7^b

* Value (mean ± SD) without a common superscript letter in the same column is significantly different at $p<0.05$.

deionized water, weighed, and stored at –20°C. The iron content of every liver was determined afterward by the Ferrozine technique *(11).*

The following parameters were calculated as described previously *(12):* dietary iron content (DIC), total iron intake (ToFeIn), hemoglobin iron (HbFe), iron bioavailability (BioFe), relative biological value (RBV), weight variation (ΔW), and liver iron content (LIC), which are given in Tables 1 and 2.

AU: weight variation (ΔW) ok?

Statistical analysis of the results was carried out by one-way analysis of variance (ANOVA) followed by the Scheffé test *(13).* The differences among data (mean ± SD) were considered significant at $p<0.05$.

Toxicity

Two groups of 70 female and 70 male Sprague–Dawley rats respectively were used to carry out the acute toxicity studies by administration of increasing doses of iron from SFG. The lethal dose 50% (LD_{50}) was determined by the method proposed by Litchfield and Wilcoxon *(14).*

RESULTS AND DISCUSSION

The DIC of the administered diets and the ToFeIn per animal are listed in Table 1; both parameters were significantly lower ($p<0.05$) for group 3. Table 1 also shows that the weight increase of the animals of group 3 was significantly lower ($p<0.05$) than the weight increase of the other animals. Taking into account that with the exception of the lack of iron, the control diet had the same composition as the fortified diets, it is evidenced that the iron provided by SFG and ferrous sulfate has a positive and similar effect on the animals growth.

Table 2 shows that the animals belonging to group 1 (SFG) and 2 (ferrous sulfate) presented a similar increase in the HbFe values during the treatment. In both cases, the results were significantly higher ($p<0.05$) than those corresponding to the animals of group 3 (control). Thus, it can be observed that the iron from SFG and ferrous sulfate is similarly incorporated

Table 2
Hemoglobin Iron Variation (ΔHbFe), Iron Bioavailability (BioFe), Relative Biological Value (RBV) of Iron Source, and Liver Iron Content (LIC) for Each Group

Group	ΔHbFe (mg)*	Bio Fe (%)*	RBV (%)	LIC (mg Fe)*
1. SFG	1.5 ± 0.3^a	36.6 ± 6.2^a	103	3.4 ± 0.8^a
2. Ferrous sulphate	1.3 ± 0.3^a	35.4 ± 8.0^a	100	2.7 ± 0.8^b
3. Control	0.2 ± 0.1^b	11 ± 7.2^b	---	1.3 ± 0.5^c

* Value (mean ± SD) without a common superscript letter in the same column is significantly different at $p<0.05$.

Table 3
Acute Oral Toxicity in Rats, values of LD_{50} and Confidence Limits for SFG

LD_{50}*	Lower Limit*	Upper Limit*	N° of animals	Sex
1775	1332	2053	70	Female
1831	1387	2108	70	Male

* Expressed as milligram of SFG per kilogram of body weight.

into the hemoglobin of the studied animals, which, in addition, explains the similar BioFe values obtained for both iron sources (*see* Table 3). Finally, in Table 3, it is observed that the LIC of group 1 is significantly higher ($p<0.05$) than the LIC of groups 2 and 3, pointing to a higher iron-storing capability for SFG than for ferrous sulfate at the administered doses.

Table 3 shows the results of the acute oral toxicity studies in rats for SFG. The mean LD_{50} values of 1775 and 1831 mg SFG/kg body wt were respectively obtained in female and male Sprague–Dawley rats. These results are almost six times higher than the LD_{50} reported for ferrous sulfate *(15),* evidencing that SFG can be considered as a safe compound from a toxicological point of view.

REFERENCES

1. J. R. Boccio, M. B. Zubillaga, R. A. Caro, et al., New procedure to fortify fluid milk and derivatives with iron: a comparative study in mice, *J. Nutr. Sci. Vitaminol.* **41,** 619–626 (1995).
2. J. R. Boccio, M. B. Zubillaga, R. A. Caro, et al., Bioavailability and stability of microencapsulated ferrous sulfate in fluid milk: studies in mice, *J. Nutr. Sci. Vitaminol.* **42,** 233–239 (1996).
3. M. B. Zubillaga, R. A. Caro, J. R. Boccio, et al., New procedure to fortify fluid milk with iron: metabolic and biochemical study in rats, *Nutr. Res.* **16(1),** 131–137 (1996).
4. J. R. Boccio, M. B. Zubillaga, R. A. Caro, et al., A new procedure to fortify fluid milk and dairy products with high-bioavailable ferrous sulfate. *Nutr. Rev.* **55,** 240–246 (1997).

5. J. R. Boccio, M. B. Zubillaga, R. A. Caro, et al., Microencapsulated ferrous sulfate to fortify cow milk: absorption and distribution in mice, *J. Nutr. Sci. Vitaminol.* **44,** 381–389 (1998).
6. J. R. Boccio, M. B. Zubillaga, R. A. Caro, et al., Bioavailability, absorption mechanism, and toxicity of microencapsulated iron (II) sulfate. Studies in mice, *Biol. Trace Element Res.* **62,** 65–73 (1998).
7. A. E. Lysionek, M. B. Zubillaga, M. J. Salgueiro, et al., Bioavailability of microencapsulated ferrous sulfate in powdered milk produced from fortified fluid milk: a prophylactic study in rats, *Nutrition* **18,** 279–281 (2002).
8. A. E. Lysionek, M. B. Zubillaga, M. J. Salgueiro, et al., Bioavailability studies of a new iron source by means of the prophylactic–preventive method in rats, *Biol. Trace Element Res.* **84,** 123–128 (2001).
9. H. J. Bernard, Hematology and coagulation, in *Clinical Diagnosis and Management by Laboratory Methods,* 17th ed., Todd, Sanford, and Davidsohn, eds., WB Saunders, Philadelphia, pp. 721–773 (1988).
10. P. G. Reeves, F. H. Nielsen, and G. C. Fahey, 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.* **123,** 1939–1951 (1993).
11. A. E. Lysionek, M. B. Zubillaga, G. P. Calmanovici, et al., Modification of the Ferrozine technique to analyze iron contents in different foods. Comparative study by the use of an internal standard as reference methodology, *Biol. Trace Element Res.* **65,** 87–94 (1998).
12. A. E. Lysionek, M. B. Zubillaga, M. J. Salgueiro, et al., Study of industrial microencapsulated ferrous sulfate by means of the prophylactic–preventive method to determine its bioavailability, *J Nutr. Sci. Vitaminol.* **46,** 125–129 (2000).
13. R. R. Sokal and F. J. Rohlf, *Biometry,* WH Freeman, San Francisco (1981).
14. J. T. Litchfield and F. Wilcoxson, A simplified method of evaluating dose-effect experiments, *J. Pharmacol. Exp. Ther.* **96,** 99–113 (1949).

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