

Double-blind randomized controlled trial of rolls fortified with microencapsulated iron

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SUMMARY

Objective: To evaluate the impact of the fortification of rolls with microencapsulated iron sulfate with sodium alginate on the hemoglobin levels in preschoolers as compared to controls. **Methods:** Double-blind randomized controlled trial comprised of children aged 2 to 6 years with initial hemoglobin exceeding 9 g/dL from four not-for-profit daycares randomly selected in the city of São Paulo – Brazil. Children of 2 daycares (n = 88) received rolls with fortified wheat flour as the exposed group (EG) and children of 2 daycares (n = 85) received rolls without fortification as the control group (CG) over a 24-week period. Rolls with 4 mg iron each were offered once a day, five days a week. Hemoglobin concentrations were determined in capillary blood by HemoCue® at three moments of trial: baseline (M1), after 12 and 24 weeks of intervention (M2, M3). **Results:** Hemoglobin concentration presented significant increase up to M3 in EG (11.7-12.5-12.6 g/dL) and in CG (11.1-12.4-12.3 g/dL) with higher elevations in children initially with anemia. There was significant reduction in the occurrence of anemia from 22% to 9% in EG and from 47% to 8.2% in CG at M3. **Conclusion:** Rolls fortified with microencapsulated iron sulfate were well tolerated, increased hemoglobin levels and reduced the occurrence of anemia, but with no difference compared to the control group.

Keywords: Food, fortified; iron; child; anemia; flour.

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RESUMO

Estudo duplo-cego randomizado controlado com pães fortificados com ferro microencapsulado

Objetivo: A deficiência de ferro é a carência nutricional mais frequente no Brasil, tendo como principal etiologia a baixa ingestão do mineral. A fortificação alimentar é medida sustentável e de melhor custo-benefício para a prevenção e controle. O objetivo foi avaliar o impacto da fortificação de pães com sulfato ferroso microencapsulado com alginato de sódio sobre os níveis de hemoglobina em pré-escolares, comparados com controles. **Métodos:** Estudo duplo-cego randomizado controlado incluindo crianças de 2 a 6 anos com hemoglobina inicial maior que 9 g/dL procedentes de 4 creches filantrópicas selecionadas aleatoriamente, São Paulo – Brasil. Crianças de 2 creches (n = 88) receberam pães com farinha de trigo fortificada, constituindo o grupo exposto (EG), e de outras 2 creches (n = 85) receberam pães não fortificados, compondo o grupo-controle (CG), durante 24 semanas. Pães contendo 4 mg de ferro foram oferecidos uma vez ao dia, cinco dias por semana. As concentrações de hemoglobina foram determinadas no sangue capilar por HemoCue® em três momentos do estudo: momento inicial (M1), após 12 e 24 semanas da intervenção (M2, M3). **Resultados:** A concentração de hemoglobina apresentou aumento significativo até M3 no EG (11,7-12,5-12,6 g/dL) e no CG (11,1-12,4-12,3 g/dL) com maiores elevações nas crianças inicialmente anêmicas. Houve redução significativa na ocorrência de anemia de 22% a 9% no EG e de 47% a 8,2% no CG em M3. **Conclusão:** Os pães fortificados com sulfato ferroso microencapsulado foram bem tolerados, elevando os níveis de hemoglobina e reduzindo a ocorrência de anemia, porém sem diferenças em relação ao grupo-controle.

Unitermos: Alimentos fortificados; ferro; criança; anemia; farinha.

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INTRODUCTION

Presently, iron deficiency anemia is considered as the major nutrition deficiency inserting infants and preschoolers as groups of major risk, especially in the developing countries. Improper diet due to low iron bioavailability is its main etiology¹.

The development of efficient strategies for prevention and control of iron deficiency anemia is still a great challenge in several regions of the world. Such strategies include nutrition education, medicine supplementation for age groups at major risk, and food fortification that should be combined and associated to public health strategies. Individually, fortification is considered to be the most feasible and best cost-effective strategy^{1,2}.

In several countries and in Brazil, studies on children's feeding with a wide variety of fortified foods such as milk and dairy products, cookies, spices, sugar, fruit beverages and juices, cereals and instant noodles, in addition to bakery products, have indicated good results for a wide segment of this population in short term³⁻⁹. Cereal flours have been used traditionally as vehicles for iron fortification. In Brazil, studies indicate that foods made from wheat flour are an important contribution as nutrition resource for general population, and when fortified they become an important alternative to cover iron deficiency, mainly among the low-income population¹⁰.

Among the compounds used for food fortification, the group including the so-called protective compounds has the advantage of being more resistant to the environment influence, (b) reducing interactions with other nutrients or ingredients of diets, and (c) improving the mineral retention in the final product, thus enabling improvements in bioavailability^{1,11}. According to the available literature, the major advantage of fortification with microencapsulated iron regards to the possibility of adding compounds with relatively high bioavailability to food vehicles of difficult fortification such as cereal flours, without causing the usual alterations of color and taste. Preliminary studies have demonstrated good iron absorption originating from microencapsulation and significant increase in hemoglobin levels after its utilization¹²⁻¹⁴.

Considering the high national prevalence of iron deficiency anemia and the difficulties to assess the existing control measures, the present trial aimed at using the wheat flour fortification and the rolls baking with this innovative introduction of microencapsulated iron sulfate, as well as the evaluation of the impact of consumption on the hemoglobin levels and the occurrence of anemia in preschoolers of daycare centers in São Paulo, SP, Brazil, as compared to controls.

METHODS

STUDY POPULATION AND DESIGN

The study was conducted in four not-for-profit daycares associated to the Municipal Secretary for Education of

São Paulo – SP, Brazil, selected by similarity criteria. A fifth daycare was eligible for the pilot trial that lasted two weeks. The studied population consisted of children aged 2 to 6 years who attended daycares from 7 a.m. to 5 p.m. during working days and received five meals per day.

The study was a 24-week (100 working days) double-blind institutional randomized controlled trial resulting in prevalent cases of anemia and variation of hemoglobin levels. The trial population was stratified so that the children of the two daycares receiving fortified rolls with ferrous sulphate microencapsulated with sodium alginate formed the Exposed Group (EG) and the children of the other two daycares receiving the rolls without fortified wheat flour formed the Control Group (CG). The head of the Department of Nutrition and responsible for the preparation of ITAL and delivery of bread participated in the randomization of the subjects and had knowledge of the groups until the end of the study.

For sample size calculation it was used the Epi-Info version 6.04, whereas after the institutional trial, double-blind randomization by cluster (two nurseries were randomly assigned to receive enriched bread and two to receive non-enriched bread), the prevalence of anemia in the intervention group was 10% and in the control group 25%, we would have with $\alpha = \beta = 0.05$ and 0.20, the sample size for each group would be 112. To restore any losses and correcting for the fact that randomization was not a sampling unit, but cluster, we adopted 20% larger sample size of 134 in each group.

The individual consumption of rolls was properly registered on a card everyday by a member of the field team of each daycare center. Consumption was measured in half-roll servings with a maximum of three rolls per day.

After the intervention, children who had ingested less than one roll per day on average were excluded.

The children from the daycares had similar baseline socioeconomic characteristics and EG and CG presented no statistical differences for the means of age, gender and iron content in diets. Children were evaluated for hemoglobin levels at three moments of the trial: baseline (M1), after 12 weeks (M2) and after 24 weeks (M3).

No other programmed intervention took place during the trial period.

This study was approved by the Universidade Federal de São Paulo, Ethics Committee for Research, number 0173/03, and was initiated only after the informed written consent was obtained from the adults responsible for each child and from the board of directors of the not-for-profit entities.

EXCLUSION CRITERIA

Children with chronic diseases taking iron medication with hemoglobin < 9.0 g/dL when first evaluated or in week 12 of study were excluded from the trial.

FORTIFICATION AND CHARACTERISTICS OF THE FORTIFIED FOOD

The small rolls used in this trial weighed 20 g and were programmed for a 4 mg basic iron content per unit as microencapsulated iron sulfate equal to approximately 30% of Dietary Reference Intake (DRI). The microcapsules with iron sulfate microparticles covered with sodium alginate (natural polysaccharide extracted from seaweed) were developed and prepared by the Laboratório de Tecnologia de Partículas do Instituto de Pesquisas Tecnológicas do Estado de São Paulo (IPT) (Particle Technology Laboratory of the São Paulo Institute for Technologic Research) using spray drying technique¹⁵. No other iron fortified food product was served at daycares during the trial and all preschool children received the regular daycare diet. Rolls were baked and packed weekly at the Instituto de Tecnologia de Alimentos do Governo do Estado de São Paulo (ITAL) (State of São Paulo Government Institute for Food Technology) and labeled with each daycare's name in order to be offered at breakfast. A maximum of 3 rolls/day per child was allowed. Bread offered to children in the control group were prepared identically, except for the non-addition of ferrous sulphate.

A pilot project trained field team comprised of four graduate students stayed at each daycare every morning for registering the roll consumption, morbidity, absences and interurrences.

QUALITY CONTROL OF ROLLS AND FOOD ANALYSIS PROPERTIES

A package of 12 rolls was collected from each daycare every week in order to determine the iron content by atomic absorption and threefold reading. Food analysis was made using one sample of roll received on the same day from all daycares by an independent laboratory (not the one that produced the rolls). Microbiologic analysis showed proper patterns for consumption of rolls with and without fortification. A taste panel with trained adults was utilized for sensory analysis to verify for texture, appearance, taste and flavor which have shown no modifications.

DETERMINATION OF IRON CONCENTRATION

A package of rolls from each daycare center was collected weekly as sample to be analyzed at the Research Laboratory of the Discipline of Pediatric Gastroenterology, Universidade Federal de São Paulo (UNIFESP).

Rolls were crushed inside their own wrappings. Three 1 g samples from each wrapping were weighed. Then, 1 g of roll suffered $\text{HNO}_3/\text{HClO}_4$ digestion and the volume was completed up to 50 L with Milli-Q water. The iron dosage was determined using Perkin-Elmer 5.100 atomic absorption spectrophotometer; hollow-cathode lamp; wave-length: 248,3 nm; slit: 0,2 nm; energy: 60; air/acetylene oxidizing flame: 10:3; nonlinear calibration; integration time: 3 seconds; standard Fe Tritisol – Merck diluted in Milli-Q water in concentrations 0,1; 0,2; 0,3; 0,5 and 1,0 $\mu\text{g}/\text{mL}$. Readings were made three times.

DIETARY IRON CONTENT IN MEALS

The dietary iron content offered at daycares was calculated using Mونسен's¹⁶ method based on the mean analysis for the 5-meal menu served everyday during 5-week days¹⁷. An estimate intake of 1 small roll per day for children aged 2 to 4 years and 1-1/2 small roll for older children was considered.

HEMOGLOBIN EVALUATION

A portable Hemocue photometer (Hemocue, Angelholm, Sweden) was used to measure hemoglobin using electric power. Capillary blood samples were obtained using disposable lancets EZ-Lets II Red (Palco Labs Incorporated, USA) and collected in disposable HemoCue® microcuvettes by a unique trained member, always in the morning period. Hemoglobin < 11.0 g/dL was considered as anemia¹⁸. Equipment calibration was checked using the equipment cuvette control in the beginning of all collection days and at every 10 readings.

The capillary blood samples were obtained by digital puncture of right hand's ring fingertip previously cleaned with alcohol 70%, followed by gently milking and collecting a drop of blood, and always in the morning period.

The result expressed in g/dL was digitally read in 30 seconds and immediately noted on a proper card. Every equipment were admeasured at IPEM/INMETRO – Instituto de Pesos e Medidas/Instituto Nacional de Metrologia (Institute of Weights and Measures/National Institute for Metrology).

STATISTICAL ANALYSIS

Data were double entered in database using Epi Info 6.04 software with subsequent validation. For the analysis of results StataCorp 2003 and Epi-Info 6.04 softwares were used. The following statistic tests were used: G-Cochran test was used to compare the occurrence of anemia in the three study moments in the two groups; Student's *t*-test was used to assess the hemoglobin mean differences for the EG and CG; $p < 0.05$ was adopted for significance level.

RESULTS

The studied population was comprised of 324 children whose initial prevalence of anemia was 39.2% and at the end of trial 11.7%.

Significant increase on the average hemoglobin levels for both groups, EG e CG, was observed at the end of the 24 weeks in children who had ingested an equal or higher amount of one roll per day in average (Table 1).

The analyses of rolls showed no differences between samples with and without fortification except for light darkening of fortified rolls. The laboratory analysis demonstrated that the iron content of the fortified roll (4 mg) was adequate to the present trial proposal being much higher to that of CG rolls (0.7 mg).

Table 1 – Means, standard deviations, lower and higher values of hemoglobin (g/dL) at M1, M2 and M3 for children of exposed and control daycare centers, São Paulo, SP, Brazil, 2007

Groups	Moment			p-value
	M1	M2	M3	
	Means (SD) (Minimum-maximum)	Means (SD) (Minimum-maximum)	Means (SD) (Minimum-maximum)	
EG	11.7(1.0) (9.2 - 13.8)	12.5(1.4) (9.0 - 12.6)	12.6 (1.1) (9.9 - 15.0)	0.0001
CG	11.1 (1.1) (9.1 - 14.1)	12.4(1.2) (10.0 - 15.1)	12.3 (1.1) (9.7 - 15.6)	0.0001

EG, exposed group; CG, control group; M1, baseline moment of study; M2, after 12 weeks; M3, after 24 weeks; SD, standard deviation; p, descriptive level of Student's *t*-test.

Due to the fact that approximately half of studied children had no anemia when they were first evaluated, the evolution of hemoglobin was measured separately either in children with anemia and without anemia. Hemoglobin increase was higher in children with initial anemia ($p < 0.0001$) for both groups (Table 2).

By the time of the evaluation of the estimate offer of iron in diets at daycares, all children in CG were already in borderline situations. Nevertheless, with the inclusion of fortified rolls in EG, the mineral contribution met the recommended daily intake (Table 3).

DISCUSSION

The initial 39.2% prevalence of anemia was considered to be high. Although the existing researches on prevalence of iron deficiency anemia are regional or restrict to certain population groups in part due to the size of the national territory, the studies published indicated anemia in preschoolers ranging from 30% to 50%, thus confirming that the anemia in this age group is an important public health issue to be prevented and fought against^{19,20}.

It is likely that the characteristics of the daycares studied that receive children of low socioeconomic conditions coming from the northern outskirts and southern slums of the city have influenced the results. It is well known that the decrease in the hemoglobin levels does not reflect an iron deficiency anemia condition only; however, it is also known that in population where iron deficiency presents a high prevalence, this parameter is considered as an estimate for prevalence of iron deficiency anemia in this

Table 3 – Average content of iron (mg/day) in diet of exposed and controls daycares for consumption of 1 roll/day at age 2-4 years and 1-1/2 roll/day at age 4-6 years, São Paulo, SP, Brazil, 2007

Age (years)	Iron (mg/day)	
	EG	CG
2-4	12.6	7.4
4-6	18.4	10.6

DRI (Dietary Reference Intake) 1997-2002 for 2 to 6 years = 10 mg⁽²⁵⁾. EG, exposed group; CG, control group.

population. Parents' low education degree and the limited access to health care services are, in general, risk factors for the development of anemia¹⁹.

The present trial assessed a new technology for wheat flour fortification in which the protection of iron sulfate in microcapsules might provide better stability of salt in the food. The option for small rolls was based on the acceptance of the product by preschoolers, as well as the technical and economic feasibility for baking and distributing them^{1,15}. The iron content added (4 mg per unit) was agreed upon likewise other studies^{6,17,21}. However, a wide range of iron amounts used for fortification has been observed in the literature^{7,22,23}.

Regarding the acceptance, children under 36 months old ingested minor amounts in relation to older children, both in EG and CG, confirming that this food as a fortification vehicle shows better results in older children¹.

There was no clinical intolerance as already reported in the literature that states no adverse effects were found

Table 2 – Hemoglobin means (g/dL) at baseline and 24 weeks after for anemic (Hb < 11.0) and non-anemic (Hb ≥ 11.0) children at baseline by exposed group and control group, São Paulo, SP, Brazil, 2007

Hemoglobin baseline (g/dL)	EG			CG		
	M1	M3	p-value	M1	M3	p-value
< 11.0	10.2	12.2	0.0001	10.1	12.2	0.0001
≥ 11.0	12.1	12.7	NS	12.0	12.5	NS

Hb, hemoglobin; EG, exposed group; CG, control group; Student's *t*-test; EG x CG n.s.

for food fortification with iron in healthy people, once the amount of mineral addition is lower to those levels used for therapeutic supplementation or to the highest daily limit established by the current recommendations^{24,25}.

Average hemoglobin values rose significantly by the end of the 24-week trial period. As demonstrated in Table 1 the present trial began with average hemoglobin values within the limits of normality as required for a study aiming basically at prevention. Concerning hemoglobin behavior the population responded rapidly to the intervention elevating significantly the average hemoglobin values after 12 weeks, demonstrating the efficiency of the fortification. Even when starting from values considered adequate, the hemoglobin status could be improved, confirming the existence of children at borderline condition.

Our results are similar to those obtained by Giorgini et al.⁸ when using fortified sweet rolls for preschoolers obtaining a hemoglobin average increase of 1.1 g/dL after 6 months of supplementation. The results are also similar to the study of Sazawal et al.² using fortified milk, which improved hemoglobin by 1.3 g/dL, and Sari et al.²⁶, in which hemoglobin had an average increase of 1.0 g/dL after 12 weeks of fortified candies with elemental iron. However, the elevation in hemoglobin levels exceeded those obtained by Paula et al.⁴ using sugar fortified with iron amino acid chelate for children between 10 to 48 months of age, in a population with characteristics similar to ours, or by Moretti et al.²² using fortification of extruded rice in a primary school in Bangalore, India. In the latter, the average hemoglobin levels remained unchanged at the end of seven months.

Previous studies on iron fortification without a control group presented similar increase of average values for hemoglobin by the end of the intervention^{3,8}. Such improvement could be further justified by the fact that the shortage scenario was more frequent in CG in the beginning of intervention (Table 1), thus enabling children to become more receptive to iron absorption.

By the time we selected children according to the presence of initial anemia, the best response to fortification occurred in those initially diagnosed with anemia (Table 2), same as in other studies as expected, thus confirming the regulatory role of iron absorption mechanism in the intestinal mucosa²⁶. When organic needs are intensified a higher proportion of mineral is absorbed^{27,28}. The elevation of 2 g/dL in the hemoglobin levels of children initially anemic in the present study was similar to the study conducted by Miglioranza et al.²⁶, which found an increase in the hemoglobin levels of 2.2 g/dL in anemic students who received whey drink fortified with ferrous bisglycinate.

CG also presented significant increase in hemoglobin during the period of observation. The reason for this increase is not clear and there are several factors that may contribute for the final results. The present study tried not to perform any health actions at daycares during the pe-

riod of intervention except for those strictly necessary as required by Ethics, however the absence of communication with relatives, employees and children proved to be impossible, once they were involved with and aware of our field team observations. Thus, it is impossible to fully disregard this factor on the results obtained in relation to controls (intervention bias)²⁹. We emphasize that the destination of the fortified bread was unknown by all which could lead to undesirable individual initiatives encouraging a greater consumption. We believe that some previous studies on food fortification with iron might have presented a sample bias due to the lack of control group, and then, not discussing the possibility that mothers and daycare employees might have modified their behavior and improved their attention to the meals. A greater stimulus to feed may have led to increased consumption of dietary sources of iron enough for children to stay in balance, once part of the children already had initially normal levels of hemoglobin.

Although there were no significant differences between the fortified and control groups in the hemoglobin levels, it is suggested that ferritin, in a dosage unanticipated in the methodology, would have allowed a better assessment of iron status and could have indicated the difference between the two groups, because we think iron reserves were higher in CG as a result of the additional consumption of iron. This condition was observed by Chen et al.⁶ in a 18-month study with supply of iron-fortified soy sauce to Chinese children older than three years old. The authors obtained a significant increase in hemoglobin levels in both exposed and control groups, but serum levels of ferritin showed an increase only in the fortified group. Likewise, in the double-blind controlled study conducted by Moretti et al.²², body iron stores were greater in the iron-fortified group at the end of seven months compared with the unfortified group, while hemoglobin levels showed no significant difference.

Some studies on fortification are similar to the present trial when they indicate elevation in hemoglobin also in controls, however diverging when attributing this fact to actions such as the general supply of vermifuges or control of infections^{6,7,22}. Daily record of morbidity in our trial enabled to exclude possible interferences of such a condition between the two groups. In randomized controlled interventions, Sari et al., in Indonesia, with iron fortified candies³⁰ and Bagni et al. in a study with rice fortified with iron chelate in children of daycares in Rio de Janeiro³¹ have also found an elevation in hemoglobin levels around 0.4 g/dL similar in fortified and control groups. In a six-month study of Almeida et al. on children from day-care centers in Southeast Brazil, the group which used water fortified with elemental iron and ascorbic acid had a significant increase in hemoglobin of 0.56 g/dL whereas the comparison group, that received water containing only ascorbic acid had a significant increase in hemoglobin of 0.82 g/dL³².

Other possible reasons might have been taken into consideration as factors that contributed for the present results. We know that prevalence of anemia reduces with age diminishing after the 24 months of life. Older children diversify their diets and increase their intake quantitatively while the speed of their growth reduces. It should be pointed out that a great number of studies on fortification that obtained positive response were carried out in closed communities taking several types of programmed simultaneous actions such as educational work, meetings with parents and caregivers, nutrition guidance, vitamin supplementation (vitamin A and C) among others^{3,18,20}.

In addition, a marginal adjustment of iron content in diets offered at daycares most likely made the action of additional iron supplied with rolls disappear (Table 3). A previous study evaluated the diets offered at daycares of the same institution for children aged 13 to 24 months and revealed that the total iron content, as well as bioavailable iron has not reached the recommendations³³. It is well known how difficult it is to evaluate the iron content actually absorbed; however the diets in our country have a high content of inhibiting factors for iron absorption that might contribute for the high prevalence of anemia observed³⁴.

The present study enhances our knowledge mainly regarding the design of investigations on the impact of food fortification, and highlights the need to compare the effects of the fortification with those of control group carefully matched. Several studies are deprived of control group considering their positive results as representative of the direct action of intervention. Without the control group the elevation in hemoglobin and reduction in occurrence of anemia leded us to conclude, by the exclusive effect of fortified rolls without pointing to the actual multiplicity of factors, that perform synergic role when the impact of a fortified food product is assessed. Further researches are needed to provide direct evidence for this asserption.

As previously mentioned, studies have indicating the positive action of field teams in motivating employees of health care services or daycares, as well as increasing the attention to children, thereby helping to improve their hemoglobin levels in this kind of interventions^{28,29}. Iron fortification, however, is also recognized as a measure of medium term action easy and effective to perform in the control of iron deficiency, especially in childhood.

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