

Efficacy of Iron-Fortified Rice in Reducing Anemia Among Schoolchildren in the Philippines

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Abstract: *Background:* Iron deficiency anemia (IDA) in the Philippines is a serious public health problem. Fortifying rice offers a great opportunity to control IDA. However, information on other types of fortificants that can be used is scarce.

Objective: To compare the effects of two types of iron fortificants in rice in improving the hematological status of schoolchildren.

Design: 180 randomly selected 6-to 9-year-old anemic children were randomly allocated to three groups in a double-blinded manner: One group received iron-enriched rice (IER) with extruded iron premix rice (IPR) using *ferrous sulfate* as fortificant (*ExFeSO₄*); the second group received IER with extruded IPR using *micronized dispersible ferric pyrophosphate* (*ExFeP80*); and the third group received non-fortified rice (Control). These were administered daily for 5 days a week for 6 months. Blood samples were collected at baseline after 3 and 6 months.

Results: At baseline, one child in the *ExFeP80* group was suffering from IDA; at 3 months, no IDA was found in any groups; while at 6 months, one child in the *ExFeP80* developed IDA. The baseline prevalence of anemia in all groups, which was 100%, was significantly reduced to 51%, 54%, and 63% in the *ExFeSO₄*, *ExFeP80* and Control groups respectively. After 6 months, further significant reductions were observed in the *ExFeSO₄* (38%) and *ExFeP80* (33%) but remained at 63% in the Control group. Greater, significant increases were also observed in plasma ferritin in the fortified groups than in the Control group from baseline to 6 months. The predictors of change in hemoglobin (Hb) and plasma ferritin were group allocation and basal values.

Conclusion: The consumption of rice fortified with *FeP80* using extrusion technology has similar effects as that of *FeSO₄* in reducing the prevalence of IDA among schoolchildren.

Key words: Ferrous sulfate, micronized dispersible ferric pyrophosphate, iron-fortified rice, extrusion, anemia, schoolchildren

Introduction

The prevalence of iron deficiency anemia (IDA) remains high across population groups in the Philippines despite efforts to reduce its occurrence. The results of the latest National Nutrition Survey revealed that anemia prevalence among the study population, 6-to-12 year-olds, is 37.4% [1]. Numerous published studies on the negative consequences of IDA in children have shown impaired school performance [2], delayed motor and mental development [3], and slowed growth [4].

Factors that might have contributed to this high prevalence are: low dietary intake of iron (60% of recommended daily allowance; RDA); low bioavailability of dietary iron due either to the low intake of iron enhancers such as vitamin C (75% of RDA) and/or the presence of iron inhibitors in the diet. The high-phytate meal pattern of Filipinos consisting of rice, fish, and vegetables reduces the bioavailability of iron from foods. About 360 g per day (41%) of the total food intake come from cereals and cereal products and about 300 g (34%) from rice and rice products. Vegetables account for 110 g of the total food consumed. Heme iron accounted for 28% of the total iron intake of 10 mg [1]. Poverty could be another factor because it limits the availability and choices of foods. In 2003, about 1.664 million (10.4%) Filipino families did not earn enough income to provide for their basic food requirements [5].

In many earlier studies, food fortification with iron has been shown to improve iron status [6, 7]. Fortifying staple foods is a viable approach to reach the at-risk segments of the population at a minimal cost. However, in designing a satisfactory food fortification strategy, the choice of vehicle, the choice of suitable iron compound as fortificant, and appropriate technology should be critically considered [8, 9]. Filipinos are consuming about 282 g of rice daily [1] regardless of income; hence, rice is an appropriate vehicle for fortification.

The Food Fortification Law of the Philippines (Republic Act 8976) requires mandatory fortification of staple foods like rice with iron based on standards set by the Bureau of Foods and Drugs, Department of Health [10]. The mandatory fortification of rice with iron commenced in November 2004. Considering the capability of manufacturers, millers, and concerned agencies, a phased implementation of the Law has been allowed. To date the government is importing rice fortified with ferrous sulfate after which it is distributed to identified, high-risk areas. To hasten the implementation of the Law, there is an urgent need to show evidenced-based effects of different appropriate fortificants for rice to provide alternative choices for consumers.

While ferrous sulfate ($FeSO_4$) is an excellent, low-cost

source of bioavailable iron, its absorption is inhibited by phytates and tannins. Ferric pyrophosphate, on the other hand, has been widely used to fortify cereals and pasta products owing to its tendency to produce less discoloration and less metallic taste. However, its absorption is extremely poor [11]. A new technology enables the bioavailability of ferric pyrophosphate to be substantially increased, comparable to ferrous sulfate, by micronizing the fortificant to a particle size of 0.5 μ m and mixing with glycerides to minimize the interaction. In this study the micronized dispersible ferric pyrophosphate (SunActive® FeP80; Taiyo Kagaku Co., Ltd. Yokkaichi, Japan) was used to take advantage of less discoloration and less metallic taste [12]. A study reported that the relative bioavailability (RBV) of micronized dispersible ferric pyrophosphate (MDFP) varied according to the food vehicle, from 62% in a wheat-milk cereal to only 15–25% in a rice meal. RBV varied markedly with food matrix and iron status. Therefore, using RBV of poorly soluble compounds in evaluating their suitability in food fortification might be of limited value [13]. Another study on iron absorption showed that *Fe* absorption from MDPF (Sunactive Fe^{TM}) is similar to that of ferrous sulfate from a fortified infant cereal as well as from a fortified yoghurt drink [14].

The present study aimed to assess and compare the effects of two types of iron fortificants that could be used in rice to improve the hematological status of schoolchildren.

Subjects and Methods

Study site and subjects

This study was conducted from July to December 2004 in San Joaquin Elementary School, one of the more thickly populated government elementary schools in Metro Manila in the Division of Pasig City. We selected the Division of Pasig because of the high prevalence of IDA based on past surveys [15] and because of its close proximity to the Food and Nutrition Research Institute (FNRI) where blood samples were analyzed. On the basis of the inputs from the officials of the Department of Education, officials in this school were cooperative and supportive.

Figure 1 shows the selection process of subject children. The total number of enrollees of children aged 7 to 8 years in this school was 1041. Considering that the prevalence of anemia in this age group was 36%, 700 randomly selected children were invited for screening. However, only 653 children gave back the written consent signed by their parents. These children were clinically assessed by the school physician as not suffering from any sort of ill-

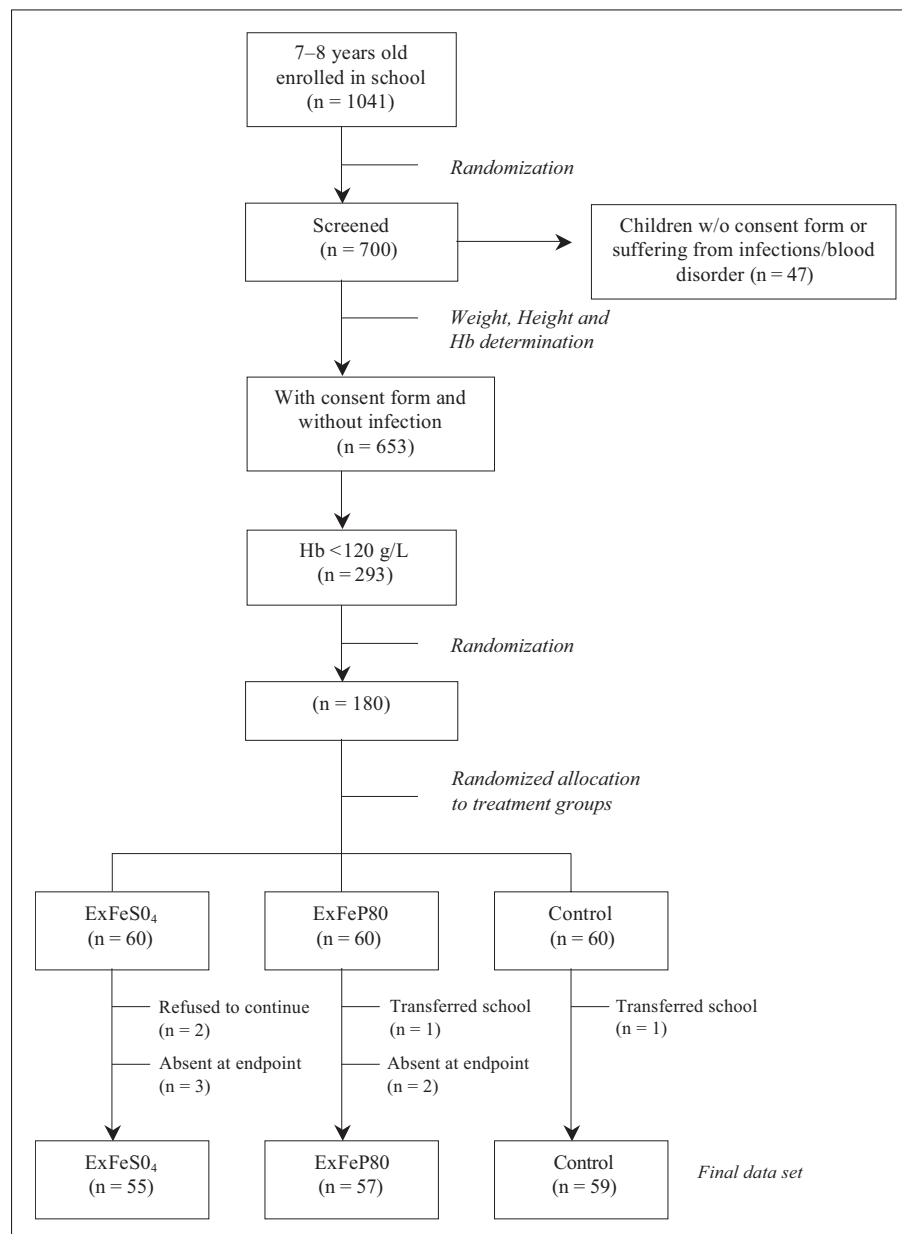


Figure 1: Operational flow of the study.

ness; e.g. fever, diarrhea, stomachache, cough, or cold at the time of examination. Further assessment was done by taking baseline hemoglobin (Hb) concentrations. All anemic children (Hb < 120 g/L) were included in the study, while children with severe anemia (Hb < 70 g/L), who reported history of blood disorders and other hemoglobinopathies, were excluded. A total of 293 children served as the sampling frame. Since the calculated sample size for the intervention study, based on a minimum change of Hb concentrations of 7 g/L (SD of 9.5 g/L, CI of 95%, power of 90%, and attrition rate of 30%) was only 180 anemic children, random selection of the final sample was conducted [16,17].

Study design

Using a double-blinded, randomized, controlled design, the 180 anemic children were randomly allocated to three groups (60 children in each group). Group 1 received iron-enriched rice (IER) with extruded iron premix rice (IPR) using ferrous sulfate powder as fortificant (*ExFeSO₄*); Group 2 received IER with extruded IPR using *micronized ferric pyrophosphate* as fortificant (*ExFeP80*); and Group 3 received unfortified rice (Control). Rice was served during a two-week cycle with standard dishes during lunch, daily for 5 days a week for 120 days (6 months) under a supervised regimen.

The intervention

Fortified rice

The Nutritional Guidelines for Filipinos recommends 4 to 5 cups (640 to 800 g) per day of cooked rice and alternates for children aged 6–10 years [18]. Following this recommendation, this study provided 160 g (1 cup) of cooked rice either fortified or unfortified which is equivalent to 66.72 g of uncooked rice.

The extruded IPR was prepared by blending ordinary rice flour with fortificant, binder, solvent, and water. The two fortificants used were $FeSO_4$ powder and micronized dispersible ferric pyrophosphate ($FeP80$). These separate mixtures were subjected to extrusion to form iron/rice-like grains premix (IPR). This premix was blended with ordinary rice at a ratio of 2.5:100. The aim was to attain 10 mg Fe per day to provide 90% of the recommended intake (11 mg) for this age group [19]; a ratio of 25 g of iron premix to 1 kg of ordinary rice was used. The study used 5 kg of uncooked ordinary rice per day per group of 60 children. This was mixed with 125 g of iron premix, which was packed in color-coded polyethylene bags. The field researchers, including the primary investigator, had no access to the preparation, development, and packaging of the IFR in color-coded polyethylene bags.

The process

The rice was withdrawn from FNRI by the catering service every two weeks and was safely stored in a safe area as instructed.

The meals (rice and dish) were cooked in the catering service. Color-coded packages of uncooked rice were placed in respective color-coded kettles by a staff at the catering service. Washing of rice was performed in the usual Filipino way; i.e. washed twice with slight agitation. For this study, we maintained a standard amount of water for washing and for cooking, and these activities were done by the same Research Assistant from the start to the end of the study.

A standard two-week cycle menu was developed, consisting of meat, vegetables such as potatoes, sweet potatoes, green papaya, and bananas (saba). Each child received 40 g meat, 20 g vegetables, and 40 g sauce. Weighed cooked rice (160 g) and dish (100 g) were packed in color-coded lunch boxes with partitions.

The iron content of the cooked fortified rice was analyzed at baseline by the dry ashing method using an atomic absorption spectrophotometer [20,21], while moisture was analyzed by AOAC 925.10 using the air oven method or forced draft oven (ULE 500) [22]. Composite nutrient content of the dishes was also analyzed using the same methods used in rice for iron, while for vitamin A was analyzed by a modified AOAC 974.29 method using high-performance liquid chromatography (HPLC) [23].

The Research Assistants, with the assistance of the teachers, distributed the meals at 12:00 noon daily, as per the color-coded identification cards (IDs) of the children. This was conducted under strict supervision in permanently designated room assignments. The School Officials and the Researchers were blinded as to the type of rice the children were eating.

Plate Waste Collection

Plate wastes on rice and dishes were collected, weighed, and recorded every 2 random days of the week. The mean plate waste (g) per child was deducted from the total required amount of rice to be consumed (160 g/day). This represented the amount of intake of either fortified or non-fortified rice. Amount of iron intake was computed as the proportion of the actual iron content of cooked fortified rice as a result of the nutrient analysis vs. the amount consumed. These values were expressed as a percentage of the Recommended Energy and Nutrient Intake (RENI). Compliance rate was the percentage of the number of days the children ate lunch divided by the number of feeding days (120 days).

Acceptability test

A pre-tested Hedonic Rating Scale score sheet was administered to children by the same teacher at 3 months and 6 months of the study to measure the degree of liking and acceptability of the rice [24].

Deworming

Earlier studies of soil-transmitted helminthiasis revealed a high prevalence, [25] hence a blanket administration of deworming drugs was given before the feeding activity, although no fecal examination was done. The School Physician and Nurses directly administered a 400-mg chewable albendazole tablet to all targeted children. A second dose was given at 12th week of the feeding [26].

Biochemical assessment

The trained Medical Technologists from FNRI conducted blood collection and processing for Hb, plasma ferritin, plasma retinol, and C-reactive protein (CrP) analyses. Hb, plasma ferritin, and CrP were analyzed at baseline, at 3 months and at 6 months, while plasma retinol was measured only at baseline and 6 months.

Blood samples were collected in the morning (7:30 am–11:30 am) from all subjects by finger-prick using sterile disposable lancets. For hemoglobin analysis, 20 μ L of blood was pipetted into a screw-capped tube containing 5 mL cyanmethemoglobin reagent, after discarding the first drop. Further, about 1.5 mL of free-flowing blood was drawn by capillary method into two 0.75-mL heparinized capillary tubes for plasma ferritin, plasma

retinol, and CrP analyses [27]. Blood samples in the capillary tubes were allowed to stand for 10–15 minutes at room temperature (30°C) before they were packed in an ice chest with wet ice. These were then transported to FNRI laboratory, requiring about 30 to 45 minutes travel by air-conditioned taxi. In the laboratory, the blood samples were taken from the ice box and were allowed to stand for 30 minutes at room temperature (22°C) and were centrifuged at $1245 \times g$ for 15 minutes at 20°C. Plasma was transferred into separate, labeled, polypropylene microcentrifuge tubes and were stored in a –20°C freezer without thawing until the end of the study for plasma ferritin, plasma retinol, and CrP analyses.

Hb was measured using the cyanmethemoglobin method. Absorbance was read in a portable spectrophotometer (Odyssey 2400, Hach Company, Loveland, CO, U.S.A.) at 540 nm and converted to equivalent hemoglobin concentration using the regression curve generated from Hemoglobin Reference preparation [28]. Anemia was defined as Hb <120 g/L [29].

An immunoradiometric test kit (Coat-A-Count Ferritin® IRMA, DPC Cat. # IKFE1) was used to analyze plasma ferritin where radioactivity was measured using a gamma counter (Packard) [30]. Iron deficiency was considered if plasma ferritin was < 12 µg/L [31,32]. IDA in this study is defined as plasma ferritin < 12 µg/L and Hb <120 g/L.

To determine the presence or absence of infections among study subjects, C-reactive protein (CrP) was measured using the Humatex CrP test kit (Murex Diagnostic, Norcross, GA) [33]. This is a semi-quantitative latex agglutination assay wherein results are reported as an approximate mg/L concentration of CrP. A positive test result indicates a CrP concentration greater than 6 mg/L. In addition, a clinical examination was conducted by the School Physician before the start of the study to screen children with symptoms of infection. During the intervention, duration and episodes of fever, cough and colds, and diarrhea were recorded by the Research Assistants. Absences and their causes and duration were recorded.

Plasma retinol was determined using HPLC [34]. Deficient plasma retinol was indicated by a concentration < 0.35 µmol/L, while low concentrations were defined as plasma retinol = 0.35 to 0.69 µmol/L. Acceptable levels were 0.70 µmol/L to 1.74 µmol/L, and high levels were determined to be ≥ 1.75 µmol/L [35].

For quality assurance, accuracy was checked using commercially available tri-level control blood samples (Human, Germany), with values as follows: level 1 = 5.7 g/dL ± 0.3, level 2 = 12.5 g/dL ± 0.4, and 16.2 g/dL ± 0.6. Precision was monitored using an internal quality blood control sample prepared from pooled blood sam-

ples. Red blood cells were washed twice with normal saline solution, concentration of which was established (11.2 g/dL ± 0.30) using the total hemoglobin standard (Sigma, USA). These control blood samples were in aliquots of one run per tube, stored frozen in a –20°C freezer. For plasma retinol, the accuracy of the methods was assessed using a standard reference material for fat-soluble vitamins in human serum (SRM 968c). Inter-run precision for all assays was also done using the pooled serum sample (also in aliquots of one run per tube, stored inside in –20°C freezer). Random subsamples of plasma were sent to Japan for confirmation of results of ferritin (PRF; Shuichi Kimura Laboratory, Showa Women's University, Tokyo, Japan).

Anthropometric measurements

Anthropometric measurements were conducted at the start of, and after 6 months of the study. The weight of children was measured and recorded to the nearest 0.1 kg using a Detecto weighing scale (Webb City, MO, U.S.A). Subjects were in lightweight clothing and no shoes. Height was measured barefoot and was recorded to the nearest 0.1 cm using a microtoise (Depose, France) posted flat against a wall. Anthropometric Z-scores were computed for all children (180) relative to the World Health Organization/National Center for Health Statistics (WHO/NCHS) reference population using the Epi-Info Nutrition (2005). In this study, the cut-off of weight for age Z-score (WAZ) < –2SD; height for age Z-score (HAZ) < –2SD; weight-for-height Z-score (WHZ) < –2SD was used to define underweight, stunting, and wasting, respectively [36, 37].

Dietary Assessment

A two-day 24-hour food recall was collected at the start and end of the study to determine the nutrient intakes of the children [36]. Food intake was transformed to nutrient intake using the Individual Dietary Energy System (IDES) developed by FNRI.

Ethical Considerations

This study was presented to and sought approval of the Technical Committee of FNRI – DOST and was approved by the Institutional Ethics and Review Committee [38]. Signed consent was sought from the parents of each of the selected children. The purpose and details of the study were explained in a meeting with all concerned parents and School Officials. Anemic children not included in the study were referred to the School Physician for management while all children who remained anemic after the study were given a bottle of 120-mL iron supplement, with a dose of 30 mg elemental Fe/day on a take-home basis, with detailed instructions. Proper referral to the School

Physician for further management was conducted when necessary.

Statistical methods

Statistical analyses were performed using SPSS version 9.0 (SPSS Inc, Chicago). All variables considered were checked for normal distribution using the One-Sample Kolmogorov-Smirnov test. Plasma ferritin data was not normally distributed, hence data was transformed on a log scale. The mean hemoglobin, plasma ferritin, and plasma retinol concentrations of children of the different treatment groups were compared using analysis of variance (ANOVA). Pair-wise comparison of the treatment groups was done using Tukey's HSD, and within-group comparison was done by paired *t*-test to determine if there was a significant increase or decrease in the mean of hemoglobin and plasma retinol from baseline to 3 months, 3 months to 6 months, and baseline to 6 months [16, 17].

Prevalence of anemia was analyzed by determining the percentage of children with hemoglobin values below the cut-off value of Hb < 120 g/L at 3 months and after 6 months. Chi-square test for independence was done to determine if the prevalence of anemia was dependent on the treatment groups to which the pupils were assigned. Baseline prevalence of anemic pupils was not tested because all the children included in the study were initially anemic.

Backward elimination regression analysis was done to determine the main predictors of change in hemoglobin and plasma ferritin concentrations [16, 17].

Comparison of the mean intake of energy, iron, calcium, vitamin A, and vitamin C between groups was done by ANOVA or Kruskal-Wallis test depending on the distribution of the data. Unpaired *t*-test was used to determine significant differences in iron retained in cooked rice between the fortified groups [16, 17].

To evaluate the efficacy of the different fortificants in rice, a significant difference of mean Hb between groups at baseline and 6 months, and a significant change in the prevalence of anemia between groups, were used as indicators. Levels of significance were set at $p < 0.05$ [16, 17].

Results

The screening for possible subjects revealed that anemia prevalence among these children was 45% (293). The 180 randomly selected anemic children who were randomly allocated into the three groups showed that mean values of age in months (*ExFeSO₄* = 94.5 ± 7.6 , *ExFeP80* = 94.4 ± 8.0 , and *Control* = 94.3 ± 7.2 , $p = 0.983$), Hb (*ExFeSO₄* = 112.1 ± 5.9 g/L, *ExFeP80* = 113.1 ± 4.8 g/L, and

Control = 113.6 ± 4.4 g/L, $p = 0.259$), weight (*ExFeSO₄* = 20.4 ± 4.0 kg, *ExFeP80* = 20.7 ± 3.8 kg, and *Control* = 20.9 ± 3.5 kg, $p = 0.798$), height (*ExFeSO₄* = 118.6 ± 5.7 cm, *ExFeP80* = 118.2 ± 6.7 cm, and *Control* = 118.2 ± 5.8 cm, $p = 0.924$), and gender (*Males*: *ExFeSO₄* = 36, *ExFeP80* = 33, and *Control* = 30; *Females*: *ExFeSO₄* = 24, *ExFeP80* = 27, and *Control* = 30, $p = 0.545$) allocation were not significantly different between groups.

The final data set for this study was 171 children, incurring only a 5% drop-out rate in the three groups. Common reasons of dropping out were: incomplete blood sample because of refusal to submit themselves to repeated blood collection (7) and transfer to other schools (2) (Figure 1). The mean Hb levels (g/L) of children before drop-out did not affect the values of these different indicators in the remaining subjects (*ExFeSO₄* = 111.9 ± 6.1 , *ExFeP80* = 113.1 ± 4.8 , and *control* = 113.5 ± 4.4). The gender allocation (*Males*: *ExFeSO₄* = 32, *ExFeP80* = 32, and *control* = 30; *Females*: *ExFeSO₄* = 23, *ExFeP80* = 25, and *control* = 29, $p = 0.717$) and mean age in months (*ExFeSO₄* = 94.3 ± 7.7 , *ExFeP80* = 94.7 ± 8.1 , and *control* = 94.0 ± 7.1 , $p = 0.904$) were homogenous between groups.

Food intake

Children's mean energy, vitamin A, vitamin C, and iron intakes were similar in all groups at the start of the intervention. After 6 months, similarly significant increases in energy intakes were observed in the fortified groups. The values were significantly higher than the control group, which had decreased by 150 kcal from baseline value (1467 ± 273). Mean vitamin A intake decreased in all groups; the mean intake in the *ExFeP80* group was significantly higher than in the Control group ($p < 0.05$) but similar to the *ExFeSO₄* group. A significant increase in mean vitamin C intake was observed in the Control group, which was significantly higher than in the fortified groups, which furthermore had similar slight decreases as compared with their basal values. For iron intake, the fortified groups had similar slight increases that were significantly different compared to the control group, which had a significant decrease (Table I).

Nutrient content of the Intervention

Laboratory nutrient analysis showed that the iron content of cooked IFR with *ExFeSO₄* yielded 12.88 mg Fe/160 g (117% RNI), while the iron content of cooked IFR with *ExFeP80* was 12.24 mg Fe/160 g (111.3% RNI). The non-fortified cooked rice contained 0.10 mg Fe/160 g.

The iron content of the dishes ranged from 0.4 mg to 1.0 mg Fe/100 g with a mean of 0.63 mg/100 g. Vitamin A ranged from 3 to 62 µg/100 g.

Table I: Mean one-day, per capita energy and micronutrient intakes of schoolchildren¹

	ExFeSO ₄ (n = 55)	ExFeP80 (n = 57)	Control (n = 59)	P
Energy (kcal)				
Baseline	1355 ± 285	1381 ± 319	1467 ± 273	.105
Endpoint	1482 ± 333 ⁴	1539 ± 354 ⁴	1317 ± 112 ⁵	.000 ²
Change	127 ± 404 ^{3, 4}	157 ± 444 ^{3, 4}	-150 ± 282 ^{3, 5}	.000
Vitamin A (µg/RE)				
Baseline	330 ± 323	386 ± 219	338 ± 235	.351
Endpoint	235 ± 844	291 ± 435 ⁴	224 ± 163 ⁵	.035 ²
Change	-95 ± 927 ³	-95 ± 469 ³	-114 ± 295 ³	.968
Vitamin C (mg)				
Baseline	15.3 ± 31.9	15.9 ± 33.2	12.1 ± 24.0	.640
Endpoint	9.8 ± 20.9 ⁴	12.7 ± 33.7 ⁴	26.5 ± 13.8 ⁵	.000 ²
Change	-5.5 ± 37.2 ⁴	-3.2 ± 44.4 ⁴	14.4 ± 23.3 ^{3, 5}	.028
Iron (mg)				
Baseline	7.69 ± 3.55	8.57 ± 2.84	8.60 ± 2.71	.200
Endpoint	8.20 ± 2.64 ⁴	9.35 ± 3.41 ⁴	6.58 ± 1.56 ⁵	.000 ²
Change	0.51 ± 4.54 ⁴	0.78 ± 4.31 ⁴	-2.02 ± 3.38 ^{3, 5}	.000

¹ Means ± SD (Vitamin A and C are presented as geometric mean)² Significantly different between groups (ANOVA or Kruskal-Wallis test, $p < 0.05$)³ Significantly different within groups (Paired *t*-test or Wilcoxon signed rank test, $p < 0.05$)^{4, 5} Significantly different between groups (Tukey's HSD or Mann-Whitney *U* test, $p < 0.05$)

Hemoglobin and the prevalence of anemia

At baseline, mean Hb concentrations in the *ExFeSO₄*, *ExFeP80* and control groups were similar. After 3 months, mean Hb concentrations in all groups were increased. However, no significant difference was observed between groups. The mean Hb change after 3 months in the *ExFeSO₄* (6.8 ± 7.7) and the *ExFeP80* (6.8 ± 7.2) groups were similar but were significantly higher than (3.2 ± 6.2) the control group. After 6 months, further similar significant increases were observed in mean Hb concentrations in the *ExFeSO₄* and *ExFeP80* groups, but the control group remained at similar concentrations as those at 3 months, and this was significantly lower than the values in the *ExFeSO₄* and *ExFeP80* groups. The mean increase in Hb concentrations in the *ExFeSO₄* and *ExFeP80* groups were similar (9.1 g/L) and were significantly higher than the value in the control group (Table II). The predictors of change in Hb from baseline to endpoint were group allocation and basal Hb concentration (Table III).

From a basal prevalence of anemia at 100% in all groups, the prevalence was significantly reduced to 51%, 54%, and 63% in the *ExFeSO₄*, *ExFeP80* and Control groups, respectively, at 3 months. The rates of decrease were similar between groups. At 6 months, further similarly significant percentage reductions were observed in the *ExFeSO₄* (38%) and *ExFeP80* (33%) groups and these were significantly higher than the Control group (63%) which was similar to the prevalence rate at 3 months ($p = 0.003$).

Plasma ferritin

Basal geometric mean of plasma ferritin (PF) was similar between groups. Mean levels at 3 months and mean increments between baseline and at 3 months in the fortified groups were similar but the mean level in the *ExFeP80* was significantly higher than in the control group. The control group had a significant reduction ($-7.6 \mu\text{g/L}$) as compared with its basal values. At 6 months, mean plasma ferritin levels in the *ExFeSO₄* and *ExFeP80* were not significantly different between groups but the mean in the *ExFeP80* was significantly higher than the values observed in the Control group. Further significant increments were observed in the *ExFeSO₄* group ($10.4 \mu\text{g/L}$) and in the *ExFeP80* group ($13.4 \mu\text{g/L}$) as compared with basal values. The Control group had levels similar to its basal values (Table II). The predictors of change in PF from baseline to endpoint are group allocation and basal plasma ferritin levels (Table III). Using the cut-off of PF $< 12 \mu\text{g/L}$, only one (1.8%) child belonging in the *ExFeP80* group was suffering from IDA at baseline; at 3 months, no iron deficiency was found in all groups. At 6 months, one (1.8%) child (different from baseline) in the *ExFeP80* group had IDA. Figure II shows that the correlation between Hb and ferritin is weak at baseline ($r = .082$, $p = .287$) and endpoint ($r = 0.206$, $p < 0.01$).

Table II: Concentrations of hemoglobin, plasma ferritin, and plasma retinol at different periods of measurement

	ExFeSO ₄ (n = 55)	ExFeP80 (n = 57)	Control (n = 59)	P ³
Hemoglobin (g/L) ¹				
Baseline (HB0)	111.9 ± 6.1	113.1 ± 4.8	113.5 ± 4.4	.267
3-mo (HB3)	118.8 ± 8.5	120.0 ± 7.9	116.6 ± 7.8	.078
6-mo (HB6)	121.0 ± 8.5 ⁵	122.3 ± 7.3 ⁵	116.5 ± 8.2 ⁶	.000
HB3-HB0	6.8 ± 7.7 ^{4, 5}	6.8 ± 7.2 ^{4, 5}	3.2 ± 6.2 ^{4, 6}	.005
HB6-HB0	9.1 ± 7.3 ^{4, 5}	9.1 ± 7.8 ^{4, 5}	3.0 ± 7.3 ^{4, 6}	.000
Plasma Ferritin (μg/L) ²				
Baseline (PF0)	55.3 [48.2,63.5]	55.3 [47.3,64.6]	54.0 [46.5,62.7]	.968
Midline (PF3)	57.3 [50.2,65.5]	60.4 [53.3,68.5] ⁵	46.4 [40.4,53.4] ⁶	.013
Endpoint (PF6)	65.7 [57.5,75.0]	68.7 [59.9,78.7] ⁵	55.1 [48.8,62.1] ⁶	.039
PF3-PF0	2.0	5.1 ⁵	-7.6 ^{4, 6}	.045
PF6-PF3	8.4 ⁴	8.3 ⁴	8.7 ⁴	.841
PF6-PF0	10.4 ⁴	13.4 ⁴	1.1	.137
Plasma Retinol (μmol/L) ¹				
Baseline (PR0)	1.24 ± 0.27 ⁵	1.15 ± 0.45 ⁵	1.40 ± 0.25 ⁶	.001
6-mo (PR6)	1.39 ± 0.31	1.25 ± 0.19 ⁵	1.42 ± 0.29 ⁶	.028
PR6-PR0	0.15 ± 0.33 ⁴	0.10 ± 0.48	0.02 ± 0.28	.181

¹ Mean ± SD² Geometric mean [95% CI of the mean], analysis was done on log-transformed values³ P-value of the ANOVA⁴ Significantly different within groups (Paired *t*-test, *p* < 0.05)^{5, 6} Significantly different between groups (Tukey's HSD, *p* < 0.05)

Table III: Predictors of change in hemoglobin and plasma ferritin concentrations of schoolchildren after 6 months

Variable	Regression coefficient	SE	P	R-squared
Hemoglobin ¹				
Constant	50.310	14.349	.001	.197
Basal hemoglobin	-0.327	0.110	.003	
ExFeSO ₄ (member = 1)	5.544	1.426	.000	
ExFeP80 (member = 1)	5.982	1.484	.000	
Age at baseline (months)	-0.117	0.074	.118	
Sex (male = 1)	0.803	1.127	.477	
Iron intake at endpoint	0.053	0.214	.807	
Plasma Ferritin ²				
Constant	1.073	.178	.000	.410
Baseline log ferritin	-0.649	.054	.000	
ExFeSO ₄ (member = 1)	0.083	.033	.012	
ExFeP80 (member = 1)	0.101	.032	.002	
Age at baseline	0.006	.002	.715	
Sex (male = 1)	0.021	.027	.434	
Rice plate waste (g)	0.001	.000	.882	

¹ Dependent variable: change of hemoglobin concentration over the study period² Dependent variable: change in log ferritin over the study period

Plasma retinol

The basal mean plasma retinol concentrations were high in all groups (> 0.70 μmol/L) but was significantly higher in the Control group than in the *ExFeSO₄* and *ExFeP80* groups. At 6 months, mean levels in the *ExFeSO₄* and *ExFeP80* groups were similar but the level in the *ExFeP80* was significantly lower than in the control group. How-

ever, increments in the fortified groups and the Control group were similar (Table II).

Six (11.3%) in the *ExFeP80* group, one (1.9%) in the *ExFeSO₄* group, and none in the control group had low levels of plasma retinol at baseline. At 6 months, only one child in the *ExFeP80* and one in the control group had low levels, but none had low retinol levels in the *ExFeSO₄* group.

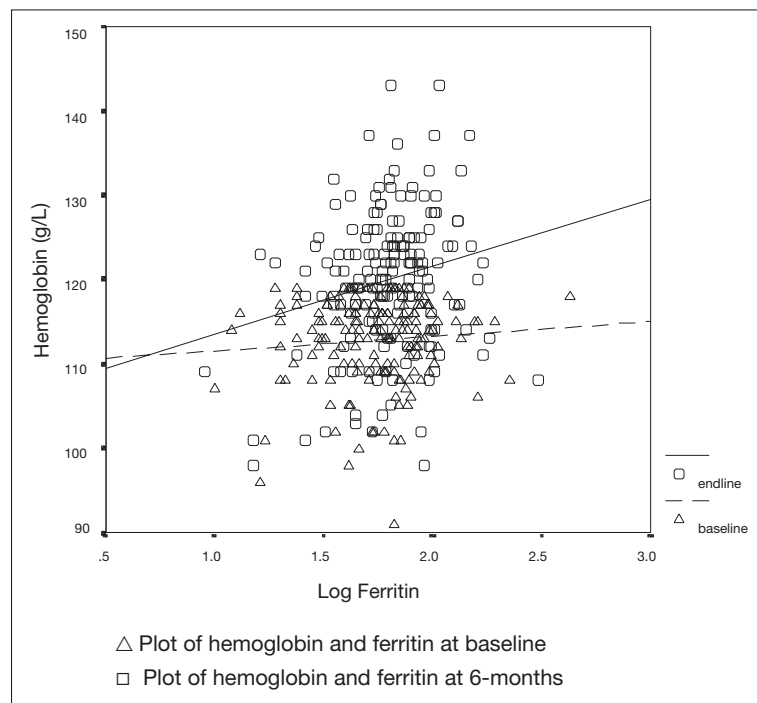


Figure 2: Relationship of hemoglobin and plasma ferritin.

Effects on weight and height

Basal values on mean weight: *ExFeSO₄* (20.38 ± 4.09); *ExFeP80* (20.76 ± 3.85); Control (20.84 ± 3.55) and mean height: *ExFeSO₄* (118.48 ± 5.91); *ExFeP80* (118.44 ± 6.75); Control (118.06 ± 5.81) were similar at start of the study. The mean weight-for-age Z-score (WAZ), weight-for-height Z-score (WHZ), and height-for-age Z-score (HAZ) were also similar in all groups at baseline. At 3 months mean weight in the *ExFeSO₄* group was 21.01 ± 4.15 kg; *ExFeP80* group, 21.40 ± 4.08 kg; and Control group 21.41 ± 3.64 kg. For height, the *ExFeSO₄* group (119.49 ± 5.93 cm); *ExFeP80* group (119.73 ± 6.82 cm); and Control group (119.22 ± 5.90 cm) had significantly increased over time within groups, but these values were not significantly different between groups. The mean changes in weight between baseline and at 6 months were: *ExFeSO₄* (1.16 ± 0.77 kg); *ExFeP80* (1.47 ± 1.18 kg); and Control (1.29 ± 0.94 kg). These values were not significantly different between groups, while the mean change in height between baseline and at 6 months; *ExFeSO₄* (2.44 ± 0.68 cm); *ExFeP80* (2.70 ± 2.02 cm); and Control (2.84 ± 1.83 cm) were also not significantly different between groups.

The prevalence of underweight decreased from 34.5% (*ExFeSO₄*) 33.3% (*ExFeP80*), and 23.7% (Control) at baseline to 30.9%, 21.1%, and 15.3%, respectively, at 6 months. The rates, however, were similar within groups and not significantly different in between-group compar-

isons. The prevalence of wasting at the start of the study was 12.7% (*ExFeSO₄*), 3.5% (*ExFeP80*), and 3.4% (Control). At 6 months, no significant change was observed both within and between groups: 10.9% (*ExFeSO₄*), 3.5% (*ExFeP80*), and 1.7% (Control). The basal prevalence of stunting was 27.3% (*ExFeSO₄*), 28.1% (*ExFeP80*), and 28.8% (Control) and these rates showed no significant change between groups at 6 months: 25.5% (*ExFeSO₄*), 28.1% (*ExFeP80*), and 23.7% (Control).

Acceptability of the iron-fortified rice

The Hedonic scale test revealed that about 93% of the children in the two iron-fortified groups and 90% in unfortified group answered that they liked the rice "very much". About 98% of all the children in all groups said that they were simply eating ordinary (unfortified) rice.

Compliance and Plate waste

Children with plate waste were similar between groups (2.3%) and were only observed during the first 8 weeks of feeding in all study groups. Children who took lunch for at least 100 feeding days were: *ExFeSO₄* (60%), *ExFeP80* (56%), and Control (76%). The estimated amounts of iron intake after deducting the plate waste were: *ExFeSO₄* (12.805 mg = 116.4% RENI), *ExFeP80* (12.19 mg = 110.8%), and Control group (0.10 mg = 0.9%).

Illnesses and CrP

Children with CrP values >6 mg/L at baseline were: *ExFeSO₄* (2) and Control (2); at 3 months: *ExFeSO₄* (3) and *ExFeP80* (2); and at 6 months: *ExFeSO₄* (1) and Control group (1). The most common illnesses experienced by the children in the three groups during the study period with only one episode per illness per child were: fever, cough, and cold. The percentage of children who had fever was significantly higher in the Control group than in the fortified groups: *ExFeSO₄* (53%), *ExFeP80* (65%), and the Control group (75%). Group designation was associated with the occurrence of fever. The duration of each episode was 2 days for fever and 4 days for cough and cold, and was similar between groups.

Discussion

In this randomized, placebo-controlled, community trial, the observation on the significant increments in Hb concentration at three months in the *Control* group (3.2 g/L) could be due to the effect of the deworming given at baseline. The administration of deworming has been proposed as an additional intervention to reduce anemia [39, 40]. A systematic review of randomized controlled trials on the effects of the administration of intestinal anthelmintic drugs on Hb revealed that a marginal increase in Hb (1.71 g/L) could translate on a public health scale into a 5% to 10% reduction in the prevalence of anemia in populations with a relatively high prevalence of intestinal helminthiasis [41]. It is a fact that soil-transmitted helminth infections (hookworm, *trichuris*, *ascaris*) contribute to anemia by causing blood loss directly through ingestion and mechanical damage of the mucosa, and indirectly by affecting the supply of nutrients necessary for erythropoiesis [42–44]. Apart from this, high retinol concentration might have influenced iron metabolism [45]. Cross-sectional studies among children have shown associations between retinol and iron status indicators [46–48]. These cross-sectional studies were followed up by a number of intervention studies which showed that increased vitamin A may lead to an improvement in iron status [47, 49, 50]. However, the significantly higher increments in both the fortified groups (6.8 g/L) than in the Control group could be attributed to the effect of the added iron in rice. Extending the feeding duration to 6 months enabled the *fortified* groups to attain further increases in Hb concentrations, reflecting an additional 2.3 g/L to the values at 3 months. The Control group remained at a concentration similar to the value at 3 months. Apart from an increasing Hb concentrations from 3 to 6 months in the fortified

groups, this study has shown that supplementary feeding of iron-fortified rice for 6 months further significantly reduced the prevalence of anemia from 100% at baseline to 51% and 54% in the *ExFeSO₄* and *ExFeP80* groups, respectively, at 3 months to 38.2% (*ExFeSO₄*) and 33.3% (*ExFeP80*) at 6 months. The Control group remained at a prevalence of 63%, which was similar to the rate at 3 months. The beneficial effects found in this present study are in agreement with the findings of previous fortification studies that showed improvements in iron status [7, 51, 52].

The findings on higher reduction rates in anemia prevalence at 6 months than at 3 months implies a programmatic consideration on the duration of supplementary feeding programs aimed at controlling anemia among anemic schoolchildren; i.e. that fortification should be carried out for at least 6 months.

Furthermore, the increases in Hb concentrations in both the fortified groups indicated a general similar improvement in the Hb concentrations; hence it may be deduced that ferrous sulfate powder or MDFP could be used in rice as fortificants. An earlier study on iron-fortified rice using coating technology in the Philippines also showed that both the fortified and the Control groups had significant increases in Hb before and after 6 months of feeding, but no significant difference was observed between groups. The authors attributed the minimal effects on the low dose of iron in raw IFR (5.33 mg/100 g) representing 38% RDA, which could be reduced in cooked rice due to iron losses during washing [53]. The present study provided a higher dose of iron and used an extrusion technology which could have prevented iron losses in cooked rice.

The insignificant increase in ferritin concentrations between baseline and 3 months, but a significant increase at 6 months, amounting to an average of 11.5 μ g/L in both the *fortified* groups, showed that providing iron-fortified rice for only 3 months might not yet be adequate to cause iron to be stored, because the first response to iron supplementation is the increase in Hb concentrations as observed in this study. Longer duration of fortification (6 months) should therefore be considered to show a greater beneficial effect on iron stores. A study on oral iron supplementation given weekly revealed that ferritin concentration was significantly higher at 12 weeks than at 8 weeks [54].

The presence of anemia despite normal concentrations of plasma ferritin implies that the children had low-grade anemia so as not to cause the utilization of ferritin for Hb formation [49]. WHO/UNICEF (1997) determined the cut-off level of anemia as 115 g/L plasma ferritin for children aged 5–11 years [56], which might be a more appropriate cut-off for lowered plasma ferritin concentrations. Several epidemiological studies have related high

iron stores with anemia as a result of vitamin A deficiency (VAD) because of the role of vitamin A in iron metabolism [45]. However, in this study, data showed that only one child at the study's endpoint had a low plasma retinol concentration (plasma retinol < 70 $\mu\text{mol/L}$). Others have postulated that this is due to the presence of parasitic infections [57]. A study in Tanzanian and Mexican preschoolers revealed a lack of hemoglobin response to iron-fortified beverages and iron supplementation after 6 and 12 months of intervention, respectively. Lack of response was attributed to presence of infections and dietary quality [52, 58]. In this present study, the children were dewormed twice during the course of 6 months; once at month 1 and once at month 5. This is in agreement with the re-infection rate (6–8 months) reported earlier [59]. CrP results (> 6 mg/L), which were found in only 2 children, seem inadequate to have caused the non-utilization of iron stores for Hb formation. The area is considered as non-endemic of malaria. Since anemia responded to the iron intervention, it could be deduced that the main cause was a deficiency in iron. However, the presence of anemia despite normal plasma ferritin levels in this study might be due to the short duration of the intervention. Other possible contributory factors might be a deficient intake of other heme-forming nutrients like vitamins B₁, B₂, B₁₂, C, and folic acid, which were not included as fortificants in this study [58].

Children with higher basal levels of Hb and ferritin had lower incremental changes after the study. This is in agreement with the study among preschool children in the Philippines. Moreover, this previous study showed that not all anemic children were iron-deficient [60]. Although there was a weak positive linear correlation between Hb and plasma ferritin, the plasma ferritin of children increased as Hb concentrations increased (Figure 2). It should be noted that iron absorption increases and decreases in response to body iron stores [61]. Children who received iron-fortified rice had higher increments in hematological status. Studies evaluating the effects of iron intervention would show greater beneficial effects if the hematological profile of the study population was low [55].

The increases in weight and height of children ranged from 1.0 kg to 1.5 kg and 2.3 cm to 3.0 cm, respectively. A normal child should increase in weight by 0.7 to 2.45 kg and by 2.3 to 3.2 cm in height in a span of 6 months [37]. Hence, the IFR had minimal effects on weight and height of children. The same results were obtained in another study among infants and young children given iron fumarate sprinkles [55]. On the other hand, in an Indonesian study, iron supplementation markedly increased linear growth of children [4, 62]. Conflicting results on the impact of iron supplementation and fortification can be at-

tributed to the different growth-limiting nutrient deficiencies in the same child, the type of food given, doses of iron, and duration of intervention.

The children generally liked and accepted the cooked rice primarily because of no change in taste. Color and appearance of cooked iron-fortified rice and cooked unfortified rice were similar and differences were not visibly recognizable because the field researchers, including the primary investigator, had no access to the preparation, development, and packaging of the IFR in color-coded polyethylene bags. The packing of allocation of cooked rice into the lunch boxes was done by color codes, hence, there was no point of comparison with the other cooked rice. However, after the study and data analysis, a thorough scrutiny was conducted by the researchers and noticed that brown-colored rice grains were visible in the uncooked rice fortified with ferrous sulfate. The concentration of premix, which was 1 g mixed with 200 g ordinary rice, however, made it difficult to really visibly see the brown-colored rice grains in uncooked iron-fortified rice with ferrous sulfate. The cooked rice fortified with ferrous sulfate yielded a somewhat very light greenish color; however, this was not recognizable when isolated from the other cooked rice as had been done in the study. The rice fortified with micronized ferric pyrophosphate was indistinguishable from the unfortified rice both in uncooked and cooked rice in terms of taste, color, appearance, texture, and odor. In the Philippines, dark colored uncooked rice grains are usually considered as dirt, hence are thrown away. Since this study took place in a catering service, packed uncooked rice was cooked as it was instructed. For future nationwide implementation of fortified rice using ferrous sulfate, a strong social marketing and advocacy strategy should be in place.

Conclusion

This study has shown that rice fortified with either FeSO_4 or FeP80 is similarly efficacious in improving the hemoglobin concentrations and reducing iron-deficiency anemia among schoolchildren when fed over a period of at least 6 months. It should, however, be considered that the add-on cost of fortification should be kept at a low price. A strong advocacy and marketing strategy should be in place for optimum benefits.

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