



## 1 **Abstract**

2 The effect of iron fortification is generally assumed to be less than iron supplementation, however, the  
3 magnitude of differences in effect is not known. The present study aimed to compare the efficacy of  
4 these two strategies on anaemia and iron status. 425 anaemic children in six primary schools in  
5 Tamnong district were included in a randomized, placebo-controlled trial comparing 2 groups  
6 receiving iron fortified instant noodles or iron supplementation for 6 months, and a control group, with  
7 all groups being dewormed. Blood samples were collected before and after intervention for  
8 haemoglobin, Serum Ferritin (SF), Serum Transferrin Receptor (TfR), C – reactive Protein (CRP),  
9 haemoglobinopathies analysis. Regression analysis was used to assess the effect of iron fortification  
10 and iron supplementation on haemoglobin concentration, SF, TfR, body iron and anaemic status as  
11 outcome variables. The improvement of haemoglobin, SF, and body iron level in the group receiving  
12 iron fortification was 42% (2.6 g/l versus 6.2 g/l); 20% (23.5 µg/L versus 117.2 µg/L) and 31.3% (1.4  
13 g/kg versus 4.4 g/kg) of that in the iron supplementation group. The relative risk of anaemia in the iron  
14 fortification group compared to the iron supplementation group was 1.19 (95% CI 0.30-4.72, p=0.80).  
15 At the end of intervention, 8 children (11%), all in the supplementation group, showed SF above  
16 normal range of 30-300 µg/L. In conclusion, the efficacy of iron fortification based on the change in  
17 haemoglobin level is 58% less than that of supplementation. To avoid iron overload, in a population of  
18 anaemic children with mild iron deficiency, iron fortification will be the preferred strategy to combat  
19 anaemia.

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22 **KEY WORDS:** Anaemia; iron deficiency; iron fortification; de-worming; children; Vietnam

## 1 INTRODUCTION

2  
3 Anaemia is a significant public health problem in Vietnam. The 2000 National Nutrition Risk Factor  
4 Survey in Vietnam showed an anaemia prevalence of 34% in children under five and 25% in women (1).  
5 No nationally representative data are available on the prevalence of anaemia among primary  
6 schoolchildren in Vietnam, however, a few local studies showed an anaemia prevalence of approximately  
7 30% (2,3). Iron deficiency is considered as the major cause of anaemia, due to low intake and bio  
8 availability of iron in the diet (4,5). The National Nutrition Survey showed that the mean iron intake of  
9 Vietnamese people, which is mainly non-haeme iron, only reaches 72% of the RDA (6). While iron  
10 supplementation in itself is highly effective in reducing iron deficiency anaemia, the implementation has  
11 been characterized with low coverage (15-20%) and non compliance (1). Food-based strategies are  
12 recommended as long-term interventions to address the malnutrition problem in the country (7). Although  
13 it is generally accepted that the increase of consumption of animal products would increase iron intake in  
14 the long term, the consumption of animal products in developing countries is sincerely hampered by low  
15 socio-economic status (8). Food fortification is often suggested as one of the most effective and  
16 sustainable strategies for increasing iron intake in the general population (4). Studies on the effect of iron  
17 supplements (9-11) (12,13) or iron-fortified foods (14-18) on indicators of iron deficiency have been  
18 carried out but few studies compared the effect of iron fortification with iron supplementation on the  
19 improvement of iron and anaemia status. It is generally known that fortification is less effective than  
20 supplementation due to differences in iron dose, and bioavailability of iron (19). However, the extent of the  
21 differences in effect is unknown. In a previous study Baltussen et al suggested that fortification would be  
22 50% less effective than supplementation but this assumption was not based on a intervention study (19).  
23 Aim of the present study is to compare the effect of iron fortification and iron supplementation on the  
24 changes in haemoglobin and iron status in order to assist public health nutritionists in making an informed  
25 choice for an appropriate strategy to combat iron deficiency and anaemia among schoolchildren in rural  
26 Vietnam.

## 1 SUBJECT AND METHODS

### 2 Study design and population

3 The study was implemented from November 2004 to May 2005 in six primary schools in Tamnong  
4 district, Phutho province, situated 90 km from Hanoi. Selection was based on high prevalence of  
5 anaemia and absence of interventions to control iron deficiency anaemia in schoolchildren. Children  
6 recruited into the study were in grade one to grade three with haemoglobin concentrations < 110g/l  
7 and >70 g/l in an initial haemoglobin – screening study. The study concerns a randomized, placebo-  
8 controlled double blind parallel trial with a 2×2 factorial design plus standard treatment (iron  
9 supplementation and de-worming) and an intervention period of six months. A total of 425 eligible  
10 children were randomly assigned to one of five groups (85 per group) receiving: I) iron-fortified  
11 noodles and mebendazole (Fe+MEB); II) noodles without iron fortificant and mebendazole (MEB);  
12 III) iron-fortified noodles and placebo (Fe) IV) noodles without iron fortificant and placebo (placebo)  
13 and V) iron supplementation and mebendazole (Fe tablet+MEB) (**figure 1**). Sample size was  
14 estimated based on an alpha error of 0.05, a statistical power of 95%, a between-group difference in  
15 treatment effect of 5 g haemoglobin/L in haemoglobin concentration being clinically relevant, a  
16 standard deviation of 11 g/l (2) and accounting for 10% of children being lost in the course of the  
17 intervention. In this article we only concentrate on the effect of the iron fortified noodles (Fe+MEB)  
18 compared to that of the standard treatment (Fe tablet+MEB). For this reason, three groups were  
19 included in this analysis: (Fe+MEB); (Fe tablet+MEB) and (MEB) as control group. The effect of  
20 iron-fortification and de-worming on iron and anaemia status of schoolchildren is discussed in another  
21 paper (20). Children were invited for the study and their parents were asked for a written informed  
22 consent. The study was approved by the Scientific Committee of the National Institute of Nutrition  
23 and the Ethics Committee of Hanoi Medical University - Ministry of Health.

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## 1 **Products and field procedures**

2 Fortified instant noodles were produced at the Hanoi Food Company. Noodles were fortified with a water  
3 soluble, highly bioavailable iron compound (NaFeEDTA: Ferrazone®, Akzo Nobel Chemicals Pte Ltd  
4 Velp) to a fortified level of 10.7 mg iron per 52 gram of noodles calculated based on the JECFA 1974  
5 recommendation of an acceptable daily intake of 2.5 mg EDTA/kg body weight and an average body  
6 weight of 29 kg (21). Before intervention retention of iron after production and preparation of fortified  
7 instant noodles was checked in laboratories at the National Institute of Nutrition Hanoi, Wageningen  
8 University and Akzo Nobel Chemicals Pte Ltd Velp. Capillary zone electrophoresis analysis (22) showed  
9 that 70% of the NaFeEDTA dissolves within 5 minutes into the soup independent of extraction time. No  
10 degradation products of NaFeEDTA were found.

11 Noodles were prepared in school by care-takers trained by the field staff and given to children at break  
12 time (9:00 am) five days per week during six months under the supervision of teachers and field staff.  
13 Children were encouraged to consume all the noodles and water.

14 Mebendazole 500 mg were given to children at the beginning of intervention and at three months  
15 following. Children, care takers, teachers and researchers were blinded to the treatment.

16 Iron supplementation in the form of ferrous fumarate 200 mg (equal to 65 mg elemental iron) were taken  
17 with a glass of water at break time every school day (five days a week). Ingestion of the supplements was  
18 supervised by a teacher and field staff and recorded in a monitoring book.

19

## 20 **Data collection.**

21 Capillary blood samples were taken from children's fingers during screening for haemoglobin  
22 measurement by cyanmethaemoglobin method. Venous blood (5 ml) was collected in the morning at  
23 baseline (T0) and after intervention (T6); 20µl whole blood was pipetted immediately before coagulating  
24 into a tube containing 5.0 ml of Drabkin's reagent with a Sali pipette for haemoglobin measurement. An  
25 aliquot of whole blood was taken for analyzing haemoglobinopathies. The remaining blood was allowed  
26 to clot for 30 minutes at room temperature, centrifuged at 3000 x g for 15 minutes and transferred to five

1 plastic labeled vials (Eppendorf tubes 0.5ml). The vials were stored in a box protected from sun-light and  
2 put into an ice box for transfer to the laboratories and kept at  $-80^{\circ}\text{C}$  until serum ferritin (SF), serum  
3 transferrin receptor (TfR) and C-reactive protein (CRP) analysis at the end of the intervention.

4 For assessment of intestinal parasite infection before and after intervention, containers for collection of  
5 stools were distributed to each class and children were asked to collect and deliver a sample of their  
6 faeces to school the next day. In case some children were unable to return a sample, one of the field  
7 workers returned the next day to collect the rest of the samples.

### 9 **Laboratory analysis**

10 Haemoglobin concentration was measured in whole blood within 12 h of sampling by  
11 cyanmethaemoglobin method using Sigma KIT in the Tamnong District Health Centre. The CV of intra-  
12 assays and inter-assays was  $4.0 \pm 1.2\%$  and  $5.0 \pm 2.0 \%$  respectively. SF and TfR, and CRP analysis was  
13 carried out at the same time for both samples of baseline and after intervention at the National Institute of  
14 Nutrition and the laboratory of Hanoi Medical University in May and June 2005. Concentrations of SF  
15 and TfR were analyzed by an Enzyme - Linked Immuno Sorbent Assay (ELISA) method (Ramco  
16 Laboratories, Inc, Houston, TE, Catalogue numbers S-22 and TF-94), with inter-assay variability of 4-7%  
17 and 4-8%, respectively. Serum CRP was measured by nephelometry using Epress plus, with an inter-  
18 assay variability of 4-8 %. A 10% sub samples was re-examined for quality control.  
19 Haemoglobinopathies analysis was performed by using the Variant Beta-Thalassemia Short program  
20 (Bio-Rad laboratories Inc, Hercules, CA) within 24 h of sampling in the Children Hospital, Hanoi,  
21 Vietnam. Stools samples were examined before and after intervention by using the Kato - Katz Technique  
22 –cellophane faecal thick smear method (23). Hookworm, *Trichuris*, and *Ascaris* eggs were counted. A  
23 10% sub sample of smears was re-examined for quality control.

## 1 **Data analysis**

2 Anaemia was defined as haemoglobin concentrations <115g/L (24). Iron deficiency was defined as SF  
3 concentrations <12 µg/L (24), and tissue iron deficiency was defined as TfR concentration >8.5 mg/L  
4 (25). Body iron content was calculated using the following formula: body iron (mg/kg) =  $-(\log(\text{TfR/SF}$   
5  $\text{ratio}) - 2.8229)/0.1207$  (26).

6 CRP concentration were considered to be elevated when  $\geq 8$  mg/L (27). Haemoglobin type was  
7 determined in each subject on the basis of haematological indexes: HbAA (normal haemoglobin type),  
8 HbF, HbA2 (Beta thalassemia); Hb AE (trait for haemoglobin E disease), or HbEE (haemoglobin E  
9 disease). Severity of intestinal worm infections was expressed as the number of eggs/g faeces using the  
10 WHO classification system (28).

11 We excluded all the children with thalassemia and haemoglobin E (HbF, HbA2, HbAE) (n= 15) and CRP  
12 elevated (n= 5) from the analysis to prevent confounding.

13 Data was entered into the computer, cleaned and managed using Epi Info version 6, (29) and analyzed  
14 using SPSS 11.0 for windows (SPSS Inc., Chicago IL, USA)(30). Data was checked for normality by  
15 visual observation. One-way ANOVA with post hoc analysis (LSD significant difference) was used to  
16 determine differences in haemoglobin concentration and other biochemical indicators between groups.  
17 Paired t-test was used to assess the difference in haemoglobin and other biomarkers within group before  
18 and after intervention. Chi-square test and Wilcoxon test were used to assess the differences between and  
19 within groups in proportions. To assess the association between iron fortification, iron supplementation  
20 and indicators of iron status, we compared children with and without iron fortification and children with  
21 and without iron supplementation with respect to their change in haemoglobin concentration, SF, TfR,  
22 and body iron, respectively by using multiple linear regression analysis. Logistic regression was used to  
23 study the effect of iron fortification and iron supplementation on anaemia prevalence.

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## 1 RESULTS

2 At baseline, the mean age of children was  $87.3 \pm 10.3$  months. The three groups did not significantly  
3 differ in age, haemoglobin concentration, iron status (SF, TfR, and body iron) (**Table 1**) nor parasite  
4 infection (**Table 2**). The prevalence of iron deficiency was very low as 0.9% of children showed SF  
5 concentration below  $12 \mu\text{g/L}$  and 3.2% of children showed TfR above  $8.5 \text{ mg/L}$ . Mean body iron was  
6 around  $6.3 \text{ mg/kg}$  body weight (**Table 1**). 66%, 71% and 9% of children were infected with *Ascaris*,  
7 *Trichuris*, and hookworm respectively (**Table 2**).

8 Haemoglobin concentration increased in all three groups; however, larger significant increase was seen in  
9 the group receiving iron supplementation:  $21.2 \pm 10.7 \text{ g/L}$  compared to  $17.8 \pm 7.6 \text{ g/L}$  and  $14.5 \pm 8.5 \text{ g/L}$   
10 in iron fortified and control groups (**Table 2**). Prevalence of anaemia significantly decreased in all three  
11 groups with a larger reduction observed in the two groups receiving iron fortified noodles and iron  
12 supplementation (73.6% and 77.6% reduction respectively) compared to the control group (68.5%  
13 reduction); however, no significant difference was found between groups (**Table 2**).

14 SF concentration increased significantly in the two groups receiving iron fortification and iron  
15 supplementation ( $18.5 \pm 30.9 \mu\text{g/L}$  and  $111.7 \pm 76.5 \mu\text{g/L}$  respectively) compared to the control group  
16 where SF concentration even decreased ( $-6.5 \pm 27.1 \mu\text{g/L}$ ) (**Table 2**). TfR concentration was very limited  
17 improved after six months of intervention in all three groups, however, the group receiving iron  
18 supplementation showed largest improvement ( $-0.8 \pm 0.9 \text{ mg/L}$ ) compared to iron fortification and control  
19 groups ( $-0.4 \pm 0.9 \text{ mg/L}$  and  $-0.4 \pm 0.9 \text{ mg/L}$ ). There were no significant differences between groups  
20 (**Table 2**). Body iron significantly increased in the two groups receiving iron fortification and iron  
21 supplementation ( $1.5 \pm 1.9 \text{ mg/kg}$  and  $4.2 \pm 1.9 \text{ mg/kg}$  respectively) compared to the control group ( $-0.1$   
22  $\pm 1.6 \text{ mg/kg}$ ).

23 Prevalence of *Ascaris* and *Trichuris* and hookworm infection fell significantly in all three groups (**Table**  
24 **2**).

25 Iron supplementation showed a larger change in haemoglobin and iron status indicators than iron  
26 fortification after taking into account haemoglobin at baseline, age and sex (**Table 3**). The improvement

1 of haemoglobin, SF, and body iron level in the group receiving iron fortification was 42% (2.6 g/l  
2 compared to 6.2 g/l); 20% (23.5 µg/L compared to 117.2 µg/L) and 31.3% (1.4 mg/kg compared to 4.4  
3 mg/kg) of that in the iron supplementation group (**Table 3**).

4 Compared to the control group, the prevalence odds ratio (relative risk) of anaemia in the iron  
5 fortification group and iron supplementation group was 0.46 (95% CI 0.15 -1.43; p= 0.18) and 0.36 (95%  
6 CI= 0.11 - 1.26; p = 0.11) respectively (**Table 4**). Relative risk of anaemia of fortification compared to  
7 supplementation was 1.19 (95% CI 0.30-4.72, p=0.80) after adjustment for haemoglobin at baseline, sex  
8 and age.

9

## 10 **DISCUSSION**

11 Results from the present study showed that in anaemic school children iron fortification was 58% (based  
12 on change in haemoglobin level), 80% (based on SF level) and 68.7% (based on body iron) less effective  
13 than iron supplementation . However, the risk of being anaemic in iron fortification relative to  
14 supplementation was only slightly and not significantly increased (OR =1.19, p=0.80).

15

16 Data collection in our study was carried out carefully. Blood samples were collected, transported and  
17 stored under standard conditions. Serum samples before and after intervention were analyzed at the same  
18 time after intervention to avoid variation between different measurements. In house quality control was  
19 carried out regularly during serum analysis at the laboratories. Randomization was successful, as the  
20 groups were comparable in the key indicators at baseline. De-worming was effective as shown by a  
21 significant reduction of intestinal parasite infection in all three groups.

22

23 In the present study, the control group also improved haemoglobin and anaemia status after 6 months of  
24 the intervention which might be explained by the effect of de-worming. Although de-worming reduced  
25 worm infection prevalence, however, no effect of de-worming on the anaemia and iron status was  
26 observed (20). Moreover, a previous analysis suggested that in absence of other major causes of anaemia

1 (like vitamin A deficiency, malaria and haemoglobinopathies), probably (chronic) inflammation could  
2 have played a role, but this needs to be further addressed (20)

3

4 A large part of the study population was anaemic at baseline (84%) but showed very low iron deficiency  
5 as indicated by the low prevalence of elevated SF and TfR indicators (0.9% and 3.2%). However, as in  
6 general an improvement of haemoglobin levels in an anaemic population through iron supplementation is  
7 seen as an indicator of the presence of iron deficiency (31), the improvement of haemoglobin levels in our  
8 anaemic population still indicates (mild) iron deficiency although not confirmed by the SF and TfR  
9 levels.

10

11 Compared to the control group, the improvement of haemoglobin concentration was 2.6 g/l and 6.2 g/l,  
12 and reduction of anaemia was 5.1% and 9.1% in fortification and supplementation respectively. The  
13 improvement of haemoglobin and reduction of anaemia in our study population is lower compared to  
14 results from other studies. In a study among anaemic Vietnamese women consuming daily 10ml fish  
15 sauce containing 10mg elemental iron from NaFeEDTA during 6 months, haemoglobin improved with  
16  $5.7 \pm 10.3$  g/L and  $-2.8 \pm 8.7$  g/L in the intervention and control group respectively (17). A study in  
17 children 12-17 years with mild or moderate anaemia in Malaysia showed that after 22 weeks receiving  
18 weekly iron supplementation of 60 mg elemental iron (as ferrous sulfate) and 3.5 mg folate showed an  
19 improvement of haemoglobin concentration of 21.4 g/L compared to 9.3 g/L in the control group  
20 (receiving 3.5 mg folate only) (32). A review of fortification and supplementation studies in Indonesia  
21 demonstrated that iron supplementation can reduce anaemia prevalence in pregnant women by 20 to 25 %  
22 and iron fortification (adding 10mg of elemental iron) can reduce anaemia by 20% among those  
23 consuming the fortified foods (33). As the amount iron absorbed and, hence, the magnitude of  
24 improvement of haemoglobin and reduction of anaemia, depends on the iron and anaemia status of the  
25 individual (32), the lower improvement in our study may indicate a mild iron deficiency compared to the  
26 study in Vietnamese women (69.9% with iron deficiency anaemia) (17).

1

2 On the basis of the change in haemoglobin, in this population with anaemia but mild iron deficiency, iron  
3 fortification is 58% less effective than iron supplementation. This reduced efficacy can be explained by  
4 the difference in amount of iron given being lower in fortification than in supplementation. Most of the  
5 supplementation programs for women, school-age children or adolescents usually use 60mg iron/day  
6 (34). However, although in our study the daily amount of iron received from iron-fortified noodles (10.7  
7 mg/day) is 6 times less than from iron supplementation (65mg/day), the improvement of haemoglobin  
8 level in the group receiving iron fortification reaches almost half of the improvement seen in the iron  
9 supplementation group (2.6 g/L compared to 6.2 g/L) (Table 4). Also, the risk of being anaemic in iron  
10 fortification relative to supplementation was only slightly and not significantly increased. However, our  
11 study population were anaemic children with low prevalence of iron deficiency, therefore the effect of  
12 iron fortification compared to iron supplementation may differ from a population where iron deficiency  
13 exists.

14

15 Data from our study showed that iron stored, as indicated by SF, was 5 times higher in the group  
16 receiving supplementation than in the group receiving iron fortification (117.3 $\mu$ g/L and 23.5 $\mu$ g/L). After  
17 the intervention, there were 8 children have SF higher than 300  $\mu$ g/L (the normal range being 30 – 300  
18  $\mu$ g/L) (35) and all of them were in the supplementation group. SF levels outside the normal range indicate  
19 an increased risk of iron overload in the supplementation group (36). While iron supplementation has a  
20 larger impact on population health than fortification (19), it may also be harmful for individuals with  
21 haemoglobinopathies. The potential benefit of iron supplementation to a predominantly iron deficient  
22 population is likely to vastly outweigh any risk this may pose for a few individual (37), but when the  
23 population is mildly iron deficient like in our study, the risk may not be neglectable. Food fortification  
24 with iron has not significantly increased the prevalence of iron overload as shown in previous studies in  
25 the USA (38) and in Sweden (39). Iron fortification poses little, if any, risk to individuals with  
26 thalassemia minor or other haemoglobinopathies (40).

1

2 In conclusion, in our anaemic schoolchildren with mild iron deficiency, the efficacy of iron fortification  
3 based on the change in haemoglobin level is 58% less than that of supplementation, but the risk of  
4 anaemia is only slightly increased. To avoid iron overload in this population, iron fortification may be the  
5 preferred strategy to combat anaemia.

6

7 **Abbreviations used:** SF, Serum ferritin; TfR, Serum transferrin receptor; CRP, C-reactive protein; IgE,  
8 (immunoglobulin E); (ELISA), Linked Immuno Sorbent Assay; (Fe+MEB), iron-fortified noodles and  
9 mebendazole; (MEB), noodles without iron fortificant and mebendazole; (Fe), iron-fortified noodles and  
10 placebo; Placebo, noodles without iron fortificant and placebo; (Fe tablet+MEB), Iron supplementation  
11 and mebendazole.

12

### 13 **Contributors**

14

15 HLT was responsible for all aspects of protocol development, study coordination, data collection, data  
16 analysis and report writing. BID was involved in protocol development, data analysis and report writing  
17 and obtaining funds for the study. BJ supported in statistical analysis. KNC was involved in supervision  
18 of data collection. KFJ was involved in protocol development, data analysis, reporting and obtaining  
19 funds for the study.

20

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22

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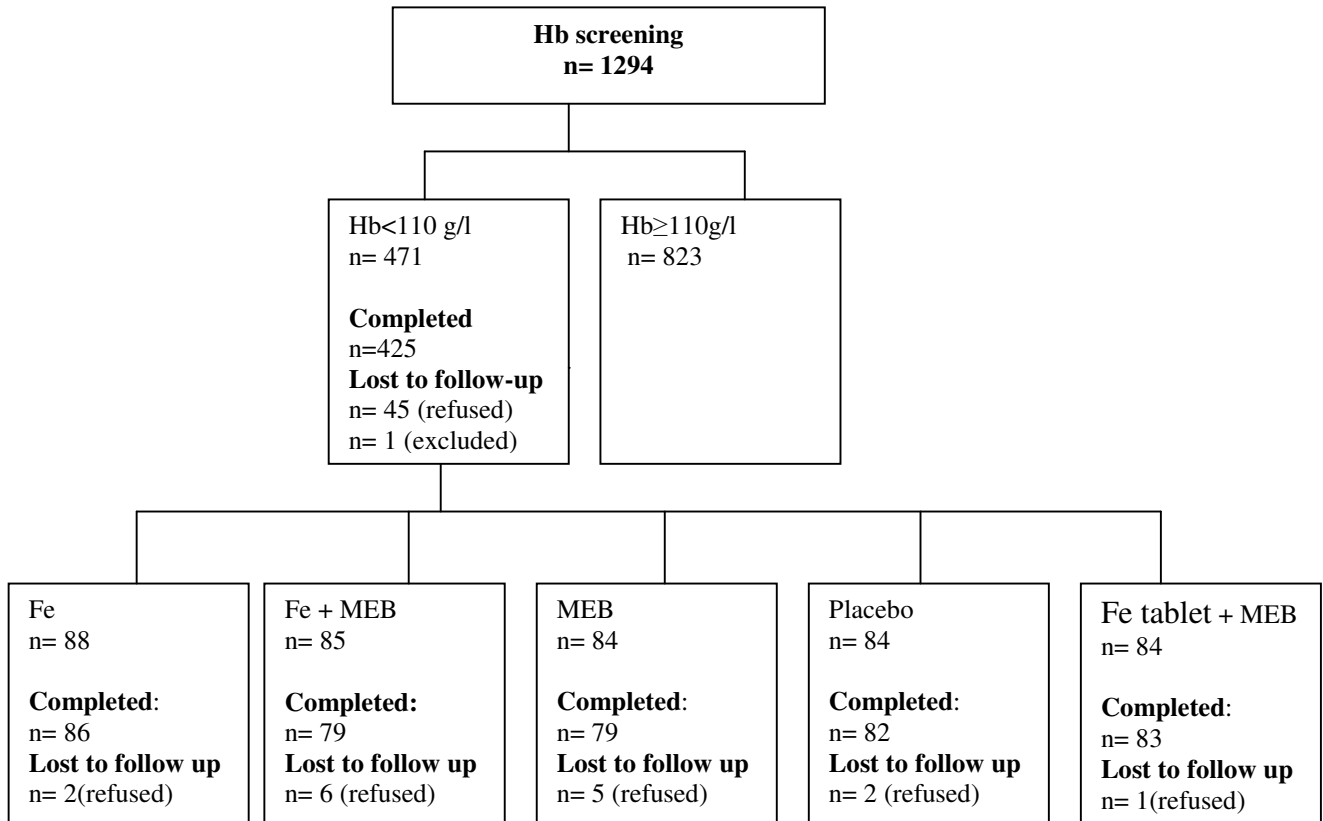
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3 **Figure 1:** Study profile: initial screening to enroll anaemic children in the study, followed by a 6 months  
 4 intervention.

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**Table 1: Baseline Characteristic of Vietnamese schoolchildren by group after random assignment (n=221)**

|                                  | Fe + MEB<br>(n= 72) | Fe tablet + MEB<br>(n= 76) | MEB (Control)<br>(n= 73) |
|----------------------------------|---------------------|----------------------------|--------------------------|
| Male sex (%)                     | 48.6                | 51.3                       | 46.6                     |
| Age in month <sup>1</sup>        | 87.3 ± 11.6         | 86.4 ± 9.8                 | 87.9 ± 10.2              |
| Haemoglobin (g/L) <sup>1,3</sup> | 107.6 ± 6.9         | 108.4 ± 6.7                | 108.9 ± 6.5              |
| SF (µg/L) <sup>2,3</sup>         | 46.8 (33.3- 66.4)   | 54.2 (39.7 -72.4)          | 54.5 (37.8-79.7)         |
| TfR (mg/L) <sup>1,3</sup>        | 6.0 ± 1.3           | 5.9 ± 1.1                  | 6.2 ± 1.4                |
| Body iron (mg/kg) <sup>3</sup>   | 6.0 ± 2.3           | 6.6 ± 1.9                  | 6.3 ± 2. 7               |
| SF < 12 (µg/L)%                  | 1.4                 | 0                          | 1.4                      |
| TfR> 8.5 (mg/L)%                 | 2.8                 | 1.3                        | 5.5                      |

1. Mean ± SD

2. Geometric mean (25 and 75 percentile)

3. No significant different between groups (One –way Anova)  $P>0.05$

**Table 2: Change in haemoglobin, iron status indicators and worm infection after and before intervention among Vietnamese schoolchildren**

|  | Fe + MEB<br>(n=72)       | Fe tablet + MEB<br>(n=76) | MEB (Control)<br>(n=73) |
|--|--------------------------|---------------------------|-------------------------|
| Change in Haemoglobin (g/L) <sup>1</sup> | 17.8 ± 7.6 <sup>2</sup>  | 21.2 ± 10.7 <sup>2</sup>  | 14.5 ± 8.5 <sup>2</sup> |
| Change in SF (µg/L) <sup>1</sup>         | 18.5 ± 30.9 <sup>2</sup> | 111.7 ± 76.5 <sup>2</sup> | - 6.5 ± 27.1            |
| Change in TfR (mg/L)                     | -0.4 ± 0.9 <sup>2</sup>  | -0.8 ± 0.9 <sup>2</sup>   | -0.4 ± 0.9 <sup>2</sup> |
| Change in Body iron (mg/kg) <sup>1</sup> | 1.5 ± 1.9 <sup>2</sup>   | 4.2 ± 1.9 <sup>2</sup>    | - 0.1 ± 1.6             |
| <b>Anaemia (%)</b>                       |                          |                           |                         |
| T0                                       | 83.3 <sup>3</sup>        | 84.2 <sup>3</sup>         | 83.6 <sup>3</sup>       |
| T6                                       | 9.7                      | 6.6                       | 15.1                    |
| <b>SF &lt;12(µg/l) (%)</b>               |                          |                           |                         |
| T0                                       | 1.4                      | 0                         | 1.4                     |
| T6                                       | 0                        | 0                         | 0                       |
| <b>TfR &gt;8.5 (mg/L) (%)</b>            |                          |                           |                         |
| T0                                       | 2.8                      | 1.3                       | 5.5                     |
| T6                                       | 2.8                      | 0                         | 0                       |
| <b>Ascaris (%)</b>                       |                          |                           |                         |
| T0                                       | 62.5                     | 67.1                      | 68.5                    |
| T6                                       | 41.7 <sup>5</sup>        | 47.4 <sup>4</sup>         | 41.1 <sup>4</sup>       |
| <b>Trichuris(%)</b>                      |                          |                           |                         |
| T0 <sup>1</sup>                          | 77.8                     | 72.4                      | 63.0                    |
| T6 <sup>5</sup>                          | 15.2 <sup>3</sup>        | 47.4 <sup>3</sup>         | 47.9 <sup>5</sup>       |
| <b>Hookworm(%)</b>                       |                          |                           |                         |
| T0                                       | 8.3                      | 10.5                      | 8.2                     |
| T6                                       | 0 <sup>5</sup>           | 1.3 <sup>5</sup>          | 1.4 <sup>5</sup>        |

1 Significant different between group (one way Anova): <sup>1</sup>p<0.001;

2 Significant within group after and before intervention (T test); <sup>2</sup>p<0.001;

3 Significant within group after and before intervention (Mc Nemar); <sup>3</sup>p<0.001; <sup>4</sup>p<0.01; <sup>5</sup>p<0.05;

**Table 3: Linear regression models of haemoglobin, SF, TfR, and body iron at the end of intervention by intervention group, compared to control group.**

| Outcome variables                  | Group                 |       |                          |       |
|------------------------------------|-----------------------|-------|--------------------------|-------|
|                                    | Iron fortification*   | p     | Iron supplementation*    | p     |
| End Haemoglobin (g/L) <sup>1</sup> | 2.59 (-0.22 – 5.40)   | 0.07  | 6.19 (3.42- 8.96 )       | 0.001 |
| End SF (µg/L) <sup>2</sup>         | 23.53 (6.82-40.25)    | 0.006 | 117.25(100.86 - 133.64 ) | 0.001 |
| End TfR (mg/L) <sup>3</sup>        | - 0.04 (-0.32 – 0.23) | 0.76  | -0.51 (- 0.78 - -0.24)   | 0.001 |
| End body iron (mg/kg) <sup>4</sup> | 1.37(0.85 – 1.89)     | 0.001 | 4.37 (3.86 - 4.88)       | 0.001 |

*1 Adjusted for Hb baseline, sex and age*

*2 Adjusted for SF baseline, sex and age*

*3 Adjusted for TfR baseline, sex and age*

*4 Adjusted for body iron baseline, sex and age*

*\* Regression coefficients (95% CI)*

**Table 4: Logistic regression model of anaemia status at the end of intervention by intervention group, compared to control group.**

| Logistic regression   |                                    |       |
|---|------------------------------------|-------|
| Outcome variable: Anaemia status at the end of intervention |                                    |       |
| Variable  | Prevalence Odds Ratio <sup>1</sup> | p     |
| Iron fortification  | 0.46 (0.15 -1.43)                  | 0.18  |
| Iron supplementation  | 0.36 (0.11 - 1.26)                 | 0.11  |
| Hb baseline   | 0.85 (0.79 – 0.92)                 | 0.001 |
| Sex (male vs female)  | 2.90 (1.01- 8.32)                  | 0.047 |
| Age   | 1.02 (0.58 - 1.79)                 | 0.95  |

*1. Prevalence Odds Ratio (95% CI)*