Anemia among school children in Vietnam:

The efficacy of iron fortification

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Propositions

1. For an anemic population with mild iron deficiency like Vietnamese school children, iron fortification, not supplementation is the preferred strategy to combat anemia. (This thesis)

2. Contrary to general assumptions, worm infection does not and iron deficiency does only partly cause anemia in primary school children in rural Vietnam. Chronic infection may have a much larger role in the anemia prevalence in Vietnam. (This thesis)

3. Hunger during schooltime may prevent children in developing countries from benefiting from education. (Grantham-McGregor. Food Nutr Bull 2005; 26 supp 2: 144-158)

4. Even in a situation of hunger, consuming a low variety of food for a long period could lead to a decrease in palatability and in acceptability of these foods (Rolls & de Waal, *Physiol Behav* 1985; 34 (6) 1017-1020).

5. Integrated interventions, which include a school feeding program combined with health and nutrition education for children and parents, are a good way to maximize benefit from education.

6. A day of travelling will bring a basketful of learning (translation of a Vietnamese proverb).

Propositions belonging to the thesis
"Anemia among schoolchildren in Vietnam: the efficacy of iron fortification"

Le Thi Huong
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Abstract

The present thesis aimed to determine the efficacy of a school-based food fortification program to improve hemoglobin concentrations and iron stores of intestinal parasites-prone school children. Furthermore this thesis also compares the effect of iron fortification and iron supplementation on the changes in hemoglobin and iron status.

A cross-sectional study among school children (6-8 years) in Tam Nong district Phu Tho province indicated a high prevalence of anemia (25%) with low iron deficiency as indicated by SF (0.5%) and TfR (2%). More than 90% of children were infected by intestinal parasites, especially *Trichuris* infection was associated with a double risk of anemia. Subsequent studies evaluate the acceptability and efficacy of iron fortification on anemia in an infection prone population. The acceptability study showed that instant noodle was an appropriate vehicle for iron fortification; noodles were consumed by half of the children at least once a week and were mainly associated with positive attributes. Although children and adults were able to differentiate between iron fortified and non-fortified noodles, fortified instant noodles were accepted by children. Boredom for noodles increased slightly over time, the acceptance ratings remained high. In the intervention study we used a randomized placebo controlled double blind trial with iron fortified noodles and de-worming plus standard treatment (iron supplementation and de-worming) to test our hypothesis that de-worming is more effective than iron fortification in an anemic infection prone population that was not considered iron deficient. However, de-worming did not show an effect on hemoglobin level and iron status, while iron fortified noodles significantly improved hemoglobin, SF and body iron (2.6 g/L, 16.2 μg /L and 1 mg/kg respectively). A seasonal improvement of hemoglobin levels and anemia status took place in all groups, also in the placebo group. The efficacy of iron fortification based on hemoglobin change was 58% less than that of supplementation. It is concluded that anemia is partly caused by iron deficiency, intestinal parasite infection does not play a role but chronic infection may be considered as a cause of anemia among our children. Iron fortification rather than iron supplementation is recommended as intervention to reduce anemia in a population with low iron deficiency. Future research should be focused on the role of chronic infection on anemia in school age children.

Contents

Chapter 1: Introduction

I. ANEMIA

Anemia occurs when bodily functions are impaired as a result of low hemoglobin concentrations. It develops when the rate of red cell destruction exceeds the capacity of the bone marrow to mount a compensatory increase in production. In children aged 5 to 11 years, anemia is defined by hemoglobin concentration (Hb) below 115 g/L when measured at sea level [1]. Associations between Hb concentration and psychomotor performance have been demonstrated at all stages of life [2]. Anemia has long been known to impair work performance, endurance and productivity [3, 4]. Severe anemia during pregnancy is thought to increase the risk of maternal mortality [5].

The World Health Organization estimates that about 40% of the world's population (more than 2 billion individuals) suffers from anemia. The groups with the highest prevalence are: pregnant women and the elderly (50%); infants and children aged 1-2 years (48%); school children (40%); non-pregnant women (35%); adolescents (30-55%); and preschool children (25%) [6]. The current estimate for anemia in school children in developing countries is 53% [6]. In Vietnam, anemia is a significant public health problem. The 2000 Nutrition Risk Factor Survey shows that the anemia prevalence was 34% in children under five years of age and 25% in women [7]. No national representative data are available on the prevalence of anemia among primary school children in Vietnam; however, a few local studies showed an anemia prevalence of around 30% [8, 9].

II. CAUSES OF ANEMIA IN SCHOOL CHILDREN

1. IRON DEFICIENCY AND ANEMIA

Red blood cell development and regulation

The life expectancy of red blood cells is normally about 120 days, and in adults the marrow must normally produce 200 billion red cells every day to replace those lost to senescence or damage [10]. Erythropoiesis is the continuous process whereby primitive erythroid progenitor cells proliferate and differentiate into mature, circulating red cells [11]. This process is principally regulated by erythropoietin, a hormone predominantly produced in the kidney in response to hypoxia. In normal conditions, increased production of erythropoietin results in erythroid hyperplasia and reticulocytosis. The biological effects of erythropoietin and others growth factors are mediated through specific receptors on target cells. One action of erythropoietin is to stimulate survival of erythroid progenitor cells by inhibiting apoptosis (gene-directed cell death).

The uptake of serum iron that is required for heme synthesis within the erythroblast is mediated and regulated by a specific receptor located on the outer cell membrane [12]. The increased expression of these receptors on the membrane of the developing red cells follows the increased expression of erythropoietin receptor but precedes the onset of cellular heme synthesis. Erythropoietin is not entirely efficient, because 10-15% of developing erythroplasts normally die within the marrow without producing mature cells, whereupon they are ingested by marrow macrophages. Red cells are taken out of circulation via phagositosis by macrophages belonging to the mononuclear phagocytosite system and resident mainly in the spleen but also in the liver, marrow and muscle.

Iron metabolism

Body iron comprises metabolically active iron that is required for normal functioning. Most body iron is incorporated into hemoglobin. About 25% of total body iron is present as stored iron, found primarily in the liver, that serves as a reservoir to supply cellular needs. The remainder of the metabolically active iron is found as myoglobin iron in muscles and as iron-containing or iron-dependent enzymes through out the cells in the body. Iron released by macrophages from degraded hemoglobin into serum is bound to the transport protein transferrin and can be recycled for erythropoiesis or used for other functions. Alternatively, macrophages can hold iron in storage by binding it to the proteins ferritin or hemosiderin. Storage iron in the mononuclear macrophage system is derived almost entirely from phagocytosis of senescent red cells or defective developing red cells. The liver also holds ferritin-bound iron in

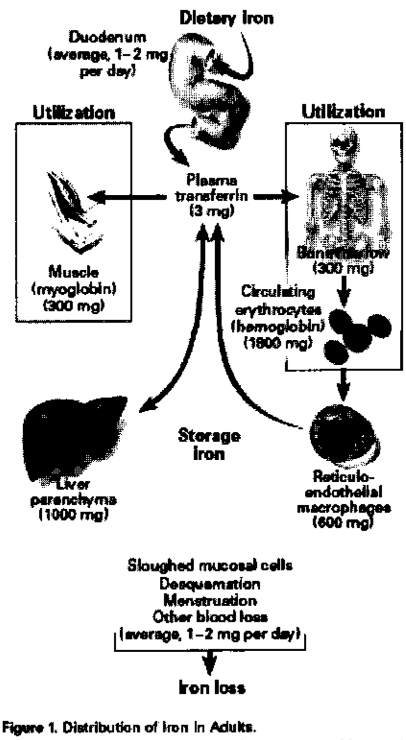

Figure 1. Distribution of Iron in Adults.
In the balanced state, 1 to 2 mg of iron enters and leaves the body each day. Dietary iron is absorbed by duodenal enterocytes. It circulates in plasma bound to transferrin. Most of the iron in the body is incorporated into hemoglobin in erythroid precursors and mature red cells. Approximately 10 to 15 percent is present in muscle fibers (in myoglobin) and other tissues (in enzymes and cytochromes). Iron is stored in parenchymal cells of the liver and reticuloendothelial macrophages. These macrophages provide most of the usable iron by degrading hemoglobin in senescent erythrocytes and reloading ferric iron onto transferrin for delivery to cells.

reserve in hepatocytes. Most of the iron used for erythropoiesis in the bone marrow is derived from recycled iron, and iron supplied by absorption through the intestine makes up only a small fraction of the total amount of iron that is used for this purpose. No physiological means of iron excretion exist. Iron absorption from the gastrointestinal tract is the sole means of regulating iron stores. ***Figure 1*** illustrates the distribution of iron in adults [13].

Iron deficiency

Iron depletion, the first stage of iron deficiency, is characterized by a progressive reduction in the amount of storage iron in the liver. At this stage, the supply of iron to the functional compartment is not compromised, so the level of transport iron and hemoglobin are normal. However, the progressive depletion of iron stores will be reflected by a fall in serum ferritin concentrations.

Iron-deficient erythropoiesis, the second stage of iron deficiency, is characterized by the exhaustion of iron stores and is also referred to as "iron deficiency without anemia." At this stage, the iron supply to the erythropoietic cells is progressively reduced, and decreases in transferrin saturation occurs. At the same time, there are increases in serum transferrin receptor and erythrocyte protoporphyrin concentration. Hemoglobin level may decline slightly at this stage, although it usually remains within the normal range.

Iron-deficiency anemia, the third and final stage of iron deficiency, is characterized by exhaustion of iron stores, declining levels of circulating iron, and presence of frank microcytic, hypochromic anemia. The main feature of this stage is a reduction in the concentration of hemoglobin in the red blood cells, arising from the restriction of iron supply to the bone marrow. Decreases in the hematocrit and red cell indices also occur [14].

Iron deficiency anemia is most prevalent and severe in young children and women of reproductive age [15]. Iron deficiency impairs children's cognitive abilities, and interventions to prevent and correct iron deficiency may enhance children's learning potential in school [16]. In an intervention study of rural primary school children in Indonesia, supplementation with 10mg ferrous sulfate per kilogram body weight per day for 3 months resulted in an apparent improvement in anemic status and learning achievement score [16]. A study conducted by Pollit et al in school children in Thailand found that there is evidence of a positive association between iron status and IQ and the results of a language achievement test in school [17]. Improving the iron status of school children will also increase their fitness and work capacity, and improving iron status of girls during their school years may help to prevent anemia during their reproductive years.

Measurement of iron status

Serum ferritin

Serum ferritin (SF) is the only measure of iron status that can reflect a deficient, excess, or normal iron status [14]. A serum ferritin concentration of 1 μg/L is said to be equivalent to approximately 10 mg of storage iron [18]. Iron deficiency leads to lower iron stores: serum ferritin values fall progressively as the stores decline, before the characteristic changes in serum iron and total iron–binding capacity (TIBC). Low serum ferritin concentrations almost always have a fairly straightforward interpretation, as concentration <15μg/L are highly predictive for absent iron stores [19].

In acute and chronic infections, inflammatory diseases, certain neoplastic diseases, and liver disorders elevate serum ferritin because ferritin is an acute-phase protein. These disorders can lead an increased rate of ferritin synthesis in the reticulo-endothelial system, which is reflected by an elevated concentration of ferritin in the serum. Hence, serum ferritin is inappropriate as an indicator of iron status where infection and inflammation are widespread and coexist with iron deficiency because serum ferritin value may be within the normal range or even increased despite the presence of iron deficiency [20-22]. In such case, an alternative measure of iron status, preferably serum transferrin receptor, should be used [14].

Transferrin receptor

Transferrin receptor (TfR) is an iron-related protein that regulates the uptake of tranferrin iron into all body cells [23]. The surfaces of cells express TfR in proportion to their requirement for iron. A soluble form of TfR circulates in human serum; its concentration is proportional to the total body mass of cellular transferrin receptors [18]. The level of TfR circulating in the serum is a sensitive indicator of the degree of tissue iron deficiency. In mild iron deficiency, when iron availability to tissues is compromised because iron stores are depleted, TfR expression by cells increases, allowing the cells to compete more effectively for transferrin bound-iron. As a result, serum TfR level increase in proportion to the functional iron deficit [24]. One of the main advantages of using TfR is that, unlike serum ferritin, serum TfR concentration remains normal when chronic disease, inflammation or infection is present, distinguishing iron deficiency anemia from anemia of chronic disease [22, 25]. Consequently, serum TfR concentrations can be used to help differentiate individuals with anemia of chronic disease from those with iron deficiency anemia. For patients with coexisting iron deficiency and anemia of chronic disease, serum TfR levels are elevated and comparable to those for patients with only iron deficiency anemia [14]. An elevated serum transferrin receptor concentration (TfR) (>8.5 mg/L) is an early and sensitive indicator of iron deficiency. It is, however, also raised in thalassemia and hemolytic anemias [26].

The diagnostic cut-offs for TfR used to identify iron deficiency were derived from studies in adults [27]. These cut-offs may not apply to children due to their increased erythropoeisis during growth [28].

2. Other nutrient deficiencies and anemia

Vitamin A status is considered as a major determinant of anemia [29-32]. Although the exact mechanisms are yet to be elucidated, evidence suggests that vitamin A can modulate iron metabolism [33]. The beneficial effect of vitamin A on hemoglobin or iron status has been shown in several supplementation studies providing vitamin A alone or in addition to iron [34-36]. Data from the Vietnamese National Nutrition Survey 2000 showed a modest prevalence of sub-clinical vitamin A deficiency (serum retinol <0.70 μmol/L) in children under five for Vietnam's Red River Delta region of 11% and for the national level of 12.4% [37]. There is no data available regarding vitamin A deficiency in school children in Vietnam, and no research has been conducted on the relationship between anemia and vitamin A deficiency in this age group. Other nutrient deficiencies associated with anemia include deficiencies of vitamins B-6, B-12, riboflavin, and folic acid [38], although not all of the causal pathways are clearly understood.

3. Infection and anemia

Parasite infection and anemia

Intestinal parasites remain a major health problem in many developing countries. Some of the most common human intestinal parasitic infections are hookworm, *Trichuris*, and *Ascaris* infections.

In children, the prevalence of hookworm infection increases with age, typically reaching a plateau in late adolescence, whereas the intensity of infection may continue to increase throughout adulthood. Hookworms cause chronic intestinal blood loss by attaching to the mucosa of the upper small intestine and ingesting tissue and blood. Blood loss occurs both from ingestion by the worms and through bleeding from damaged mucosa [39]. The relationship between hookworm infection intensity and hemoglobin concentration is evident in epidemiologic studies. Results from Stoltzfus et al in school children in Zanzibar [40] and Stephenson et al in coastal Kenya [41] strongly confirm the association between hookworm infection and anemia.

Infection with *Trichuris* (whipworm) usually has no symptoms. Heavy infections may cause intermittent stomach pain, bloody stools, diarrhoea, and weight loss. Although the incidence of whipworm infection is high, its intensity is usually light. In endemic areas, *Trichuris* usually is most prevalent among children between 5-14 years of age. Infection is associated with anemia due to the damage of the intestinal wall and possibly to the sucking of blood by the worm. Blood loss may occur in *Trichuris* infection but probably becomes significant only in heavy infections [42].

Ascaris infection is most prevalent in children. Peak intensity is found in the age range from 5 to 15 years, with worm burdens declining in adults [43]. *Ascaris* is associated with a small deficit in hemoglobin in some populations, for reason that are not well understood [39]. Stoltzfus et al observed in Zanzibari school children that *Ascaris* infection was associated with lower hemoglobin concentration, but not with erythropoiesis protoporphyrin (EP) and serum ferritin [40]. However, Islek et al's study found that *Ascaris* infection does not lead to iron malabsorption and iron deficiency anemia in children [44].

The association between hookworm infection and anemia in children has been reported in some studies [45-48]. Although the prevalence of parasite infection in school children in Vietnam is high, as indicated in previous studies in primary school children in suburban Hanoi and Namha provinces at 88.4% and 92.9% respectively [8, 49], the prevalence of hookworm infection is low. Whether or not there is still a relationship between intestinal parasite infections with anemia in school children in Vietnam is still to be determined.

Malaria has been estimated to cause 300-500 million symptomatic attacks and over 750,000-1 million deaths per year globally [50]. Most community-based studies have shown a malaria-associated reduction in hemoglobin concentration of 10-20g/l [51]. However, malaria does not exist in our study area.

Chronic infection/inflammation and anemia

Anemia of chronic disease, also known as anemia of inflammation, is caused by chronic or acute immune activation and is the second most common cause of anemia after iron deficiency [52, 53]. Anemia of chronic infection is commonly associated with such diseases as rheumatoid arthritis, systemic lupus erythematosus (and other autoimmune collagen disorders), Hodgkin's disease, colitis, and regional enteritis [54]. However, it can also occur as a result of seemingly common and as such less serious infections as urinary tract infections, a head or chest cold, tonsillitis or strep throat, stomach or intestinal flu, as well as bacterial infections [54]. This type of anemia reflects the body's normal physiological response to infection and does not respond to iron, folate, or vitamin B12 supplementation or to any other dietary or medical treatment. Without any intervention, the liver will eventually return iron to the blood as the infection resolves [54].

A study among school children in Alaska found that 15% of children had a hemoglobin level below 115g/L, but only 8% had a low ferritin. Chronic infection was considered as one of the causes of anemia in some children in this population [55]. There is no data available regarding chronic infection/inflammation in school children in Vietnam, and no research has yet been conducted among this population group.

4. HEMOGLOBINOPATHIES

Thalassemia and abnormal hemoglobins are common genetic disorders in Asia. Thalassemia is not only an important public health problem but also a socio-economic problem of many countries in the region [56]. A study in school children in northeastern Thailand found a high prevalence of anemia with hemoglobinopathies [57]. The carrier rate for beta-thalassemia in Vietnam varies from 1.5% to 25% depending on the ethnicity of the study population [58]. A study among Kinh children under 15 at Lien Ha commune, Dong Anh district, Hanoi, shows a prevalence of 1.5% for beta thalassemia and 1.3% for Hemoglobin E (HbE) [59]. Another study among Thai children under 15 in mountainous areas of northern Vietnam, where prevalence of anemia is 50%, shows that 50 out of 89 (56.2%) anemic children having hemoglobin disorders like thalassemia or HbE [60].

III. STRATEGIES TO REDUCE ANEMIA

1. PARASITE CONTROL

Worms are a significant cause of anemia, and worm control programs contribute to anemia reduction [61]. To control worm infection in the community, it is necessary to improve housing, sanitation and clean water.

School-aged children, a risk group for anemia, are also most at risk for worm infections. In other words, school-aged children have the most intense infections. De-worming programs administered through schools are sustainable and cost effective. The cost of a single dose of Albendazole is less than US$0.05 [61]. Mebendazole is less expensive and less efficacious against all helminthes. De-worming is effective and safe in reducing parasite loads and anemia in school children [39, 41, 49, 62]. The drugs are the most expensive component of a successful de-worming program. Delivery of de-worming drugs must be accompanied by health education and sanitation to be fully successful.

Based on epidemiological and experimental data, a mathematical model has been proposed to explain the response of human iron metabolism to hookworm infection [63]. Depending on the regulation of stored iron, this model predicts a 3-5 fold improvement of red blood cell density when heavily infested adults are de-wormed. In practical situations, the beneficial effects of de-worming interventions has been documented [40]. It can be concluded that de-worming treatments are effective and can lead to 50-90% reduction of hookworm prevalence. Although the prevalence of parasite infection is very high in school children in Vietnam, a regular de-worming program still does not exist.

2. SUPPLEMENTATION

Iron supplementation has been a key strategy for the short-term control of iron deficiency anemia for pregnant women and increasingly, for preschool-age children as well. WHO documents from 1989 and 1996 state that for adolescents and adults, the recommended dose is 60mg element iron/day in case of mild anemia or when anemia prevalence is less than 20% and double that dose when anemia is moderate/severe or prevalence is greater than 20%. In the case of preschool-age and school-age children, the dose should be 2 mg elemental iron/kg body weight/day or 30-60 mg element iron/day [64].

Iron supplementation has been used almost exclusively in antenatal clinics. In spite of its confirmed efficacy in supervised trials [65, 66], it has proven ineffective in practice in most developing countries [64]. Adherence is known to be poor, and the continuing high prevalence of anemia reveals that the programs are not working very well. These programs encounter four major problems: (1) inadequate supplies of supplements, (2) inadequate explanation and encouragement to participants to take the tablets regularly, (3) poor coverage of population at risk, especially in rural areas, and (4) in adequate adherence, usually due to side effects (including nausea and constipation) that can result from therapeutic doses of oral iron and that may occur in more than half of subjects [67].

In Vietnam, iron supplementation has been implemented since 1995, particularly for pregnant women from the beginning of pregnancy through one month post-partum, with one tablet daily (60 mg iron and 0.25 mg folate) [37]. While iron supplementation in itself is highly effective in reducing iron deficiency anemia, the implementation has been characterized by low coverage (15-20%) and non-compliance [37]. No supplementation program or research has been conducted among primary school children in Vietnam.

3. FOOD BASED STRATEGIES

Food based interventions encompass a wide variety of interventions that aim to increase the production and availability of and access to foods high in iron and other nutrients, increase the consumption of foods rich in iron and other nutrients, and increase the bio-availability of iron in the diet [68].

Dietary diversification

In many developing countries, rural diets are based predominantly on cereals and legumes or starchy roots and tubers: consumption of flesh foods such as meat, poultry, and small whole fish bones, which are readily available sources of iron, zinc, and preformed vitamin A, is often small because of economic, cultural and religious constraints [69]. In general, diets based on starchy roots and tubers have lower micronutrient content than those based on unrefined cereals and legumes. However, the later often contain high levels of phytic acid and polyphenols which inhibit zinc and/or non heme iron absorption by forming insoluble complexes in the intestine. Consequently, the bioavailability of micronutrients in diets based on cereals and legumes is often poor [70].

Food diversification requires a combination of activities. First is identification of food items with high iron content bioavailability and promotion of consumption. When the supply of these foods is low, interventions may aim to increase their availability by promoting cultivation of specific crops or keeping livestock. Promotion of income-generating activities is considered important as it improves purchasing power and allows people to buy specific nutritious food items in the market.

Food fortification

The fortification of food is often regarded as the most cost-effective long-term approach to reducing the prevalence of iron deficiency [71]. Iron fortification of staple foods and condiments has the potential to be a major intervention to deliver iron in an absorbable form to large populations on a permanent and self-sustaining basis. For over 50 years, many developing countries have been using fortified milled cereals. However, it is only over the past decade that the concept has been applied on a large scale in other parts of the world (such as in the Middle East and in parts of Africa and Asia), where iron deficiency anemia is a serious problem and where milled cereals form a major component of the diet [72]. Although iron has a potential for use in more food vehicles than iodine or vitamin A, fortification with iron is technically more difficult because iron reacts with several food ingredients.

The biggest challenge with iron is to identify a form that is adequately absorbed and yet does not alter the appearance or taste of the food vehicle. The use of NaFeEDTA as a food additive has recently been reviewed by the International Nutritional Anemia Consultative Group (INACG) and was strongly recommended as the most suitable iron fortificant for use in developing countries [73]. The major advantage of NaFeEDTA over other iron fortification compounds is that it prevents iron from binding with the phytic acid present in many cereal and legume grains. Thus, in cereal foods or meal containing a considerable quantity of phytic acid, the absorption of iron from NaFeEDTA is two-to threefold that from ferrous sulfate [74]. Contrary to ascorbic acid, EDTA stays stable when it is exposed to heat or humidity [75]. NaFeEDTA has been demonstrated several times to be efficacious for food fortification in improving the iron status of target populations consuming NaFeEDTA fortified fish sauce [76, 77], curry powder [78] infant formula [79], infant cereal [80, 81] and sugar [82]. Its disadvantages are higher cost (6 times as expensive as ferrous sulfate) and the tendency to cause unwanted color and flavor reactions [83, 84].

The selection of the iron compound, however, is only part of the problem. The other major difficulty is the selection of the vehicle. Iron fortification of fish sauce [76, 77] masala/curry powder [85] and soy sauce [86] have shown effectiveness on iron and anemia status. Cereal flours such as wheat and maize are currently the most common vehicles for iron fortification to reach the general population [87]. The presence of iron absorption inhibitors in the fortification vehicle itself or in the accompanying diet should be taken into account. The main inhibitory compound is phytic acid (myo-inositol 6-phosphate) [78], which is widely present in cereal grains and legume seeds [82]. As said above, the absorption of iron in the form of NaFeEDTA is less affected by the presence of phytic acid in the diet.

The effectiveness of iron fortification has been reported in some studies [76, 77, 86, 88]. However, how large it is compared to the effectiveness of the iron supplementation has never been indicated in a trial intervention study.

IV. THE STUDY SITE IN VIETNAM

This study was conducted in Tam Nong district in Phu Tho province, a rural agricultural area located 90 km northwest of Hanoi, Vietnam. Encompassing an area of 15,551 ha, Tam Nong includes 20 communes with a total population of 79,552 (in 2002). The average estimated per capita income at the end of 2002 is 3,100,000 Vietnamese Dong (VND). About 12.8% of the local population is classified as poor, defined as an average income under 100,000 VND, which is about $US 7 per capita per month. Located in North Vietnam, Tam Nong district has two main seasons: the rainy season from May to September (hot, heavy rain) and the dry season from October to April (cold, little rainfall). This area was selected because it is a poor area not very far from Hanoi, and there is no anemia intervention program for school children.

V. AIM AND OUTLINE OF THE THESIS

RATIONALE OF THIS THESIS

It is generally accepted that iron deficiency is a major cause of anemia in developing countries. Data from the Vietnamese National Nutrition Survey shows that the mean iron intake of Vietnamese people, which is mainly non-heme iron, only reaches 72% of the RDA [89]. There is no national representative data available on the prevalence of anemia and iron deficiency in primary school children, but anemia and intestinal parasite infection show a high prevalence in some local studies [8, 9]. Some supplementation programs have been carried out in Vietnam to reduce the prevalence of anemia in women of reproductive age and children under five. However, supplementation cannot be considered as a long-term solution for iron deficiency. Although it is generally accepted that increase in consumption of animal products would increase iron intake in the long term, the consumption of animal products in Vietnam is sincerely hampered by low socio-economic status, especially of the rural population. Therefore, food fortification with iron may be considered as a feasible long-term strategy for the prevention of iron deficiency in rural Vietnam. However, in a rural population with such a high prevalence of worm infection without a regular de-worming program, complications of worm infection should also be taken into account in a food fortification program. This thesis focuses on the relationship between parasite infection and iron deficiency anemia. Furthermore, the efficacy of de-worming, food fortification and supplementation on anemia and iron status was also investigated.

AIMS AND OUTLINE

The aim of this thesis is to determine the efficacy of a school-based food fortification program to improve hemoglobin concentrations and iron stores of intestinal parasites-prone school children. Furthermore this thesis also compares the effect of iron fortification and iron supplementation on the changes in hemoglobin and iron status in order to assist public health nutritionists in making an informed choice for an appropriate strategy to combat iron deficiency and anemia among school children in rural Vietnam.

The specific objectives are as follows:

1. To measure the efficacy of iron-fortified food to iron and anemia status of rural school children
2. To clarify to what extent intestinal parasite infection modifies the effect of iron-enriched food
3. To compare the effects of consumption of iron-fortified food with the 'golden standard' treatment (iron supplementation + de-worming) on iron and anemia status

In order to achieve the specific objectives, the following sub-objectives were added:

a. To measure the prevalence of iron deficiency anemia and intestinal parasite infection and their relationship in Vietnamese rural school children
b. To assess the dietary pattern as well as energy and nutrient intake of school children mainly focused on non-heme and heme iron intake
c. To assess the short and long term acceptability of consumption of iron-fortified food by rural school children and their parents

Our study was divided into three phases **(Figure 2)** and was conducted in Tam Nong district of Phu Tho province from October 2003 to September 2005. The first phase consisted of a cross-sectional study to pursue sub-objectives a and b. Its results are described in chapter 2 of this thesis. The second phase included an acceptability study to fulfill objective c, and its results are presented in chapter 3 and 4. The last phase comprised a randomized control trial undertaken to achieve objectives 1, 2 and 3; its results are described in chapter 5 and 6 of the thesis. Finally, the main findings and conclusions of the above studies are discussed in chapter 7, together with recommendations for health policies and further research.

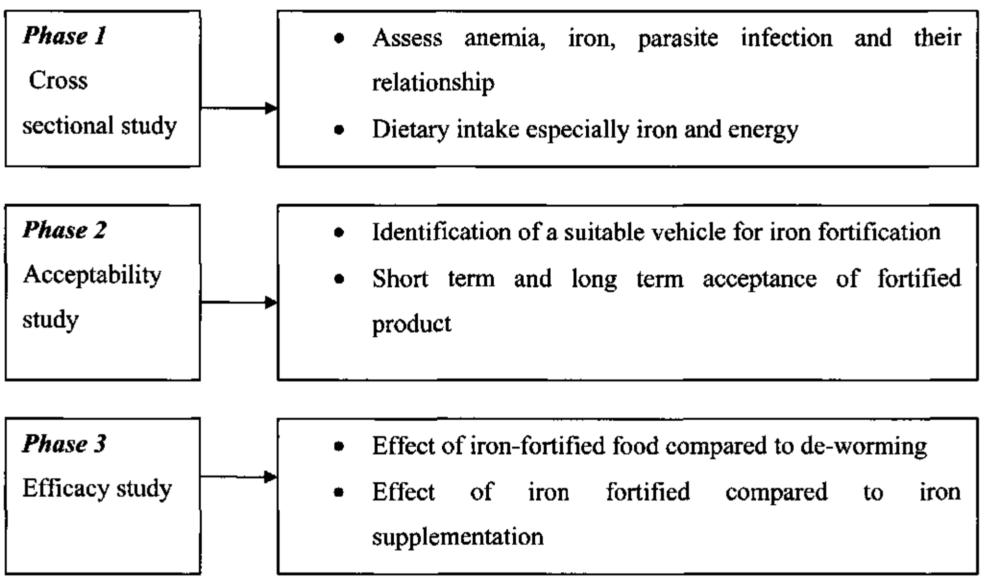

***Figure 2*: Outline of study components**

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Chapter 2: Anemia and intestinal parasite infection in school children in rural Vietnam

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ABSTRACT

The present study hypothesized that besides iron deficiency, intestinal parasites infection is also a determinant of anemia in school children in rural Vietnam. 400 primary school children from 20 primary schools in Tam Nong district, a poor rural area in Vietnam, were randomly selected from enrolment lists. Venous blood (5ml) was collected in a cross sectional study and analyzed for hemoglobin, serum ferritin (SF) and serum transferrin receptor (TfR), serum C-reactive protein (CRP) and total immunoglobulin E (IgE). Stools samples were examined for Hookworm, *Trichuris*, and *Ascaris* infection. Logistic regression was used to assess the effect of intestinal parasite on anemia. The prevalence of anemia (Hb<115g/l) was 25%. Iron deficiency (TfR >8.5mg/L) occurred in 2% of the children. The prevalence of intestinal parasites was 92% with the highest prevalence for *Trichuris* (76%) and Ascaris (71%). More than 30% and 80% of the children showed an elevated CRP (≥ 8 mg/L) and IgE (> 90 IU/ml) concentration respectively. Anemia status was borderline significantly associated with SF and not associated with TfR and CRP. The prevalence odds ratio of *Trichuris* infection for anemia was 2.02 (95% CI: 1.11- 3.68). It is concluded that anemia is highly prevalent among school children in Vietnam but not associated with iron deficiency. *Trichuris* infection is associated with a doubled risk of anemia, not mediated through iron deficiency. Chronic infection may play a role in anemia, but needs further investigation.

INTRODUCTION

Anemia is a significant public health problem in Vietnam. The 2000 Nutrition Risk Factor Survey showed that the anemia prevalence was 34% in children under five years of age and 25% in women [1]. No national representative data are available on the prevalence of anemia among primary school children in Vietnam, however, a few local studies showed an anemia prevalence of around 30% [2, 3]. Anemia in children can be caused by iron deficiency and by health factors such as parasite infection [4] or rare causes of anemia, being genetic disorders such as the hemoglobinopathies and thalassemias [5, 6]. School children carry the heaviest burden of intestinal parasitic infection [7]. Studies in primary school children in sub-urban Hanoi and Nam Ha province showed high prevalences of intestinal parasites of 88.4% and 92.9%, respectively [3, 8]. Many studies have shown that Hookworm causes chronic intestinal blood loss; [9] blood loss can also occur in *Trichuris* infection [10, 11] but probably becomes significant only in severe infection [12]. Iron deficiency is the predominant cause of anemia in all age groups [13]. However, the possibility that infection could also play an important role has received increasing attention during the last few years [13]. We hypothesize that both iron deficiency and intestinal parasite infection are associated with anemia in school children in rural area in Vietnam. Using data from a survey among school children, we assessed the iron status and parasitic infection and tested whether our data are consistent with this hypothesis.

METHODS

STUDY AREA AND POPULATION

This study was conducted in October 2003 in Tam Nong district, Phu Tho province, a rural agricultural based area located 90 km northwest of Hanoi, Vietnam. Tam Nong includes 20 communes with a total population of 79.552. The school lists of all children in grade one to three in all 20 primary schools in Tam Nong district with complete information on date of birth and sex were collected. 400 primary school children were selected from the list using systematic sampling. Children were invited for the study and their parents were asked for an informed consent. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University - Ministry of Health.

DATA COLLECTION

Interviews with parents were performed to obtain information about background characteristics of children as well as the children's past and present illnesses. Body weight and height were measured using standardized procedures [14] and recorded as the midpoint of duplicate measurements. Children's weight was measured to the nearest 100 gram using an electronic scale (Seca, 890, UK) and standing height was measured to the nearest 1 mm with a wooden stadiometer (CMS equipment Ltd, UK). The subjects wore minimum clothing and no shoes. Age was calculated from the birth date in the school records based on birth certificates.

Blood samples (5 ml) were collected by venipuncture, 20µl whole blood was pipetted immediately before coagulating into a tube containing 5.0 ml of Drabkin's reagent with a Sali pipette for hemoglobin measurement, the remaining blood was allowed to clot for 30 minutes at room temperature, centrifuged at 3000 x g for 15 minutes and transferred to 5 plastic labelled vials (Eppendorf tubes 0.5 ml). The vials were stored at -30°C until serum ferritin (SF), transferrin receptor (TfR), C-reactive protein (CRP) and immunoglobulin E (IgE) analysis.

For assessment of intestinal parasites infection, containers for collection of stools were distributed to each class and children were asked to collect and deliver a sample of their faeces to school the next day. In case some children were unable to return a sample, one of the field workers returned the next day to collect the rest of the samples. 394 children returned a faecal sample.

Food consumption of the children was investigated by a 24-h recall [15] of two non-consecutive days among a subsample of 60 children in two schools (51 included in the cross sectional study complemented with 9 random selected from the school lists).

LABORATORY ANALYSIS

SF and TfR, CRP and IgE analysis was carried out at the National Institute of Nutrition and the laboratory of Hanoi Medical University. A sample of 43 and 41 blood samples were reanalyzed in SHO (Stichting Huisartsenlaboratorium Oost, Velp, The Netherlands) for TfR and SF for quality control. Hemoglobin concentration was measured in the whole blood within 12 h of sampling by cyanmethemoglobin method using Sigma KIT. The CV of intra-essays and inter-essays was 4.0 ± 1.2% and 5.0 ± 2.0% respectively. In Vietnam, SF and TfR were performed by an Enzyme - Linked Immuno Sorbent Assay (ELISA) method (Ramco

Laboratories, Inc, Houston, TE, Catalogue numbers S-22 and TF-94), with inter-assay variabilities of 4-7% and 4-8%, respectively. For the re-analysis of these indicators in the Netherlands, an ELISA (The Access, Beckman) was used for SF and the Behring N Latex OQ TC11 method was used for TfR and run on a Behring Nephelometer (BNII). Serum CRP was measured by nephelometry using Epress plus, with an inter-assay variability of 4-8 %. Serum IgE was determined by ELISA using the Kallestad Total IgE Microplate Kit from GmbH - Germany, with an inter-assay variability of 4-6 %. Stools samples were examined by using the Kato - Katz Technique – a cellophane faecal thick smear method [16]. Hookworm, *Trichuris trichura,* and *Ascaris lumbricoides* eggs were counted. A 10% sub sample of smears was re-examined for quality control.

DATA ANALYSIS

Anthropometric indices were calculated using the WHO/NCHS reference data [17]. Being wasted, stunted and underweight is defined by z-scores <–2SD for weight-for-height, height-for-age and weight-for-age, respectively. The iron intake was calculated using the VBS BAS Nutrition Software programme [18], using a modified Nutritive Composition Table of Vietnamese foods [19]. Absorbable iron is calculated assuming heme-iron bioavailability of 23% and non-heme iron bioavailability of 5% [20]. This was compared to the FAO/WHO recommended requirement of iron [21].

Anemia was defined as hemoglobin concentrations <115 g/L [22]. Iron deficiency was defined as SF <12 µg/L [22], and tissue iron deficiency was defined as TfR>8.5 mg/L [23] measured with the Ramco test and TfR >1.76 mg/dL measured with the Behring N Latex method (reference from the laboratory). Serum CRP concentration was used as an indicator of a possible infection and/or inflammatory diseases and considered to be abnormal when ≥ 8 mg/L [24]. Serum IgE concentrations, which are elevated in cases of high fever, allergy, and presence of parasitic infection were considered to be elevated if > 90 IU/mL in children 6-9 years of age [25]. Severity of intestinal worm infections was expressed as the number of eggs/g faeces using the WHO classification system [26].

STATISTICAL ANALYSES

Data were entered into the computer, cleaned and managed using Epi Info version 6 [27], and analyzed using SPSS 11.0 for windows (SPSS Inc., Chicago IL, USA). [28] Differences in hemoglobin concentrations and anemia were assessed by age (using four age classes 5, 6, 7, and 8 years), sex, elevated/normal CRP and elevated/normal IgE by using the independent sample t-test for continuous variables and the Chi-square test for proportions. To assess the association between worm infection and indicators of iron and anemia status, we compared children with and without different types of worms with respect to their hemoglobin, TfR and SF. To further explore the association between anemia, worm infection and iron deficiency, logistic regression was used to assess the effect of intestinal worms on anemia. Anemia was modelled as a function of type of worm infection (*Ascaris, Trichuris,* Hookworm), and iron deficiency as reflected by TfR (model 1) or SF (model 2). Sex, age, and serum CRP concentration were considered as potential confounding variables.

RESULTS

Descriptive statistics for the study population are provided in **Table 1.** The prevalence of anemia (Hb<115 g/L) was 25% and mean hemoglobin concentration was 119.6 ± 8.3 g/L. Iron deficiency defined as serum transferrin receptor >8.5 mg/L occurred in 2% of the children (as assessed using the RAMCO test). The prevalence of infection with intestinal parasites was 92% with the highest prevalence for *Trichuris* (76%) and *Ascaris* (71%) with most children (52%) being infected by both parasites. Most of *Ascaris* infection was moderate (57%), and 68% of *Trichuris* infection was light. Only 6 % of the children had hookworm infection. More than 30% and 80% of the children had elevated concentrations of CRP and IgE, respectively. The prevalence of malnutrition was very high with 15.0%, 25.5% and 41.5% of the children being wasted, stunted and underweight, respectively. The daily iron intake was 7.5 ± 4.0 mg which corresponds to 46% of the Recommended Nutrient Intake (16.2 mg/day) calculated based on age distribution of study sample [21, 29]. A major part of the iron intake is provided by rice (37%) and only 15% is from animal sources. Amount of absorbable iron was 0.58 mg, just below the FAO/WHO recommended daily iron requirement of 0.63 mg for this age group [21].

The youngest group showed the highest anemia prevalence (**Table 2**). Compared to girls, boys had a higher prevalence of anemia (28.8% compared to 21.1%) and a slightly lower hemoglobin concentration (1.4 g/L, 95% CI = -3.1 to 0.19). Although not significant, children with elevated IgE showed a slightly lower hemoglobin concentration and higher prevalence of anemia (**Table 2**).

Table 1: Characteristics of the study population

Variables	Population estimate (n=400)
Age in months[1]	87.8 ±10.5
Sex (Boys)%	50.3
Hemoglobin concentration[1], g/L	119.6 ±8.3
Serum transferrin receptor[1], mg/L	5.5± 1.27
Serum ferritin[2], μg/L	50.2 (33.9-73.2)
Prevalence of anemia (Hemoglobin concentration <115g/L), %	25
Prevalence of iron deficiency	
(Serum ferritin <12μg/L), %	0.5
(Serum transferin receptor >8.5 mg/L), %	2.0
Prevalence of iron deficiency (excluded CRP> 8 mg/L)	
(Serum ferritin <12μg/L), %	0.6
(Serum transferin receptor >8.5 mg/L), %	1.9
Prevalence of children with inflammation (Serum C-reactive protein ≥ 8 mg/L), %	30.5
Prevalence of IgE elevated (Serum IgE >90 IU/mL), %	80.3
Nutrition status	
WAZ[1]	-1.7 ± 0.7
HAZ[1]	-1.4 ± 0.9
WHZ[1]	-1.3 ± 0.7
Prevalence of malnutrition	
Wasting (WHZ<-2SD), % (95% CI)	15.0 (11.6-18.4)
Stunting (HAZ<-2SD), % (95% CI)	25.5 (21.3-29.7)
Underweight (WAZ<-2SD), % (95% CI)	41.5 (36.7-46.3)
Parasite infection (n= 394)[3]	92 (89-94)
Ascaris only %	15 (12-19)
Trichuris only%	19 (15-23)
Ascaris and Trichuris%	52 (47-57)
Ascaris, Trichuris and Hookworm, %	4 (2-6)
Trichuris + Hookworm, %	1 (1-2)
Hookworm only, %	1(1-2)
Daily iron intake mg (n= 60)[1,4]	7.5 ± 4.0
Contribution to iron intake, %	
Rice	37
Animal food	15

[1] *Mean ± SD*
[2] *Geometric mean (25 and 75 percentile)*
[3] *Data available for 394 children*
[4] *Data available for 60 children*

Table 2: Hemoglobin concentrations and anemia prevalence by age, sex, inflammation and iron deficiency status among primary school children in rural Vietnam

	Variable	Hb-concentration, g/L (mean ± sd)	Hb < 115g/L (%)
Age, yr			
	5 (n=26)	119.0 ± 6.9	38.0
	6 (n=125)	120.1 ± 8.7	24.4
	7 (n=126)	118.9 ± 7.5	24.6
	8 (n=123)	119.9 ± 8.9	23.6
Sex			
	Boys (n=201)	118.9 ± 8.4	28.8[1]
	Girls (n= 199)	120.3 ± 8.2	21.1
C-reactive protein			
	Elevated (≥ 8 mg/L)	119.2 ± 8.2	27.0
	Normal	119.7 ± 8.3	24.1
IgE			
	Elevated (>90 IU/mL)	119.3 ± 8.5	25.0
	Normal	120.9 ± 7.5	19.8

[1] $p=0.047$

Table 3: Serum transferrin receptor and serum ferritin by elevated and non-elevated of CRP and IgE

	Serum transferrin receptor	Serum ferritin
C-reactive protein		
Elevated (≥ 8 mg/L)	5.3 ± 1.3	55.6 ± 33.9[1]
Normal	5.5 ± 1.3	66.7 ± 38.2
IgE		
Elevated (>90 IU/mL)	5.5 ± 1.3	60.7 ± 36.9[2]
Normal	5.3 ± 1.1	52.1 ± 28.9

[1] $p= 0.004$
[2] $p= 0.05$

Children with elevated IgE had a higher concentration of SF compared to those with a non elevated IgE while children with elevated CRP had a lower SF than non elevated children. No differences were found in TfR between elevated and non elevated CRP and IgE groups (**Table 3**).

Table 4: Iron status and inflammation by type of parasite infection among school children in rural Vietnam

	Parasite		*Ascaris*		*Trichuris*		Hookworm	
	Infection (n= 361)	No infection (n= 33)	Infection (n= 281)	No infection (n= 113)	Infection (n= 298)	No infection (n= 96)	Infection (n= 23)	No infection (n= 371)
Hemoglobin concentration, g/L[5]	119.5±8.3	120.0±8.4	119.5±8.4	119.8±8.1	119.1±8.5	121.0±7.6[1]	118.4±7.1	119.6±8.4
Anemia (hemoglobin <115 g/L), %	25.5	24.2	24.9	26.5	28.2	16.7[2]	30.4	25.1
Serum transferrin receptor, mg/L[5]	5.5±1.3	5.5±1.1	5.5±1.4	5.4±1.1	5.5±1.3	5.5±1.3	5.1±1.2	5.5±1.3
Serum ferritin, mg/L[6]	58.2 (33.3-72.2)	67.1 (42.7-93.5)	57.2 (33.0-72.2)	63.3 (38.0- 77.3)	58.9 (34.4-73.0)	58.9 (32.3-79.9)	50.1 (30.3-72.2)	59.5 (34.4-75.6)
CRP elevated, % (>8 mg/L)	30.2	30.3	29.2	32.7	29.5	32.3	34.8	29.9
IgE elevated, % (>90 IU/mL)	81.4	63.6[3]	81.9	75.2	82.6	71.9[4]	87	79.5

[1]*p = 0.037(Difference between infected and not infected, independent sample t-test)*
[2]*p= 0.015 (Difference between infected and not infected, Chi-square test)*
[3]*p= 0.017 (Difference between infected and not infected, Chi-square test)*
[4]*p= 0.019 (Difference between infected and not infected, Chi-square test)*
[5]*Mean* ±SD
[6]*Geometric mean (25th,75th percentile)*

Children infected with Hookworm and *Trichuris* showed a lower hemoglobin concentration and a higher prevalence of anemia **(Table 4),** although not statistically significant for Hookworm **(Table 4).** This was further supported in the logistic regression model where *Trichuris* infection doubled the risk of anemia (OR = 2.02 with 95% CI = 1.11-3.68). **(Table 5).** *Ascaris* and hookworm were not significantly associated with hemoglobin concentrations or anemia prevalence. No association was found for TfR, SF, CRP concentration with parasite infection, but there was a relationship between *Trichuris* infection and IgE **(Table 4).** No association was found for TfR and SF concentrations with anemia after taking into account CRP concentration **(Table 5).**

Table 5: Prevalence Odds Ratio for anemia of parasite infection and indicators of iron status of Vietnamese school children

Logistic regression Outcome variable: Anemia status			Logistic regression Outcome variable: Anemia status[1]		
Varriable	Prevalence Odds Ratio[2]	p	Variable	Prevalence Odds Ratio[2]	p
Trichuris	2.02 (1.11-3.68)	0.02	Trichuris	2.05 (1.12 – 3.74)	0.02
Hookworm	1.26 (0.49-3.21)	0.62	Hookworm	1.17 (0.46 – 2.99)	0.74
Ascaris	0.84 (0.51-1.40)	0.51	Ascaris	0.83 (0.50 – 1.39)	0.48
Transferrin receptor	1.11 (0.93-1.33)	0.26	Ferritin	0.99 (0.99 – 1.00)	0.05
Sex	0.65 (0.41-1.04)	0.07	Sex	0.68 (0.42 – 1.09)	0.11
Age	0.91 (0.70-1.19)	0.50	Age	0.94 (0.72 - 1.23)	0.66

[1] *Adjusted for CRP level*
[2] *Prevalence of Odds Ratio (95% CI)*

DISCUSSION

In the present study 25% of children were found to be anemic (Hb<115g/L). The low percentages of children having SF below 12 μg/L (0.5%) and TfR above 8.5 mg/L (2%), indicate that there is very low iron deficiency anemia among our study population. This was surprising to us as to our knowledge this is the first study in Vietnam showing a high anemia prevalence of 25% with very low iron deficiency. Moreover, our food consumption data indicate that iron intake of the children was marginal and a previous study in the area showed improvement of anemia status of pregnant women and young children (from 25.4% to 12.5% and 68% to 31.7%) after iron supplementation [30]. We were concerned about our blood measurements but reanalysis of subsamples at SHO (Stichting Huisartsenlaboratorium Oost, Velp, the Netherlands) revealed similar results.

SF is shown to increase during infection, giving false negative results for iron deficiency in infection prone populations [31]. For this reason, it has been suggested to use a higher cut–off value for SF to determine iron deficiency in populations where infections and /or inflammatory diseases are highly prevalent [32, 33]. In our study, 30.5% of children showed elevated CRP indicating presence of infection which may lead to underestimation of iron deficiency using SF. However, excluding children with elevated CRP in our study did not reduce SF. Data from our study show a borderline significant association between anemia and SF after adjusting for CRP (**Table 5**), indicating that there is a small contribution of iron deficiency to anemia.

Serum TfR is considered to be a more reliable measure of iron status than SF in settings with a high prevalence of acute infections [34]. However, few large published clinical trials among adults in developing countries [35, 36] are available and even fewer studies with children. The discriminating power of TfR in presence of infection has been questioned [34, 37]. Elevated TfR differentiates iron deficiency anemia from anemia of chronic infection, but normal TfR in the presence of chronic infection can not exclude iron deficiency [34, 38].

The presence of anemia with a low prevalence of iron deficiency suggests that important causes of anemia beyond iron deficiency exist. A study in school children in North-East Thailand also found a high prevalence of anemia without iron deficiency, with hemoglobinopathies, suboptimal vitamin A status and age as the major predictors of hemoglobin concentration [39]. Another study in school-aged children in Alaska showed 15% anemia with 8% being iron deficient, with bacterial infection (helicobacter pylori) as a possible contributor to anemia [40]. Data from a sub-study among schoolchildren in the same agegroup in the same study area indicated that probably both thalassemia and vitamin A were not causes of anemia in our population as thalassemia was only present in 7 % and vitamin A deficiency in 8% in the population of this sub-study [41]. Other nutrient deficiencies associated with anemia include deficiencies of vitamins B-6, B-12, riboflavin, and folic acid [42] although not all of the causal pathways are yet clearly understood. Vitamin B12 and folic acid deficiency are associated with an increased TfR [14], but we did not observe elevated TfR levels in our study population. The role of other nutrients could not be verified in the present study. Malaria, another main cause of anemia [43, 44], does not exist in our study area.

In our study the presence of *Trichuris* infection was associated with a doubled risk of having anemia, and although our study reconfirms the role of *Trichuris* infection with anemia reported in other studies [10, 11], we surprisingly found that *Trichuris* is related to anemia not only in severe infection but also in mild infection. Although literature indicates there is indeed a strong association of hookworm with increased prevalence of anemia and reduced iron stores [9, 45, 46], our study did not find this association probably due to low prevalence of hookworm and low worm burden in the study population. *Ascaris* infection showed no association with anemia, which is in agreement with other previous studies [47, 48].

The mechanism of the association between *Trichuris* and anemia in this study is not clear. *Trichuris* is suggested to be associated with anemia mediated through iron deficiency caused by blood loss or anorexia [11]. However, in our study we also did not see an association between *Trichuris* infection and iron deficiency. This may indicate that the association between *Trichuris* infection and anemia found in this study might be due to the presence of another un-identified factor associated with both *Trichuris* and anemia. We did not see an association between *Trichuris* infection and (acute) infection as measured by CRP concentration, but there was a positive association with IgE concentration. Faulkner et al suggested that the high IgE concentration may indicate immunity to *Trichuris* infection which may explain the absence of an association with CRP [49].

In absence of an association between CRP and SF, the high SF levels in our population may indicate chronic infection as CRP is a good measure of acute infection or inflammation but less appropriate when conditions are chronic [50]. Also SF in our population was much higher compared to the 50^{th} percentile SF value (28.7 μg/L) for the age group 6-9 years in NHANES III [14], being close to the 90^{th} percentile level of 55.9 μg/L. In our study elevated IgE was associated with higher SF concentration. The high levels of SF and IgE and their association with anemia in our study may suggest that chronic infection may play a role in anemia in our population, however, it needs to be further investigated.

In conclusion, anemia is highly prevalent among school children in Vietnam but not associated with iron deficiency. *Trichuris* infection is associated with a doubled risk of anemia, but the mechanism is not clear and needs further investigation. High levels of SF and IgE may suggest that chronic respiratory infection may play a role in anemia in our children. Further research needs to be carried out to confirm this.

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Chapter 3: Instant noodles, a suitable vehicle for iron fortification to combat iron deficiency anemia among primary school children in rural Vietnam?

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ABSTRACT

The aim of the present study was to identify an appropriate vehicle for iron fortification to be used in a school feeding programme aimed at improving the iron and anemia status of school children in rural Vietnam. Children 6- 8 years old and their parents in two primary schools in Tam Nong district were included in the study. The study consisted of 3 sub studies: (1) a food consumption study comprising 24-h recalls of two non – consecutive days; (2) a food believes study, with focus group discussions (FGD), a pile sort (PS) and a food attribute and difference exercise (FAD), and a food acceptance study using noodles and biscuits fortified with NaFeEDTA. The average daily meal consumption was 3.2 ± 0.4 with an average energy and iron intake of 5.1 ± 1.7 MJ and 7.5 ± 4.0 mg respectively. Compared to biscuits and instant rice soup, instant noodles were more frequently consumed, with larger portion sizes and are more acceptable as child food in the culture of the local people. The iron-level of the fortified product did not affect the mean consumption of noodles but a higher level of iron was associated with a lower mean consumption of biscuits ($P<0.05$). The production process did not affect the NaFeEDTA level in noodles; however during preparation at least 70% is leaked to the soup. It is concluded that Instant noodle is a suitable vehicle for iron fortification for use in school based intervention to improve iron deficiency anemia among primary school children in rural Vietnam.

INTRODUCTION

Anemia is a significant public health problem in Vietnam. The 2000 Nutrition Risk Factor Survey showed a prevalence of anemia of 34% among children under five and of 25% among women [1]. No data are available from national representative surveys on the prevalence of anemia among primary school children in Vietnam, however, few local studies showed that the prevalence of anemia in primary school children was around 30% [2, 3]. To control iron deficiency, iron supplementation programs have been implemented in Vietnam since 1995, however with low coverage and compliance reducing health effect [1]. In the National Nutrition Strategy for the period 2001-2010 food fortification is considered as one of the most sustainable and long term strategies to control iron deficiency anemia in Vietnam [4]. The success of a food fortification program depends on the choice of the food vehicle. A suitable food vehicle should fulfill the following relevant criteria: it should be commonly consumed with serving sizes that are able to meet a significant part of daily dietary requirement of iron; no change in consumer acceptability (in taste, smell or colour) of the food vehicle after fortification and the preparation of the food vehicle should not alter the iron level [5]. The aim of the current study is to identify an appropriate vehicle for iron fortification to be used in a school based intervention to improve the iron and anemia status of primary school children in rural Vietnam. Three possible candidates were considered: instant noodles, instant rice soup and biscuits.

SUBJECTS AND METHODS

Study subjects and location

The research project took place in Tam Nong District in Phu Tho Province, an agricultural based rural area located 90 km in north-west direction from Hanoi. There are 20 primary schools with 8060 children from grade 1 to grade 5 in this district. For the present study, two communes, Co Tiet and Hien Quan, were selected having a high prevalence of intestinal worm infections (92% and 100 % respectively) and anemia (27% and 28% respectively) among school children aged 6-8 years [6]. Selected children and their parents were invited for the study and were asked for informed consent. The study comprised 3 sub studies: a food consumption study (n= 60), a food believes study (n=30) and a food acceptance study (n= 160). A study on the retention of the iron fortificant in instant noodles after production and preparation was carried out at the laboratories of the National Institute of Nutrition, Hanoi, the Wageningen University and Akzo Nobel Chemicals. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University - Ministry of Health.

Products

Fortified instant noodles and biscuits were produced at the Hanoi Food and Hai Ha companies in Vietnam, respectively. Noodles and biscuits were fortified with a water soluble, highly bioavailable iron compound (NaFeEDTA: Ferrazone® Akzo Nobel Chemicals Pte Ltd Arnhem) to two levels: 5.8 mg iron and 10.7 mg iron per 52 gram of noodles and 40 gram of biscuits (8 pieces of 5 gram each). The fortified level of 5.8 mg was calculated based on the WHO recommendation of a daily preventive dosage for children of 0.2 mg elemental iron per kg body weight when given as NaFeEDTA [7] and the level of 10.7 mg was calculated based on the JECFA 1974 recommendation of the acceptable daily intake of 2.5 mg EDTA/kg body weight and an average body weight of 29 kg [8]. Unfortified instant noodles contain 4 mg of iron per 100 gram of instant noodles.

Data collection techniques

The food consumption study comprised a 24-h recall [9] of two non – consecutive days combined with a structured interview, both administered by 3 well-trained interviewers from the Nutrition Department of Hanoi Medical University. The 24-h recall covered all foods and drinks consumed by the child during the previous day, from the early morning the day before the interview till the early morning of the day of the interview [9]. A common set of household measures of food were used to facilitate the estimation of portion sizes. Conversion factors from household measures to weight were determined afterwards. A structured interview with parents was carried out after the 24-h recall focused on the role of noodles, instant rice soup and biscuits in the diet of their children.

The food believes study comprised focus group discussions (FGD), a pile sort (PS) and a food attribute and difference exercise (FAD). The FGD were carried out with 2 groups of 8 parents each in the two communes after completion of the 24 hours recall to discuss attitudes and beliefs of the parents concerning instant noodles, instant rice soup or biscuits. Based on the results of the 24-h recall and of the FGD, the PS and FAD were focused on instant noodles and biscuits. The PS [10] was carried out with the use of 25 pictures of foods representing frequently consumed foods selected based on the results of the 24 h recall in the following food groups: staple food (4 products); animal food (7 products); oil/fat/nut products (4 products); fruits (3 products); vegetables (5 products) complemented with one picture of fortified biscuit and one picture of fortified noodles. Mothers were asked to sort the foods into piles of cards belonging together in whatever way she wants and to explain the reason for this grouping. The FAD [10] was used to identify in greater detail the attributes and qualities that parents in the community apply to food [10] using the same list of 25 foods.

For the food acceptance study, only children in grade one were selected assuming that the quantity consumed by this youngest group could also be consumed by the older school children. Children were randomly divided into two groups: the instant noodles group receiving one packet of instant noodles (52 grams) fortified with NaFeEDTA [11] to a level of 5.8 mg iron and of 10.7 mg iron on two consecutive days, and the biscuit group receiving 40 grams of biscuits fortified to the same two iron levels also on two consecutive days. The instant noodles were prepared by researchers at school and given to children at the morning or afternoon break time (either at 9:00 am or at 15:00 pm). At start, a known amount of instant noodles/biscuits were served in a standard bowl to each child. Researchers supervised the consumption of the noodles and biscuits by the children and weighed the left over. A factor 4 was used to convert the left over weight of soaked noodles to that of dry noodles*. Just before consumption and after consumption of the iron-fortified noodles/biscuits, the subjects were asked to rate on a 3 points Likert scale the desire-to-eat with a rate ranging from 1 (not want) to 3 (want); the pleasantness of taste, of smell, and colour with the rate ranging from 1 (dislike) to 3 (like). The subjects were asked whether they received breakfast/lunch at home before coming to school.

Samples of fortified instant noodles were analyzed in the laboratories both at the National Institute of Nutrition Hanoi and the Wageningen University to check retention of iron after the production of instant noodles. For the determination of retention of iron after preparation of the iron fortified noodles, 400 ml boiling water was added to the content of a

* The determination of the conversion factor was done with 30 packages of noodles. The instant dry noodle was weighed, then 300 ml boiling water was added and the noodles were soaked for 5, 10 and 15 minutes after which the total dish, the soaked noodle and the water left was weighed. The conversion factors after 5, 10 and 15 minutes soaking were calculated to be 2.7; 3.5 and 4 respectively. As most of children stopped eating after about 14 minutes, the conversion factor 4 was used.

package of instant noodles (about 52 grams) according to the prescription on the package. The soup was allowed to extract for 5, 10 and 15 minutes before filtration. The iron EDTA content of the filtrated was determined by capillary zone electrophoresis analysis (CZE) [12] at the laboratory of Akzo Nobel Chemicals.

Data analysis

The nutrient intake was calculated based on the 24-h recall using the VBS BAS Nutrition Software program [13], using a modified Nutritive Composition Table of Vietnamese foods [14]. The mean number of meals consumed per day, and the food and nutrient intake per day were calculated and compared with the recommended nutrient intake. The recommended intake of iron is based on a bio-availability of 5% [15]. The iron absorption-rate in the Vietnamese diet varies between 5-10% [16]. For rural areas, the lower level of 5% can be expected, because the diet in rural areas is more plant-based than the diet in urban areas and contains iron absorption inhibitors, like oxide acid in leafy vegetables [17]. Based on the structured interviews, the frequency of weekly consumption of noodles, instant rice soup and biscuits were calculated. Results from the semi-structured interviews, focus group discussions, pile sort, food attribute and differences exercise were coded according to the topics discussed. For all topics coded, the information from all the 4 methods were combined and analyzed.

Based on the results of the food acceptance study, mean amount of noodles/biscuits consumed in two consecutive days by the children (based on initial amount provided minus the left-over) were calculated as well as the percentage of children who could not finish the 52 gram of noodles or 40 gram of biscuits. Whether level of fortification and having breakfast/lunch before school affected the total amount consumed was assessed by comparing mean intake between groups of children with low and high level of iron in the food product and between groups of children with and without breakfast using the independent t-test [18] with significant levels set at $p<0.05$. Percentages of children finishing all or part of the noodles/biscuits and the frequencies of scores for desire to eat and pleasantness of taste, smell, colour were determined. Data analysis was performed using SPSS Statistical Package for Windows version 11 [18].

RESULTS

Children consume on average 3 meals a day, mainly breakfast, lunch and dinner, with only few children having an extra meal in between. The average daily energy and iron intake (5.1 ± 1.7 MJ and 7.5 ± 4.0 mg) were below the recommended nutrient intake for this age group (7,5 MJ and 16.2mg) [15]. A major part of the energy and iron intake was from rice (64% and 37% respectively), only 15% of the iron intake is from animal sources like meat, egg, fish and dairy products **(Table 1).**

Table 1: Meals consumption, energy and iron intake of 6-8 years old children in Tam Nong district, Vietnam

Characteristic	
Dietary intake (n = 60)	
Average meal consumption, mean ± sd	3.2 ± 0.4
Energy (MJ), mean ± sd	5.1 ± 1.7
Iron (mg), mean ± sd	7.5 ± 4.0
Meals consumed by children in two days (n= 120)	
Breakfast	93%
Lunch	100%
Dinner	100%
In between meal per day	27%

50% and 15% of the children respectively consumed instant noodles and biscuits at least one time per week. Only 3% consumed instant rice soup at least once a week. If children consumed instant noodles, biscuits or instant rice soup, they do this about 3 times, 2.5 times and 2 times per week respectively **(Table 2).**

Table 2: Weekly consumption of instant noodles, instant rice soup and biscuits by 6-8 years old school children in Tam Nong district, Vietnam (n = 60)

	Percentage of children consuming[1]	Mean number of portions per child per week
Instant noodles	50.0	2.9 (±1.9)
Instant rice soup	3.3	2.5 (±0.7)
Biscuits	15.0	2.1 (±0.9)

[1] *Children who consume instant noodles, instant rice soup or biscuits at least once a week.*

Results from the focus group discussions, and structured interviews with parents on the attributes of instant noodles, instant rice soup and biscuits showed that the attributes of instant noodles are mainly positive. Important positive attributes mentioned are "easy and quick to prepare", "alleviating the child's hunger" and "children like to eat them". Negative attributes mentioned were "expensive" and "lack of information about nutritional value".

Only negative attributes were mentioned about instant rice soup by parents; "children do not like to eat", "do not consider it nutritious" and "more suitable for younger children". Attributes towards biscuits are equally positive and negative: they are "available at several places", "children like to eat them" and they are considered to be "nutritious". On the other side they are "expensive". The results from the pile sort and food attribute study **(Table 3)** confirmed the positive attributes of the noodles (being "full stomach/satisfy hunger", "can replace rice", "rich of carbohydrates" and "can be used for breakfast") while biscuits were considered as "rich of sugar", "rich of fat", "sweet food" or "extra food" or "desert" and parents thought children like to eat biscuits.

Table 3: Attributes concerning the use of instant noodles or biscuits for school children in Vietnam

Atributes	Noodles		Biscuits	
	Pile sort (n=30)	Food attribute (n=30)	Pile sort (n=30)	Food attribute (n=30)
Expensive	0	0	0	2
Rarely to eat/not often to eat	0	1	0	3
Easy and quick to prepare	2	6	1	2
Children like to eat/delicious	0	1	0	6
For breakfast	0	6	0	0
Full stomach/satisfy hunger/ main food/can replace for rice	13	11	1	0
Extra food/dessert/not main food	0	0	11	6
Rich of nutrients	0	1	0	3
Rich of carbohydrate	27	10	9	1
Rich of milk	0	0	0	2
Rich of sugar/sweet	0	0	22	12
Rich of vitamin	0	2	0	0
Rich of fat	1	3	1	7

Results from biscuits and noodles consumption on two consecutive days shows that 8.8% of children refused to eat fortified biscuits while only one child (0.6%) did not want to eat noodles (data not show). Reasons for refusal were that they did not like to eat or were not hungry. Of those who did eat biscuits and noodles 80% finished the total offered amount of biscuits (40 gram) while 96.8% children finished the total amount of noodles offered (52 gram). Of those children who finished the total amount offered of biscuits, 78.4% indicated to want to eat more biscuits while only 22.7% wanted to eat more noodles.

The mean amount consumed was 37.2 gram ± 8.2 for biscuits and 52.2 gram ± 2.3 for noodles **(Table 4)**. The consumption of breakfast/lunch before going to school did not affect the mean noodle and biscuits consumption. The level of iron fortification also did not affect the mean consumption of noodles but did affect the consumption of biscuits. A higher level of iron fortification was associated with lower mean consumption of biscuits ($P<0.05$) **(Table 4)**.

Table 4: Mean consumption of biscuits and noodles by 6 - 8 years old school children in Tam Nong distric, Vietnam, on 2 consecutive days.

Indicators	Biscuits (gram) Mean ± SD (n= 145)	Noodles (gram) Mean ± SD (n= 159)
Total consumed	37.2 ± 8.2	52.2 ± 2.3
Level of fortification		
Low level	39.2 ± 5.3[1]	52.2 ± 2.3
High level	35.6 ± 9.7	52.3 ± 2.0
Breakfast/lunch before going to school		
Yes	37.9 ± 7.4	52.4 ± 1.7
No	35.0 ± 10.0	50.9 ± 4.2

[1]*p=0.007 for difference level of biscuits*

Among 145 children who consumed biscuits and 159 children who consumed noodles, high mean ratings were given for liking in general (2.8±0.5 and 2.9±0.3) and in terms of the taste (2.9±0.4 and 2.8±0.5), smell (2.9±0.4 and 2.9±0.3) and colour (2.7±0.7 and 2.8±0.4) of the iron fortified biscuits and noodles **(Table 5)**. However, the proportion of children disliking biscuits in general and in terms of taste, smell and colour was slightly higher than for noodles. There was no significant difference between noodles and biscuits in the pleasantness in general and in terms of taste, smell and colour.

Figure 1 shows that the production process of instant noodles including deep frying in oil under high temperatures (140-160^{0}C) does not affect the iron content of instant noodles.

Figure 2 shows that about 70% of the NaFeEDTA dissolves within 5 minutes into the soup independent of extraction time. No degradation products of NaFeEDTA were found indicating that NaFeEDTA is stable in the soup.

Table 5: Pleasantness of taste, smell and colour of NaFeEDTA fortified noodles and biscuits as experienced by 6-8 years old school children in Vietnam.

Indicators	Fortified biscuits			Fortified noodles		
	Low level[1] (n =68)	High level[2] (n =77)	Total (n= 145)	Low level[1] (n =80)	High level[2] (n =79)	Total (n =159)
Taste						
Like (%)	91.2	94.8	93.1	75	87.3	81.1
Not like not dislike (%)	1.5	3.9	2.8	20	12.7	16.4
Dislike (%)	7.4	1.3	4.1	5	0	2.5
Rating(Mean ±SD)	2.8±0.5	2.9±0.3	2.9±0.4	2.7±0.6	2.9±0.3	2.8±0.5
Smell						
Like (%)	94.1	92.2	93.1	87.5	91.1	89.3
Not like not dislike (%)	1.5	5.2	3.4	12.5	8.9	10.7
Dislike (%)	4.4	2.6	3.4	0	0	0
Rating (Mean ±SD)	2.9±0.4	2.9±0.4	2.9±0.4	2.9±0.3	2.9±0.3	2.9±0.3
Color						
Like (%)	79.4	90.9	85.5	81.3	88.6	84.9
Not like not dislike (%)	1.5	2.6	2.1	17.5	8.9	13.2
Dislike (%)	19.1	6.5	12.4	1.3	2.5	1.9
Rating (Mean ±SD)	2.7±0.7	2.8±0.5	2.7±0.7	2.8±0.4	2.9±0.4	2.8±0.4
In general						
Like (%)	86.8	94.8	91.0	85.0	87.3	86.2
Not like not dislike (%)	4.4	3.9	4.1	15.0	12.7	13.8
Dislike (%)	8.8	1.3	14.8	0.0	0.0	0.0
Rating (Mean ±SD)	2.8±0.6	2.9±0.3	2.8±0.5	2.9±0.4	2.9±0.3	2.9±0.3

[1]*Low level: 5.8 mg iron per 40 gram biscuits or 52 gram of noodles*
[2]*High Level: 10.7 mg Iron per 40 gram biscuits or 52 gram of noodles*

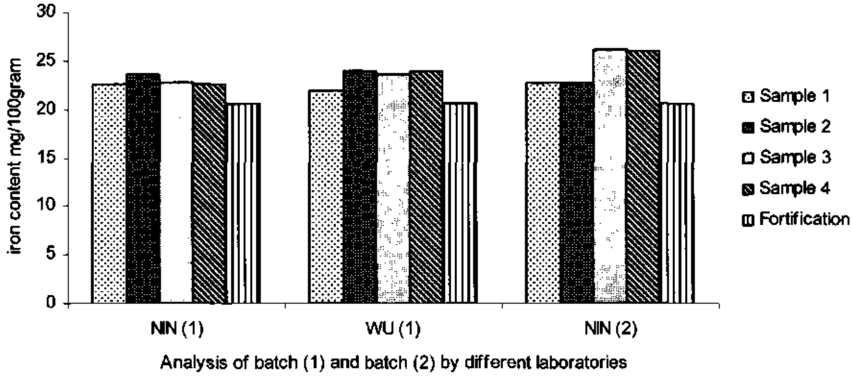

Figure 1. Retention of iron after production of noodles fortified with 20.6 mg elemental iron per 100 grams noodles in the form of FeNaEDTA

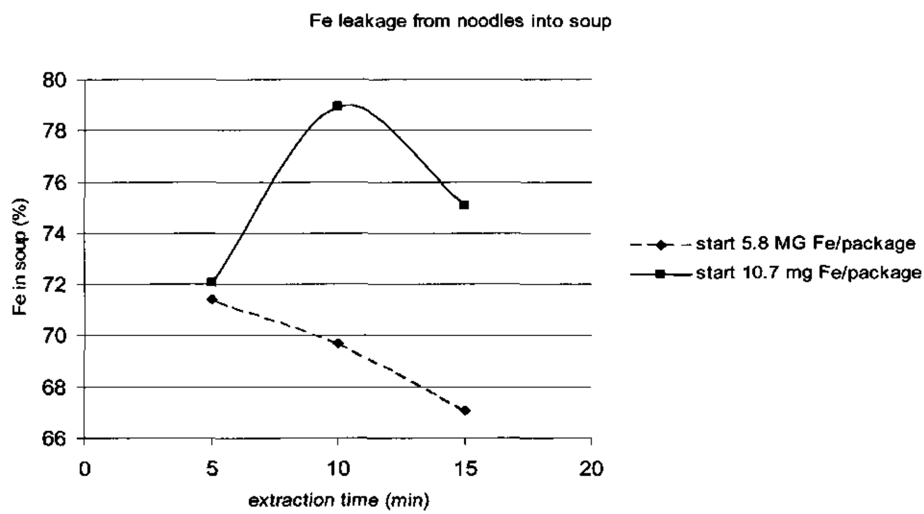

Figure 2. Iron leakage from instant noodles in soup after preparation

DISCUSSION

The present study was carried out in order to identify an appropriate vehicle for iron fortification to be used in a school based program to combat iron deficiency among school children in Vietnam. Three candidates were considered: instant noodles, instant rice soup and biscuit. There is little direct evidence that iron fortification of major staple foods, such as wheat flour or corn flour, is a useful strategy to combat iron deficiency [11, 19] Being cereal based, selection of one of the three considered food vehicles would allow to contribute to such evidence. Secondly, these products are easily to prepare and deliver in a school context.

In general, the diet of the school children in our study is characterised by a low meal frequency reflected in an energy and iron intake below the recommended intake. The energy intake (5.1 ± 1.7 MJ) only met 67% of the recommended intake of 7.5 MJ [20] and mainly comes from rice (64%) while iron intake only met 46% of the recommended iron intake of 16.2 mg [15] . This finding is in line with results from other studies. Vietnamese people usually eat 3 meals a day and during food shortage periods the number is reduced to 2 meals. Cereals are the main source of energy in the Vietnamese diet providing 78% of total energy [21] . A study among primary school children in a rural area in Ho Chi Minh city also found a low total daily iron intake (10.4 ± 3.6 mg) and heme iron intake only reached 0.75 ± 0.81mg. However, due to the large day-to-day variation of iron intake [9], the measurement of iron intake on two non consecutive days in our study may not be enough to reflect usual iron intake.

Children in our study only consume 3 meals a day. In view of the WHO recommendation that children from two years and older should have twice a day a nutritious snack in addition to three main meals [22], combined with the low energy and iron intake in our study population, an extra iron fortified snack offered at school is justified in order to improve energy and iron intake of school children in rural areas.

Key factors in the selection of the right food vehicle include that it should be consumed on a regular basis by a large proportion of the population [5]. Results of the study indicated that biscuits and instant rice soup are consumed by very few children (15% and 3% respectively) while half of the children eat instant noodles on a weekly basis. The low percentages of children consuming instant rice soup and biscuits is also reflected in the opinion of parents concerning these products: instant rice soup was considered as being not appropriate for this age group, while biscuits were considered as expensive and seen as an extra food or desert, but not as a main food. On the other hand, all respondents stated that instant noodles can alleviate hunger and can replace rice, probably explaining why more children consume instant noodles.

In selecting the food vehicle, it is also important that the food is consumed in sufficient amounts to allow for a sufficient level of fortification [5]. Our study indicates that more children (96.8%) were able to finish the portion of noodles compared to biscuits (80%). Noodles are accompanied by a soup containing water facilitating the swallowing. However, biscuits are sweet and dry making it difficult for children to consume a large portion. Concerning the noodles, only 22.7% of the children desired to eat more after finishing the total amount offered of 52 gram, meaning that the portion of 52 gram of noodle can be considered appropriate for this age group. This portion size is also the smallest portion size available for instant noodles in the markets in Vietnam.

Iron fortification can only be successful if the consumers accept the fortified product. The consumer acceptability of the food vehicle should not change after fortification [5, 11]. In general children in our study gave a very high score for liking the taste, smell and colour of the products for both instant noodles and biscuits. This indicates that the short term acceptance of the fortified products was very high. This may be caused by the culturally determined habits of the children. A study of Yeh et al (1998) indicated that compared to US consumers, Chinese, Korean, and Thai respondents avoided dislike categories of a nine-point labelled category scale [23]. The existence of hunger may also explain the high rating scores. Our children only consume 3 meals a day, no snack between meals with a mean energy intake only meeting 67% of the recommended energy intake. Cabanac suggested that a given stimulus can induce a pleasant or unpleasant sensation depending on the subject's internal state, and food intake was considered as one of the factors reflecting internal state [24]. In our study population with a low food intake, fortified instant noodles or biscuits may induce a pleasant sensation explaining the high ratings for liking.

The stability of iron fortification is also important for the success of the fortification program [5]. Data from our study showed that the production process of instant noodles including deep frying in oil under high temperatures (140-160^{0}C) did not affect the iron content of instant noodles. A previous study also mentioned that NaFeEDTA stays stable when it is exposed to heat or humidity [25].

In summary, among the three candidates considered, instant noodles seem the most suitable food vehicle for iron fortification. Also in the micronutrient control strategy in Vietnam [1], instant noodles is considered as a potential food vehicle for fortification. The 2000 National Nutrition Survey showed that on average Vietnamese people consumed 44.7gr/capita/day [1]. Moreover, another study showed that instant noodles consumption has risen steadily since 1995 in Vietnam and some others Asian countries [26] and at least three countries - Thailand, Indonesia, and the Philippines - have succeeded in fortifying instant noodles seasoning at a commercial level. Thailand and Indonesia are currently evaluating its use as a public health intervention and exploring ways to improve the formulation and effective implementation of a national fortification program using instant noodles [26].

In conclusion this study identified instant noodles as a suitable food for iron fortification which can be used in a school feeding programme aiming at combating iron deficiency and anemia in school children in rural Vietnam. Noodles are more frequently consumed, with larger proportion sizes and are more acceptable as child food in the culture of the local people. The production process did not affect the NaFeEDTA level in noodles; however during preparation at least 70% is leaked to the soup. Hence, consumers need to be advised to eat both the soup and the noodles for maximized benefit of iron fortification.

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Chapter 4: The effect of NaFeEDTA on sensory perception and long term acceptance of instant noodles by Vietnamese school children

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ABSTRACT

This study investigated the effect of NaFeEDTA fortification on taste and long term acceptance of noodles. A triangle test among 48 children (6-8 years) and 48 adults (17-30 years) showed that both children and adults could discriminate fortified from non-fortified noodles with and without seasoning added. A paired preference test by the same subjects with seasoned noodles showed no preference for fortified or non-fortified noodles. A long term acceptance study was carried out with 60 children randomly assigned to a monotony group and a free choice group, receiving five days a week, during 10 weeks fortified noodles with a fixed type of flavour or a flavour of own choice respectively. Results showed a slight but significant increase of boredom over time, but acceptance ratings remained high and boredom ratings remained low throughout time in both groups. It is concluded that NaFeEDTA fortified noodles are suitable for use in food fortification programmes.

INTRODUCTION

Anemia is a significant public health problem in school children in Vietnam. The 2000 Nutrition Risk Factor Survey showed that the prevalence of anemia was 34% in children under five and 25% in adult women [1]. No national representative data are available on the prevalence of anemia among primary school children (age 6-10 years) in Vietnam. However, two local studies in sub urban areas showed an anemia prevalence of around 30% [2, 3].

Iron fortification of food is considered to be the most cost effective [4], long term, and convenient approach to provide additional absorbable iron to populations [5]. Iron fortification can only be successful if consumers accept the fortified product. The effect of the added iron fortificant on the sensory quality of the food product should be minimal [6, 7]. Also, the fortified product should be accepted for a long period without feeling of boredom as there is ample evidence that respondents change their opinions about a food product after repeated exposure to the same food product over longer periods of time [8].

It has been shown that instant noodles are suitable as (iron) fortification vehicle [9]. However, it is unknown whether iron fortification has an effect on the sensory perception and it is also unknown whether or not iron fortified instant noodles are accepted on the long term. In order to find out whether NaFeEDTA fortified noodles is an appropriate product to be used in a program to combat iron deficiency anemia in Vietnam, the present study aimed to investigate the effect of iron fortification on the sensory perception and the long term acceptance of instant noodles by Vietnamese school children from rural areas.

METHODS

SUBJECTS

The study was carried out in Di Nau commune, Tam Nong district, Phu Tho province situated 90 km from Hanoi. Sixty primary school children from grade one to grade three, 6-8 years old (7.6 ± 0.6 years) and 48 adults ranging from 17 till 30 years (24.9 ± 0.7 years) participated in this study on a voluntary basis. The study comprised two sub studies: a short term sensory study (n = 48 children and 48 adults) focused on discrimination and short term preference, and a long term acceptance study (n= 60 children). The 48 children from the sensory study were a subgroup of the 60 children participating in the long term acceptance study. Parents from children invited for this study provided an informed consent. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University - Ministry of Health.

PRODUCTS

Fortified instant noodles were produced by the Hanoi Food Processing and Trading Company, which has a good quality control system. A package of instant noodle included noodles (± 52 gram), a small pack of (spicy) oil (± 2 gram) and a small pack of seasoning (± 4 gram). The seasoning can have different flavours such as vegetable, beef or chicken flavour.

Noodles were fortified with a water soluble, highly bioavailable iron compound (NaFeEDTA: Ferrazone®, Akzo Nobel Chemicals Pte Ltd Arnhem, The Netherlands). The Joint FAO/WHO Expert Committee on Food Additives has recently approved NaFeEDTA for government approved fortification strategies [10]. In the presence of phytic acid, NaFeEDTA is 2 to 3 times better absorbed than ferrous sulfate [7]. EDTA stays stable when it is exposed to heat or humidity [11] and has shown to be efficacious for fortification in improving iron status [12-14]. The fortified level of 10.7 mg iron per 52 gram of noodles was calculated based on the JECFA 1974 recommendation of an acceptable daily intake of 2.5 mg EDTA/kg body weight corresponding to 0.37 mg NaFeEDTA/kg body weight/day [15] and using an average body weight of 29 kg [16]. Retention of iron after production was double checked in the laboratories at the National Institute of Nutrition, Hanoi and Wageningen University. Iron leakage from noodles into soup after preparation was checked in the laboratories at Akzo Nobel Chemicals Pte Ltd Arnhem. Capillary zone electrophoresis analysis [17] showed that 70% of the NaFeEDTA dissolves within 5 minutes into the soup independent of extraction time. No degradation products of NaFeEDTA were found.

PROCEDURE

Discrimination and preference test

Preparation of the samples

The whole content of a package of instant noodles was broken into smaller pieces and put into a plastic heat resistant bowl. An amount of 200 ml of hot boiled water from a thermos flask was added to the noodles. We decided to add less water to the instant noodles than the amount of water recommended by the factory (400 ml) as to make sure that the children finish the whole dish (noodles and water) and consume all the iron content in the noodles. Based on earlier experiments it was expected, that some children could not finish all the water when adding 400 ml, and hence would consume less iron than expected. However, the package of seasoning is adequate for 400 ml, so when adding 200 ml water, half of the seasoning was added. Also no oil was added to the noodles since the children disliked the oil due to its spiciness.

The temperature of the samples being served was around 40 degree Celsius. The water after boiling stood for quite a while in the thermos flasks before it was added to the noodles, after which the noodles were soaked for 4-5 minutes in open air. We did not measure the temperature of the samples before consumption.

Triangle test

Besides verbal instruction, the children were trained using artificial fruits to get familiar with a triangle test in general and to ensure that they understood the task of the test. Artificial mangos and pineapples were chosen for the training, because both are green/yellow, are familiar for the children in the area, and have different tastes. The training was done in two stages; first, three group rounds were intended to get the children acquainted with the procedure and the score sheet. The group rounds were followed by three individual rounds. If a child made a mistake in one of the three individual rounds his/her data was not used for the statistical analysis of the triangle test. Adults only received instructions regarding the evaluation procedure in both written and verbal formats.

Both children and adults were invited to participate in two triangle test sessions on two separate occasions one week apart of each other. The first triangle test session consisted of six triangles without seasoning. In the second session, subjects tasted six triangles with seasoning added to the samples. The color differences between the samples were hidden by covering each sample by a lid, as fortified noodles were somewhat darker than non-fortified noodles as measured by the CIE-L*a*b method [18] (data not shown). Subjects were allowed to remove only one lid at a time when tasting the samples. Subjects were not forced to eat the whole sample (portions of approximately twenty-eight grams), re-tasting was allowed. Samples were swallowed and not spitted out. Six different triangle combinations of products were offered. The sequence of combinations was counterbalanced across subjects. Samples were served in white plastic disposable cups of approximately 100 ml. To minimize adaptation, a 4–5 minute break occurred between each triangle test.

Paired preference test

Paired preference tests with and without seasoning added were carried out for both children and adults. During the paired preference test, subjects received two samples, one fortified and one non-fortified, simultaneously. The samples were not covered as was the case in the triangle test to enable comparison of color between the two samples. Subjects selected the preferred sample by circling the three-digit number of the sample on a ballot. They were not forced to eat the whole sample; re-tasting was allowed. First, the two possible combinations of the noodles without seasoning were served. Hereafter, the two combinations of noodles with seasoning were served. To minimize adaptation, a 4 till 5 minute break occurred between each pair.

Long term acceptance test

The preparation of the noodles consisted of soaking a whole package of dried noodles (52 grams) in 200 ml of hot boiled water together with half a package of seasoning.

60 children 6 – 8 years old received the noodles at school at 8:30 am for 5 times per week, in a period of 10 weeks. Subjects were randomly assigned to 2 groups to observe the effect of freedom of choice [8]: (1) **Monotony** group (n=30) consistently received noodles with the same flavor (seasoning was chicken flavor). (2) **Free choice** (n=30) in variation group was allowed to choose among three flavors (seasoning could be chicken, beef and vegetable flavored) everyday.

The measurements were carried out twice a week (at the beginning and the end of the week), every two weeks (week one, three, five, seven, nine and ten). In total 12 sessions were conducted. Subjects gave their answers on 3 point and 5 point category scales [19, 20]. Just before consumption of the noodles, subjects were asked to rate desire to eat as 1 (not at all), 2 (usual) or 3 (very much). At the point of meal completion subjects were asked to rate pleasantness as 1 (dislike a lot), 2 (dislike a little), 3 (neither like nor dislike), 4 (like a little) or 5 (like a lot); boredom as 1 (not at all bored), 2 (slightly bored) or 3 (extremely bored); desire to eat as 1 (not at all), 2 (usual) or 3 (very much). Before conducting the real test we explained to the children what is meant by desire to eat (willingness to consume), and boredom with eating noodles (declined acceptance due to repetition of exposure) by using 3 different face pictures showing a smiling face, an indifferent face and a sad face. After explanation, we trained the children to familiarize them with the concepts and the method used.

STATISTIC ANALYSIS

Discrimination and preference test

The (beta) binomial statistic analysis was applied for data analysis. The binomial distribution assumes the existence of only the between subjects variability. However, in this study each subject tasted six triangles tests in each session and 2 pairs of stimuli for the preference test. The responses of one subject are not independent from each other. If subjects are not acting in an identical fashion, the variance due to differences in between-person and within-person is explained by the beta distribution [21]. This variability is known as overdispersion, which is measured by gamma (γ), a value that ranges from 0 to 1. When $\gamma = 0$, there is no overdispersion and binomial statistics may be used. When γ is significantly greater than 0, the binomial model is invalid and the beta-binomial model should be used instead [21, 22]. In this study the moment estimation method was used to calculate the panelist variability [22]. To test whether panelist variability was significant, Tarone's Z statistic [21] was used. When panelist variability was significant, the beta-binomial model was used to determine if a significant difference existed between samples; when panelist variability was not significant, the binomial model was used. To see whether the results achieved the minimum number of correct or agreeing choices (for the difference and preference tests respectively) for significance, Bi & Ennis (1999) were used for the critical values [23]. Results of both triangle tests of adults were combined and compared with the combined results of the children' triangle tests by means of a Mann-Whitney non-parametric test using SPSS 12.1 [24]

Long term acceptance test

Graphs were made to visualize the change in desire to eat and boredom over time. Percentages of subject's answers at each of the categories of desire to eat and boredom were calculated. Differences between monotony and free choice groups per measurement were tested with the Chi-square test.

RESULTS

DISCRIMINATION AND PREFERENCES

Discriminatory ability of children and adults

The simpler binomial model was used only for the triangle data with children without seasoning **(Table 1).** Both adults and children detected significant differences between the fortified instant noodles and the non-fortified instant noodles whether or not seasoning was added. **(Table 1**). There was a significant difference between the adults and children's performance (p= 0.001); children were less well able to discriminate between non-fortified and fortified instant noodles than adults.

Table 1: Ability of children and adults to discriminate iron-fortified and non-fortified noodles with and without seasoning added.

	Without seasoning		With seasoning	
	Adults (n=48)	Children (n=39)	Adults (n=48)	Children (n=39)
Panelist variability (γ)	0.23[1]	0.02	0.20[1]	0.20[1]
Appropriate model	BB	Binomial	BB	BB
No of responses	288	234	288	234
No.of correct responses needed for significance (α = 0.05)	119	91	119	97
No. of correct responses observed	197[2]	127[2]	194[2]	121[2]
$\hat{\mu}$ (Triangle Test: μ_0=1/3)	0.68	0.54	0.67	0.52

[1] = Significant (α = 0.05) using Tarone's Z statistic
[2] = Significant (α = 0.05)
BB = Beta-binomial
(γ): Panelist variability (Bi&Ennis 1999)
$\hat{\mu}$: is the moment estimate of μ in the BB model and μ is Mean of the Binomial parameter p; p being a proportion of responses that prefer non-iron fortified noodles above iron fortified noodles

Preference by children and adults

Beta-binomial model was used for all preference test data **(Table 2)**. No significant preference was found for non-fortified noodles among adults with and without seasoning added **(Table 2)**. For children however, there was a significant preference for non-fortified noodles when no seasoning was added. Sixty responses were in favor of the non-fortified instant noodles whereas fifty-nine responses in favor of the non-fortified instant noodles were needed for statistical significance. When seasoning was added to the instant noodles, the children showed no significant preference anymore for non-fortified instant noodles **(Table 2)**.

Table 2: Preference of children and adults for iron fortified or non iron-fortified noodles with and without seasoning added.

	Without seasoning		With seasoning	
	Adults (n=48)	Children (n=48)	Adults (n=48)	Children (n=48)
Panelist variability (γ)	0.49[a]	0.37[a]	0.40[a]	0.44[a]
Appropriate model	BB	BB	BB	BB
No of responses	96	96	96	96
No. of responses needed for significance (One-tailed, α = 0.05)	60	59	59	59
No. of responses observed favoring non-fortified noodles	42	60[b]	42	56
$\hat{\mu}$ (Paired preference: μ_0=1/2)	0.44	0.63	0.44	0.57

[a] = Significant (α = 0.05) using Tarone's Z statistic
[b] = Significant (α = 0.05)
BB = Beta-binomial
(γ): Panelist variability (Bi&Ennis 1999)
$\hat{\mu}$: is the moment estimate of μ in the BB model and μ is Mean of the Binomial parameter p; p being a proportion of responses that prefer non-iron fortified noodles above iron fortified noodles.

LONG TERM ACCEPTANCE

100% of children finished the whole portion of noodles served to them every day from beginning till the end of the study period and this did not differ between the free choice group and the monotony group.

Desire to eat

Subjects gave high ratings for desire to eat before consumption in both groups throughout the study period as more than 75% children rated desire to eat as "3" (very much) over the 10 weeks **(Table 3).** The desire to eat noodle slightly decreased in both groups from week three to week seven, after which it slightly increased again in the last two weeks, especially in free choice group **(Figure 1).** Subjects also gave high ratings for desire to eat after consumption: 87.7 % rated desire to eat as "3" (very much) in the first week and 81.7% in the last week, with no differences between groups (data not shown).

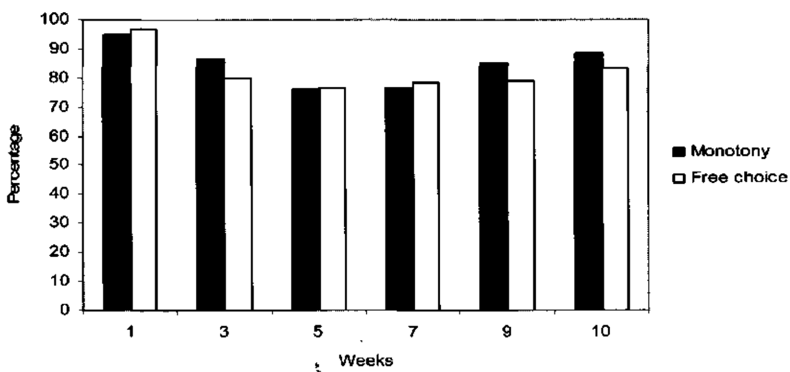

Figure 1: Percentage of children (6-8 years) who desire to eat fortified instant noodles very much (rating 3) over a 10 weeks period.

Pleasantness and boredom

The pleasantness was high throughout the study for both groups; on a 5-point category scale mean ratings were between 4 (like a little) and 5 (like a lot). No significant differences between groups were found (data not shown).

In both groups, subjects gave low ratings for boredom on the 3-point category scale; between 1: *Not at all bored* and 2: *slightly bored.* Data indicated that 92.7 % of the children were not bored at all with the noodles in the first week (**Table 4**). Boredom significantly differed between the two groups in the first week ($p = 0.02$, Chi-square test), but in week 3 to 9 about 70% indicated not to be bored at all in both groups with no significant difference between groups (**Table 4**). In the last week only 58.3% in monotony group and 78.3% in free choice group indicated not to be bored at all with noodles ($p = 0.015$ Chi–square test). For both groups together, repeated exposure to iron-fortified noodles resulted in a slightly increase of boredom over time (**Figure 2**)

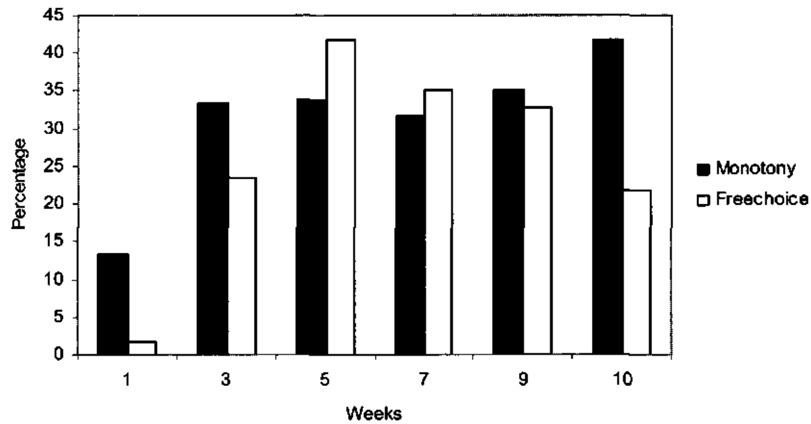

Figure 2: Percentage of children (6-8 years) who are slightly or extremely bored with fortified instant noodles (rating 2 and 3) over a 10 weeks period

Table 3: Percentages of desire to eat NaFeEDTA fortified noodles falling into each of three categories in monotony and free choice group over a 10 weeks period among children (6-8 years old).

	Desire to eat								
	Not at all (%)*			Usual (%)*			Very much (%)*		
Week	Monotony	Free choice	Total	Monotony	Free choice	Total	Monotony	Free choice	Total
1	3.4	1.8	2.6	1.7	1.8	1.7	94.8	96.5	95.7
3	1.7	0	0.8	11.7	20.0	15.8	86.7	80.0	83.3
5	1.7	3.3	2.5	22.0	20.0	21.0	76.3	76.7	76.5
7	0	1.7	0.8	23.3	20.0	21.7	76.7	78.3	77.5
9	1.9	3.8	2.8	13.0	17.3	15.1	85.2	78.8	82.1
10	0	1.7	0.8	11.7	15.0	13.3	88.3	83.3	85.8

* *No significant difference between groups (Chi-square test)*

Table 4: Percentages of boredom with NaFeEDTA fortified noodles falling into each of three categories in monotony and free choice group over a 10 weeks period among children (6-8 years old).

	Boredom								
	Not at all bored (%)			Slightly bored (%)			Extremely bored (%)		
Week	Monotony	Free choice	Total	Monotony	Free choice	Total	Monotony	Free choice	Total
1	86.5*	98.2	92.7	11.5	1.8	6.4	1.9	0.0	0.9
3	66.7	76.7	71.7	30.0	21.7	25.8	3.3	1.7	2.5
5	66.1	58.3	62.2	30.5	31.7	31.1	3.4	10.0	6.7
7	68.3	65.0	66.7	30.0	33.3	31.7	1.7	1.7	1.7
9	64.8	67.3	66.0	35.2	30.8	33.0	0.0	1.9	0.9
10	58.3**	78.3	68.3	40.0	21.7	30.8	1.7	0.0	0.8

** Difference between group $p<0.02$ (Chi-square test)*
*** Difference between group $p<0.015$ (Chi-square test)*

DISCUSSION

Results from triangle tests showed that both adults and children perceived a difference in taste between NaFeEDTA – fortified instant noodles and non-fortified instant noodles even when seasoning was added. Children showed a slight preference for non-fortified instant noodles, when they consumed it without seasoning, however, for both children and adults, non-fortified instant noodles were not preferred above fortified instant noodles when seasoning was added. The high acceptance for iron fortified instant noodles with seasoning added is also reflected in the results from the long term acceptance test, where acceptance ratings remained high and boredom ratings remained low throughout the10 weeks of the study.

Both adults and children in our study perceived the difference in taste between NaFeEDTA – fortified instant noodles and non-fortified instant noodles even when seasoning was added. Previous studies also found that fortification with NaFeEDTA leads to color and flavor changes in fortified products [7, 25]. NaFeEDTA fortification of fish sauce [12, 13] and soy sauce [26] did not lead to sensory changes probably because the very distinct and strong taste of the fortification vehicles masked the change in taste. Lamparelli et al (1987) used curry powder as vehicle and suggested that curry powder being intensily colored and spiced masks the addition of NaFeEDTA [14].

The comparison of performance in the triangle test between adults and children showed that the discriminatory ability is significant lower in children ($p<0.001$). Doty et al also suggested that taste and odour sensitivity is lower in children than in adults [27]. A logical explanation for this may be that children have a less- developed taste system [28, 29]. Another explanation might be that children are more easily distracted than adults. Since children are young and playful, it might be harder for them to stay focused especially when the task has to be repeated several times. A solution to obtain a higher focus of the children is to perform the test in a one-on-one situation [30]. A third explanation might be that young children may not have enough cognitive ability to do the triangle test. A previous study also mentioned that discriminatory ability of children changes due to cognitive development [31]. However, our children were carefully trained and moreover, we did exclude the children who were not able to run the test with the artificial fruit in a correct way.

The results of paired preference tests showed a difference between the children and adults. For adults there was no evidence that non-fortified instant noodles were preferred above fortified instant noodles, either with or without seasoning added. For the children there was a significant preference for the non-fortified instant noodles above the fortified instant noodles when no seasoning was added. However, in view of the large number of statistical tests carried out, this significant difference could have easily been caused by chance, especially because the number of preference responses (60) is very close to the number of responses needed for significance (59). When the seasoning was added there was no significant preference found. In fact, during daily use (fortified) noodles will never be consumed without any seasoning. In addition, when looking close at the results, there is only a subtle difference between the results of the two preference tests of the children (60 preference responses for non-fortified instant noodles when no seasoning is added and 56 preference responses when seasoning was added out of the maximum responses of 96). This means that although seasoning may have made some difference in preference of the children consuming instant noodles, the role of seasoning in having a preference for one type of noodle is not substantial.

In the long term acceptance test, children consumed iron fortified instant noodles with added seasoning for 10 weeks. A slight increase of boredom occurred after long term repeated in school consumption of iron-fortified instant noodles. This finding is in line with some previous studies. Repeated exposure over a longer period can lead to an increase of boredom over time for familiar generally no-novel foods [8, 32]. Although Zandstra et al (2000) measured a substantial increase in boredom and a substantial decline in acceptance ratings during a period of ten weeks, in our study we only found a small increase of boredom over time in both groups. This small increase may be explained by the existence of hunger or inadequate food intake of the study population. A previous study showed that children in the study area only consume 3 meals a day without a snack between meals. The mean energy intake only meets 67% of the recommended energy intake for this age group [33]. Cabanac suggested that a given stimulus can induce a pleasant or unpleasant sensation depending on the subject's internal state, and food intake was considered as one of the factors reflecting internal state [34]. In our study population with a low food intake, fortified instant noodles may induce a pleasant sensation and lead to small increase of boredom. However, Rolls and de Waal (1985) showed with their study on long term sensory specific satiety among refugees in an Ethiopian refugee camp, that in a situation of hunger providing a low variety of food for a long period could lead to a decrease in palatability and in acceptability of these foods [35].

The acceptance ratings remained high throughout time in both groups. This may be explained by the type of food product used. Besides rice, noodles can be considered as one of the main staple foods in Vietnamese diets. Previous studies suggested repeated consumption did not change the liking of staple foods, and appeared to demonstrate a flat time-preference curve [32, 36, 37]. In the study of Hetherington et al (2002) two groups were given either chocolate or bread and butter for 22 days on a daily basis. Pleasantness of taste and desire to eat chocolate declined significantly over time, but no such changes were observed for the staple products bread and butter [32]. However, Rolls and de Waal (1985) indicate in their study, that in a situation of low variety of food, the long-term sensory specific satiety can be so marked that it decreases the palatability even of staple foods [35].

The rating of desire to eat before and after consumption, pleasantness and boredom was almost similar in both free choice and monotony group. The increase of boredom was slightly higher in the monotony compared to free choice groups (especially in the last week). As the only difference between the monotony and free choice groups was the type of seasoning, seasoning did apparently not play a substantial role in boredom perceptions. Only 2 grams of seasoning was added. Zandstra et al found a larger increase in boredom and decline in acceptance in a monotony group where subject consistently received 285 gram of the same meat sauce once a week in 10 weeks while a free choice group was allowed to choose among 3 difference flavours: curry, sate and sweet-and-sour sauce [8]. The very low amounts of flavour used in our study may explain the small differences found between monotony and free choice groups. Therefore, the results of this study do not give reason to recommend the use of different flavours in an intervention programme in order to reduce boredom and desire to eat.

In our study children gave a high rate for desire to eat even after consumption. This may be due to the possible cultural bias related to the Asian politeness in avoiding negative responses. A study of Yeh et al (1998) indicated that compared to US consumers, Chinese, Korean, and Thai respondents avoided dislike categories of a nine-point labelled category scale [38]. However, if such a culture bias exists, children could say they want to eat the noodles but they would not finish the whole portion of the noodles. In our study all children finished the portion every day from the beginning until the end of the study, probably indicating that culture bias does not play a major role.

In conclusion, both children and adults in the present study perceived a difference in taste between the two types of noodles. However, there was no preference for non-fortified noodles when seasoning was added. Slight increase of boredom occurred especially in monotony group after long term repeated in school consumption of iron fortified instant noodles by school children. However, for both monotony and free choice groups, the decrease in acceptance ratings was small and acceptability remained high throughout time. Overall, noodles fortified with NaFeEDTA do not change consumer acceptability and can therefore be considered as a suitable food vehicle to be used in a school-based program that aims to combat iron deficiency anemia in school children.

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Chapter 5: The effect of iron fortification and de-worming on anemia and iron status in low-iron deficient Vietnamese school children

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ABSTRACT

Previous data from Vietnam show that anemia is highly prevalent among school children, who are supposed not to be iron deficient. *Trichuris* infection doubled the risk of anemia. The present study aimed to evaluate the hypothesis that de-worming is more effective than iron fortification in an anemic, infection prone- population that was not considered iron deficient. In a trial with a 2x2 factorial design, 425 anemic children aged 6 to 8 years were randomly assigned to receive either iron fortified noodles or placebo, and Mebendazole or placebo. Outcomes considered were change in haematological indicators of iron status (hemoglobin, Serum Ferritin (SF), Serum Transferrin Receptor (TfR) and hemoglobinopathies analysis); inflammations (C-reactive Protein (CRP)); parasite infection status (hookworm, *Trichuris* and *Ascaris* infection) and Immunoglobulin E (IgE). Analysis of variance and logistic regression were used to assess the effects of iron fortification and de-worming on hemoglobin, SF, TfR, body iron and anemia. Hemoglobin improved in all groups after 6 months of intervention. Iron fortification significantly improved hemoglobin, SF and body iron (2.6 g/L, 16.2 μg /L and 1 mg/kg respectively). Prevalence of elevated IgE was very high at baseline (99%) and significantly reduced to about 75% in all groups after intervention. De-worming unexpected showed no effect on hemoglobin, iron status and IgE level. It is concluded that iron fortification slightly improved anemia and iron status in anemic school children in rural Vietnam that were not considered iron deficient. Chronic infection or other un-identified factors including a limited iron uptake for heme synthesis may play an important role in the seasonal reduction of anemia seen in all treatment groups.

Anemia is a significant public health problem in Vietnam. The 2000 National Nutrition Risk Factor Survey in Vietnam showed an anemia prevalence of 34% in children under five and 25% in women [1]. No nationally representative data are available on the prevalence of anemia among primary school children in Vietnam; however, a few local studies showed an anemia prevalence of around 30% [2, 3]. Although various nutrients and cofactors are involved in maintaining the normal synthesis of hemoglobin, iron deficiency is the most frequent cause of anemia on a worldwide basis [4]. However, infection and inflammation [5, 6], malaria [7], intestinal parasite infection [8, 9], as well as hemoglobinopathies [10, 11] may play a role. Their relative importance in Vietnam is still unclear. A previous cross-sectional study conducted in Tam Nong district, Phu Tho province, a poor rural area of North Vietnam, showed a high prevalence of anemia among primary school children with low iron deficiency as measured by serum transferrin receptor (TfR) and serum ferritin (SF) with 2% of children with TfR >8.5mg/L and 0.5% of children with SF < 12 μg/L [12]. Further, *Trichuris* infection was associated with a doubled risk of anemia, probably not mediated through iron deficiency. Food fortification is often suggested as one of the most effective and sustainable strategies for increasing iron intake in the general population [13]. Also in the nutritional strategies for prevention and control of micronutrient deficiencies in Vietnam, food fortification is considered as a sustainable solution to combat iron deficiency anemia [1]. However, based on the previous study, it is hypothesized that de-worming is more effective than iron fortification in an anemic infection prone population that was not considered iron deficient. Using a trial with iron fortified noodles and de-worming, we assessed the changes in iron and anemia status among anemic school children, and tested whether our data are consistent with this hypothesis.

SUBJECT AND METHODS

STUDY DESIGN AND POPULATION

The study was implemented from November 2004 to May 2005 in 6 primary schools in Tam Nong district, Phu Tho province, situated 90 km from Hanoi. Selection was based on high prevalence of anemia and absence of interventions to control iron deficiency anemia in school children. Children recruited into the study were in grade one to grade three with hemoglobin concentrations < 110g/L but not < 70 g/L in an initial hemoglobin – screening study. We excluded children with Hb level less than 70 g/L because these children were considered as severely anemic and received treatment immediately. The study concerns a randomized, placebo-controlled double blind parallel trial with a 2×2 factorial design plus standard treatment (iron supplementation and de-worming) and an intervention period of 6 months.

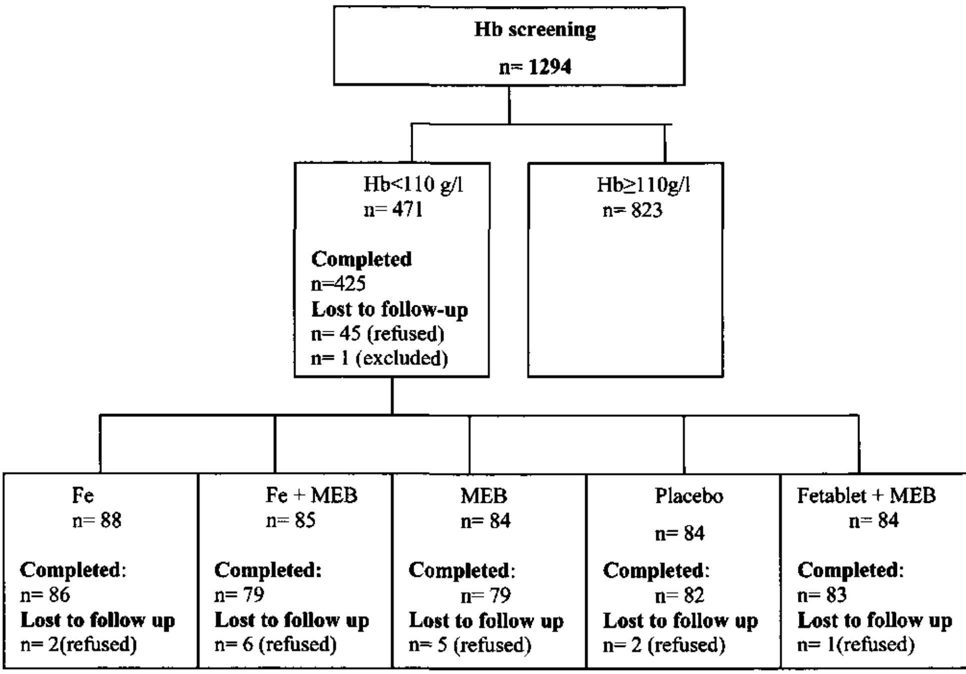

Figure 1: Study profile: initial screening to enroll anemic children in the study, followed by a 6 months intervention.

A total of 425 eligible children were randomly assigned to one of five groups (85 per group) receiving: I) iron-fortified noodles and mebendazole (Fe+MEB); II) noodles without iron fortificant and mebendazole (MEB); III) iron-fortified noodles and placebo (Fe) IV) noodles without iron fortificant and placebo (placebo) and V) iron supplementation and mebendazole (Fe tablet+MEB) **(Figure 1)**. Randomization was carried out by a researcher from the Division of Human Nutrition, Wageningen University, The Netherlands, who did not know the children and could not introduce bias in the randomization. Stratified randomization was applied based on the Hb levels and age of the children to ensure equal distribution of Hb and age across groups. Sample size was assessed to achieve a statistical power of 95%, based on an alpha error of 0.05, a between-group difference in treatment effect of 5 g hemoglobin/L in hemoglobin concentration being clinically relevant, a standard deviation of 11 g/L [2] and accounting for 10% of children being lost in the course of the intervention. In this article we focus on the placebo-controlled parallel trial with a 2×2 factorial design to assess the effect of iron-fortified noodle and de-worming on iron and anemia status of school children. The extent of the effect of the iron-fortified noodles compared to the standard treatment and its policy relevance is discussed in another article. Children were invited for the study and a written informed consent was obtained from their parents. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University - Ministry of Health.

PRODUCTS AND FIELD PROCEDURES

Fortified instant noodles were produced at the Hanoi Food Company. Noodles were fortified with a water soluble, highly bioavailable iron compound (NaFeEDTA: Ferrazone®, Akzo Nobel Chemicals Pte Ltd Arnhem) to a fortified level of 10.7 mg iron per 52 gram of noodles calculated based on the JECFA 1974 recommendation of an acceptable daily intake of 2.5 mg EDTA/kg body weight [14] and an average body weight of 29 kg [15]. Before intervention retention of iron after production and preparation of fortified instant noodles was checked in laboratories at the National Institute of Nutrition Hanoi, Wageningen University and Akzo Nobel Chemicals Pte Ltd Arnhem). Capillary zone electrophoresis analysis [16] showed that 70% of the NaFeEDTA dissolves within 5 minutes into the soup independent of extraction time. No degradation products of NaFeEDTA were found.

Noodles were prepared in school by care-takers trained by field staff and given to children at break time (9:00 am) 5 days per week during 6 months under the supervision of teachers and field staff. Children were encouraged to consume all the noodles and water.

Mebendazole 500 mg and an identical placebo were given to children at the beginning of intervention and at 3 months following. Children, teachers and researchers were blinded to the treatment.

DATA COLLECTION.

Capillary blood samples were taken from children's fingers during screening for hemoglobin measurement by cyanmethemoglobin method. Children's weight was measured to the nearest 100 gram using an electronic scale (Seca, 890, UK) and standing height was measured to the nearest 1 mm with a microtoise (Stanley Mabo No 191- France) during baseline and after intervention. Venous blood (5 ml) was collected in the morning at baseline (T0) and after intervention (T6); 20μl whole blood was pipetted immediately before coagulating into a tube containing 5.0 ml of Drabkin's reagent with a Sali pipette for hemoglobin measurement. An aliquot of whole blood was taken for analyzing hemoglobinopathies. The remaining blood was allowed to clot for 30 minutes at room temperature, centrifuged at 3000 x g for 15 minutes and transferred to 5 plastic labelled vials (Eppendorf tubes 0.5ml). The vials were stored in a box protected from sun-light and put into an ice box for transfer to the laboratories and kept at -80°C until serum ferritin (SF), serum transferrin receptor (TfR), C-reactive protein (CRP) and Immunoglobulin E (IgE) analysis at the end of the intervention.

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

Laboratory analysis

Hemoglobin concentration was measured in whole blood within 12 h of sampling by cyanmethemoglobin method using Sigma KIT in the Tam Nong District Health Centre. The CV of intra-assays and inter-assays was 4.0 ± 1.2% and 5.0 ± 2.0%, respectively. SF, TfR, CRP and IgE analysis was carried out at the same time for both samples of baseline and after intervention at the National Institute of Nutrition and the laboratory of Hanoi Medical University in May and June 2005. Concentrations of SF and TfR were analyzed by an Enzyme - Linked Immuno Sorbent Assay (ELISA) method (Ramco Laboratories, Inc, Houston, TE, Catalogue numbers S-22 and TF-94), with inter-assay variability of 4-7% and 4-8%, respectively. Serum CRP was measured by nephelometry using Epress plus, with an inter-assay variability of 4-8%. Serum IgE was determined by ELISA using the Kallestad Total IgE Microplate Kit from GmbH - Germany, with an inter-assay variability of 4-6%. A 10% sub samples was re-examined for quality control. Hemoglobinopathies analysis was performed by using the Variant Beta-Thalassemia Short program (Bio- Rad laboratories Inc, Hercules, CA) within 24 h of sampling in the Children Hospital, Hanoi, Vietnam. Stools samples were examined by using the Kato - Katz Technique –cellophane faecal thick smear method [17]. Hookworm, *Trichuris*, and *Ascaris* eggs were counted. A 10% subsample of smears was re-examined for quality control.

DATA ANALYSIS

Anthropometric indices were calculated using WHO/NCHS reference data [18]. Being wasted, stunted and underweight was defined by z-scores <–2SD for weight-for-height, height-for-age and weight-for-age, respectively. Anemia was defined as hemoglobin concentrations <115g/L [19]. Iron deficiency was defined as SF concentrations <12 µg/L [19], and tissue iron deficiency was defined as TfR concentration >8.5 mg/L [20]. Body iron content was calculated using the following formula: body iron (mg/kg) = -(log (TfR/SF ratio)- 2.8229)/0.1207 [21].

CRP and IgE concentrations were considered to be elevated when ≥ 8 mg/L [22] and > 90 IU/ml [23] respectively. Hemoglobin type was determined in each subject on the basis of hematological indexes: HbAA (normal hemoglobin type), HbF (hemoglobin F); HbA2 (Hemoglobin A2); HbAE (trait for hemoglobin E disease), or HbEE (hemoglobin E disease). Severity of intestinal worm infections was expressed as the number of eggs/g feces using the WHO classification system [24].

Data was entered into the computer, cleaned and managed using Epi Info version 6, [25] and analyzed using SPSS 11.0 for windows (SPSS Inc., Chicago IL, USA) [26]. Data was checked for normality by visual inspection. One-way ANOVA was used to determine differences in hemoglobin concentration and other biochemical indicators between groups. Paired t-test was used to compare hemoglobin and other biomarkers before and after intervention. Chi-square test and McNemar test were used to assess the differences in proportions between and within groups. To assess the association between iron fortification, de-worming and indicators of iron status, we compared children with and without iron fortification and children with and without de-worming with respect to their change in hemoglobin concentration, SF, TfR, and body iron, respectively, by using an analysis of variance model. Logistic regression was used to study the effect of iron fortification and de-worming on anemia prevalence.

RESULTS

At baseline, the mean age of children was 87.4 ± 10.2 months. The four groups did not significantly differ in age, hemoglobin concentration, iron status (SF, TfR, and body iron) and nutritional status (**Table 1**) nor in CRP, IgE status and parasite infection (**Table 3**). The prevalence of iron deficiency was very low as 1.2% of children showed SF concentration below 12 μg/L and 5.5% of children showed TfR above 8.5 mg/L. Mean body iron was around 6.3 mg/kg body weight (**Table 1**). Two third of the children were infected with *Ascaris* and/or *Trichuris*, and about 8 % infected with hookworm. Very few children showed elevated CRP levels (1.8%) and 99% of the children showed an elevated IgE level (**Table 3**). The prevalence of thalassemia (HbA2, HbAE, HbF) was around 7 % (**Table 1**).

Hemoglobin concentration increased after 6 months of intervention in all four groups; however, a larger significant increase was seen in the two groups receiving iron fortified noodles (17.8 ± 9 g/L and 17.5 ± 7.5 g/L respectively) compared to 14.6 ± 8.8 g/L in the group receiving de–worming only and 15.4 ± 8.3 g/L in the placebo group (**Table 2**). Prevalence of anemia significantly decreased in all four groups with a larger reduction observed in the two iron fortified groups; however, no significant difference was found between groups (**Table 2**).

SF concentration increased significantly in the two groups receiving iron fortification (15.0 ± 30.4 μg/L and 17.8 ± 31.8 μg/L respectively) compared to the other two groups. The group receiving only de-worming showed even a decrease in SF concentration compared with data at baseline (-7.9 ± 27 μg/L) (**Table 2**).

TfR concentration was very slightly improved after 6 months of intervention and there were no significant differences between groups (**Table 2**).

Body iron significantly increased in the two groups receiving iron fortification (1.0 ± 1.8 mg/kg and 1.4 ± 1.9 mg/kg) compared to the de-worming only and placebo groups (-0.1 ± 1.6 mg/kg and 0.4 ± 1.8 mg/kg, respectively).

Prevalence of *Ascaris* and *Trichuris* infection dropped significantly in two groups receiving Mebendazole (**Table 3**). *Ascaris* infection slightly increased in the placebo group, however, the prevalence of *Trichuris* infection also decreased in the group receiving iron fortified noodles without de-worming. Prevalence of hookworm infection decreased in all four groups; however, the prevalence of hookworm infection was very low at the baseline. The proportion of children with elevated CRP levels decreased in all three groups except the placebo group, where the number of children with elevated CRP level even increased. Prevalence of elevated IgE significantly decreased in all four groups, with no differences between groups.

Table 1: Baseline characteristics by treatment group in rural Vietnamese school children (n=409)

	Treatment groups				
	Fe (n= 86)	Fe + MEB (n=79)	MEB (n= 79)	Placebo (n= 82)	Fe tablet + MEB (n=83)
Male %	51.2	50.6	46.8	48.8	53.0
Age in month[1]	88.0 ± 10.1	87.6 ± 11.4	87.7 ± 10.1	86.9 ± 10.3	86.9 ± 9.8
Hemoglobin (g/L)[1,3]	107.5 ± 8.3	107.3 ± 6.8	108.5 ±.6.9	107.8 ± 6.2	107.9 ± 7.2
Serum ferritin (µg/L)[2,3]	52.3 (33.0- 78.8)	48.1 (33.7- 67.5)	54.9 (40.0 -85.5)	50.1 (35.4-77.6)	54.9 (40.5-72.5)
Transferrin receptor (mg/L)[1,3]	5.9 ± 3.0	6.0 ± 1.4	6.2 ± 1.5	6.2 ± 1.6	6.0 ± 1.3
Body iron (mg/kg)[3]	6.5 ± 2.3	6.1 ± 2.3	6.5 ± 2.7	6.2 ± 2.7	6.6 ± 1.9
Serum ferritin < 12 µg/L, %	1.2	1.3	1.3	1.2	0.0
Transferrin receptor > 8.5 mg/L, %	3.5	3.8	6.3	8.5	3.6
WAZ[1,3]	-1.8 ± 0.8	-1.9 ± 0.6	-1.9 ± 0.6	-1.9 ± 0.6	-1.7 ± 0.6
HAZ[1,3]	-1.6 ± 0.7	-1.6 ± 0.8	-1.7 ± 0.8	-1.7 ± 0.8	-1.6 ± 0.8
WHZ[1,3]	-1.2 ± 0.8	-1.2 ± 0.7	-1.2 ± 0.7	-1.2 ± 0.6	-1.0 ± 0.7
Thalassemia %	9.3	6.3	3.8	8.5	8.4

[1] *Mean ± SD*

[2] *Geometric mean (25 and 75 percentile)*

[3] *No significantly different between groups (One-way ANOVA) p>0.05*

Fe: Iron fortification; MEB: Mebendazole

Table 2: Change in hemoglobin, anemia and iron status before and after intervention in rural Vietnamese school children

	Treatment groups			
	Fe (n= 86)	Fe + MEB (n=79)	MEB (n= 79)	Placebo (n= 82)
Change in Hemoglobin concentration (g/L)^{1*}	17.83 ± 0.97^4	17.54 ± 0.85^4	14.60 ± 1.00^4	15.40 ± 0.92^4
Change in Serum ferritin (µg/L)^{2*}	15.05 ± 3.28^4	17.86 ± 3.61^4	-7.94 ± 3.05^5	5.37 ± 4.31
Change in serum Transferrin receptor (mg/L)*	-0.35 ± 0.10^4	-0.38 ± 0.10^4	-0.39 ± 0.10^4	-0.31 ± 0.13^6
Change in Body iron (mg/kg)^{3*}	1.00 ± 0.20	1.39 ± 0.22	$- 0.09 \pm 0.18$	0.39 ± 0.20
Anemia (%)				
Baseline	89.5^7	84.8^7	83.5^7	91.5^7
At the end	10.5	11.4	15.2	19.5
Serum ferritin <12 (µg/L) (%)				
Baseline	1.2	1.3	1.3	1.2
At the end	0	0	0	0
Serum transferrin receptor >8.5 (mg/L) (%)				
Baseline	3.5	3.8	6.3	8.5
At the end	3.5	3.8	1.3	6.1

1,2,3. Significantly different between group (on- way ANOVA): ^{1} $p= 0.037$; ^{2} $p<0.001$; ^{3} $p<0.01$

4,5,6. Significantly different within group before and after intervention (t-test) ^{4} $p<0.001$; ^{5} $p = 0.01$; ^{6} $p = 0.02$

7. Significantly different within group before and after intervention (Mc Nemar) ^{7} $p<0.001$

** Mean ± SE*

Fe: Iron fortification; MEB: Mebendazole

Table 3: Change in the parasite infection, inflammation and nutritional status before and after intervention in rural Vietnamese school children

	Treatment groups			
	Fe (n= 86)	Fe + MEB (n=79)	MEB (n= 79)	Placebo (n= 82)
Ascaris (%)				
Baseline	69.8	65.8	67.1	69.5
At the end[1]	68.6	44.3^{3}	41.8^{2}	74.4
Trichuris (%)				
Baseline	76.7	78.5	63.3	73.2
At the end[1]	47.7^{2}	15.2^{2}	48.1^{4}	72
Hookworm (%)				
Baseline	7.0	10.1	7.6	11.0
At the end[1]	4.7	1.3^{4}	2.5	3.7
CRP elevated (≥ 8 mg/L), %				
Baseline	1.2	2.5	3.8	0.0
At the end	0	1.3	0	3.7
IgE elevated (>90 IU/ml), %				
Baseline	97.7	98.7	100	98.8
At the end	76.5^{2}	73.4^{2}	78.5^{2}	72.0^{2}
Underweight (%)				
Baseline	41.9	51.9	50.6	45.1
At the end	33.7^{2}	46.8^{2}	38^{2}	35.4^{2}
Stunting (%)				
Baseline	30.2	31.6	41.8	31.7
At the end	29.1	27.8	29.1^{3}	29.3
Wasting (%)				
Baseline	9.3	16.5	13.9	12.2
At the end	5.8^{3}	17.7	13.9	13.4

1. Significantly different between group (one-way ANOVA): $^{1}p<0.001$

2, 3, 4. Significantly different within group before and after intervention (Mc Nemar); $^{2}p <, 0.001$; $^{3}p <0.01$; $^{4}p<0.05$;

Fe: Iron fortification; MEB: Mebendazole

In the analysis of variance, iron fortified noodles showed a larger change in hemoglobin level (2.6g/L, p= 0.005) than the non-fortified group after taking into account hemoglobin at baseline, thalassemia, age and sex (**Table 4**). Increase of hemoglobin level was greater in younger children compared to older children (β= -1.22, 95%CI -2.2 - -0.2) and sex showed no effect on the improvement of hemoglobin level (β= -0.49, 95%CI -2.27-1.28). There was no interaction effect of iron fortification and de-worming on the improvement of hemoglobin level (data not shown). Children with hemoglobinopathies showed a reduced improvement of hemoglobin of 4.2 g/L (data not shown).

Table 4: Univariate analysis of variance models of hemoglobin, SF, TfR, and body iron at the end of intervention by intervention group, compared to control group.

Outcome variables	Treatment			
	Iron fortification*	p	De-worming*	p
Change in Hemoglobin (g/L)[1]	2.57 (0.80 - 4.34)	0.005	- 0.59 (-2.37 - 1.18)	0.50
Change in SF (μg/L)[2]	16.24 (9.43 - 23.04)	0.001	-5.10 (-11.89 - 1.69)	0.14
Change in TfR (mg/L)[3]	-0.03 (-0.23 - 0.17)	0.70	-0.03 (-0.23 - 0.17)	0.70
Change in body iron (mg/kg)[4]	1.02 (0.66 -1.36)	0.001	-0.07 (-0.43 - 0.28)	0.68

[1] *Adjusted for Hb baseline, thalassemia, sex and age*
[2] *Adjusted for SF baseline, CRP, sex and age*
[3] *Adjusted for TfR baseline, sex and age*
[4] *Adjusted for body iron baseline, CRP, sex and age*
* *Regression coefficients (95% CI)*

In the two-way analysis of variance, iron fortification showed a significant increase of SF (16.2μg/L) while de-worming slightly decreased SF levels (- 5.1 μg/L) after excluding children with CRP≥ 10 mg/L and adjusting for serum SF at baseline, age and sex (**Table 4**). Body iron increased 1mg/kg after 6 months of intervention in children receiving iron fortified noodles (**Table 4**). Both iron fortification and de-worming showed no effect on TfR.

In the logistic regression analysis, iron fortification contributed to a considerable additional reduction in anemia prevalence (OR=0.37, 95%CI= 0.17-0.80, p=0.01), while de-worming showed no effect (OR=0.98, 95%CI= 0.46-2.05, p=0.96).

DISCUSSION

Results of our study did not support the hypothesis that de-worming is more effective than iron fortification in an anemic, infection prone population that was not considered iron deficient. Remarkably, hemoglobin concentrations increased and anemia reduced in all groups, including the placebo group, during the six months intervention period.

Data from our study reconfirmed the previous finding of existence of anemia with low iron deficiency in infection-prone school children in rural Vietnam as indicated by SF and TfR. However, a positive response of hemoglobin levels to iron treatment in anemic subjects is considered to be a clear indicator of pre-existing iron deficiency [27]. Also in our study population, children receiving iron fortified noodles showed a significant improvement in hemoglobin, which indicates iron deficiency. SF is an acute phase protein that may be elevated due to infection or inflammation masking iron deficiency during infection. Malope et al supposed that SF values between 12-100 μg/L can still indicate iron deficiency especially in the presence of infection and inflammation [27]. However, considering the low prevalence of elevated CRP in our study population, it seems unlikely that acute infection is affecting the SF levels. TfR has an advantage over SF as it is unaffected by the acute phase response [28] and is therefore considered as a sensitive indicator of iron status in children [29, 30]. However, the diagnostic cut-offs for TfR used to identify iron deficiency were derived from studies in adults [31]. These cut-offs may not apply to children due to their increased erythropoeisis during growth [32]. Based on a study conducted in Morocco and Cote d'Ivoire, Zimmerman suggests a lower cut-off point for white compared to black Africans [33] although the differences between suggested cut-off points are small. In addition, researchers indicate, regardless of diagnostic cut-offs, modest sensitivity and specificity of TfR in identifying iron deficiency in children [28, 33].

Based on the previous study indicating that *Trichuris* infection was associated with a doubled risk of anemia, we expected an improved hemoglobin level after de-worming. The results of this study however did not support this hypothesis as de-worming showed no effect on Hb levels and iron status. The *Trichuris* infection in our population was classified as mild therefore, the absence of effect is in line with the previous studies showing that blood loss can occur in *Trichuris* infection, but probably becomes significant only in severe infection [34-36]. Mebendazole treatment was given directly to children at school by researchers and field staff, and the two groups receiving Mebendazole showed a larger reduction in prevalence of all three types of worms. Surprisingly, the effects on *Trichuris* in both groups receiving iron were remarkable and indicate interplay between certain helminth infections and iron. More data are becoming available suggesting that iron (and other micronutrients) has important effects on the natural resistance to parasites and that iron supplementation may reduce parasite infection. Studying *Trichuris suis*, Pederson *et al.* [37] found that iron deficiency increased the severity of *T. suis* in pigs. It has recently been documented that adults given supplemental iron have significantly lower reinfection rates of *Trichuris trichiura*, *Ascaris lumbricoides* and *Schistosoma mansoni* infection [38]. The effect was suggested to be due to reduced risk behaviour, to improved immune function or to unfavourable host gut conditions caused by an increased oxidative stress.

Remarkably, Hb increased and anemia reduced in all groups, including the placebo group, during the intervention period. Hemoglobin levels are mainly a function of red blood cell production and turnover, affected by factors other than iron deficiency in our population. A study in school children in North-East Thailand found a high prevalence of anemia without iron deficiency, with hemoglobinopathies, suboptimal vitamin A status and age as the major predictors of hemoglobin concentration [39]. Another study in school-age children in Alaska with 15% anemia and 8% being iron deficient, showed an association between a bacterial infection (helicobacter pylori) and anemia [40]. Data from our study indicated that the prevalence of thalassemia was very low and therefore could not explain the high prevalence of anemia in our study population. Vitamin A status is considered as a major determinant of anemia [41], but sub-samples ($n = 81$) analyzed for serum retinol levels showed a very low prevalence of suboptimal Vitamin A deficiency (8% and 6% with serum retinol concentration <0.70 μmol/L at baseline and after intervention respectively). Other nutrient deficiencies associated with anemia include deficiencies of vitamins B-6, B-12, riboflavin, and folic acid [42] although not all of the causal pathways are yet clearly understood. Vitamin B12 and folic acid deficiency are associated with an increased TfR [43], but we did not observe elevated TfR levels in our study population. The role of other nutrients could not be verified in the present study. Malaria remains another important cause of anemia in tropical countries [44, 45], however, malaria was not present in our study area.

The increase of Hb levels in all groups, including placebo group might be due to an increase in iron intake due to a seasonal change in food habits or an increase in energy intake through the noodles. We do not have reason to believe there is a seasonal increase in iron intake. A food consumption study among a sub-sample of 59 children (data not shown) showed no change in iron intake between October (baseline) and January, but we did not measure iron intake at the end of intervention (May). Although the noodles intervention did increase energy intake in the sub-sample, we do not think this caused the improvement of hemoglobin levels, as the iron supplementation with de-worming group (the 'standard' treatment who did not receive noodles) also showed a hemoglobin improvement (data not shown).

In the absence of aforementioned causes of anemia and the observed improvements in the placebo group, our data may suggest the presence of anemia of inflammation. Firstly, SF in our population was much higher compared to the 50th percentile SF value (28.7 µg/L) for the age group 6-9 years in NHANES III [43], being close to the 90th percentile level of 55.9 µg/L. This may indicate the inflammation is more chronic of nature. Secondly, Malope et al (2001) suggests the use of log TfR:SF ratio to differentiate between iron deficiency anemia (ratio > 2.55) and anemia of inflammation (ratio < 2.55) although the latter ratio was not able to exclude iron deficiency [27]. In our study the ratio of log TfR:SF was 2.06, indicating the existence of anemia of inflammation but being unable to exclude iron deficiency. Children from the placebo group not receiving iron showed increased Hb levels, having a log TfR:SF ratio of 2.07, accompanied by absence of increase (in placebo) or even decrease (in de-worming only) in SF. This indicates a shift of iron from storage to hemoglobin and according to Malope et al [27] this suggests that beside iron deficiency another likely cause of anemia in our children was infection which improved during the supplementation trial. Thirdly, the seasonal burden of infections in developing countries has been recognized for many years by agricultural and health professionals [46]. Mild inflammatory conditions such as upper-respiratory infections and otitis media, which remain common in early childhood, may contribute to anemia [6]. In our population the proportion of children with elevated CRP is very low. However, CRP is a good measure of acute infection or inflammation but less appropriate when conditions are chronic [47]. Earlier a relationship between IgE and respiratory disease was found [48]. In our previous study, *Trichuris* infection was associated with IgE levels that could have confounded the association of *Trichuris* with anemia and hemoglobin concentration. De-worming apparently did not affect IgE levels and if the assumption holds true that another unknown infection reflected by IgE plays a role in the anemia in our study population, this would explain the absence of de-worming effect. In our study, the high level of IgE and the reduction of IgE levels concurrently with reduction of anemia independent of treatment may suggest that chronic infection may and intestinal parasite infection may not play a role in inflammation anemia. Finally, our data indicated that at the baseline 38.7% children reported fever or respiratory infection in the previous two weeks but at the end survey this prevalence reduced to 13.2% (data not shown). We did not find an association between fever and respiratory infection with anemia, however, the reduction in fever and respiratory infection may reflect a general reduction of infection status during the intervention period in our population. This supports our speculation that anemia is associated with chronic infection in our study population and that the anemia reduction observed in the placebo group may be due to a seasonal reduction of chronic infection. However, the role of chronic infections in anemia needs to be further investigated in our population.

Conclusions: Iron fortification slightly improved anemia and iron status in anemic school children in rural Vietnam that were not considered iron deficient. De-worming reduced prevalence of worm infection but has no effect on anemia and iron status. A positive seasonal effect was seen in all treatment groups. Chronic infection or other un-identified factors including a limited iron uptake of heme synthesis may play an important role.

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Chapter 6: Efficacy of iron fortification compared to iron supplementation among Vietnamese school children

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ABSTRACT

The effect of iron fortification is generally assumed to be less than iron supplementation; however, the magnitude of differences in effect is not known. The present study aims to compare the efficacy of these two strategies on anemia and iron status. 425 anemic children in six primary schools in Tam Nong district of Phu Tho province were included in a randomized, placebo-controlled trial comparing two groups receiving iron fortified instant noodles or iron supplementation for 6 months and a control group, with children in all groups having been dewormed. Blood samples were collected before and after intervention for hemoglobin, serum ferritin (SF), serum transferrin receptor (TfR), C – reactive protein (CRP), and hemoglobinopathies analysis. Regression analysis was used to assess the effect of iron fortification and iron supplementation on hemoglobin concentration, SF, TfR, body iron, and anemic status as outcome variables. The improvement of hemoglobin, SF, and body iron level in the group receiving iron fortification was 42% (2.6 g/L versus 6.2 g/L), 20% (23.5 μg/L versus 117.3 μg/L), and 31.3% (1.4 mg/kg versus 4.4 mg/kg) of that in the iron supplementation group. Relative risk of anemia of fortification compared to supplementation was 1.26 (95%CI= 0.34 - 4.63, p=0.73) after adjustment for hemoglobin at baseline, sex, and age. In conclusion, the efficacy of iron fortification based on the change in hemoglobin level is 58% less than that of supplementation due to the lower dose of iron used in fortification compared to supplementation. However, in a population of anemic children with mild iron deficiency, iron fortification should be the preferred strategy to combat anemia.

INTRODUCTION

Anemia is a significant public health problem in Vietnam. The 2000 National Nutrition Risk Factor Survey in Vietnam showed an anemia prevalence of 34% in children under five and 25% in women [1]. No nationally representative data are available on the prevalence of anemia among primary school children in Vietnam; however, a few local studies show an anemia prevalence of approximately 30% [2,3]. Iron deficiency is considered as the major cause of anemia, due to low intake and bio availability of iron in the diet [4,5]. The National Nutrition Survey shows that the mean iron intake of Vietnamese people, which is mainly non-heme iron, only reaches 72% of the RDA [6]. While iron supplementation in itself is highly effective in reducing iron deficiency anemia, the implementation has been characterized by low coverage (15-20%) and non compliance [1].

Food-based strategies are recommended as long-term interventions to address the malnutrition problem in the country [7]. Although it is generally accepted that the increase of consumption of animal products would increase iron intake in the long term, the consumption of animal products in developing countries is sincerely hampered by low socio-economic status [8]. Food fortification is often suggested as one of the most effective and sustainable strategies for increasing iron intake in the general population [4].

Studies on the effect of iron supplements [9-13] or iron-fortified foods [14-18] on indicators of iron deficiency have been carried out, but few studies compare the effect of iron fortification with iron supplementation on the improvement of iron and anemia status. It is generally known that fortification is less effective than supplementation due to differences in iron dose and the bioavailability of iron [19]. However, the extent of the differences in effect is unknown. In a previous study, Baltussen et al suggested that fortification would be 50% less effective than supplementation, but this assumption was not based on an intervention study [19].

The aim of the present study is to compare the effect of iron fortification and iron supplementation on the changes in hemoglobin and iron status in order to assist public health nutritionists in making an informed choice for developing an appropriate strategy to combat iron deficiency and anemia among school children in rural Vietnam.

SUBJECT AND METHODS

STUDY DESIGN AND POPULATION

The study was implemented from November 2004 to May 2005 in six primary schools in Tam Nong district, Phu Tho province, situated 90 km from Hanoi. Selection was based on the high prevalence of anemia and the absence of interventions to control iron deficiency anemia in school children. Children recruited into the study were in grade one to grade three with hemoglobin concentrations < 110g/L but not <70 g/L in an initial hemoglobin-screening study. We excluded children with Hb level less than 70 g/L, because these children were considered as severe anemic and received treatment immediately.

The study concerns a randomized, placebo-controlled double blind parallel trial with a 2×2 factorial design plus standard treatment (iron supplementation and de-worming) and an intervention period of six months. A total of 425 eligible children were randomly assigned to one of five groups (85 per group) receiving: I) iron-fortified noodles and mebendazole (Fe+MEB); II) noodles without iron fortificant and mebendazole (MEB); III) iron-fortified noodles and placebo (Fe); IV) noodles without iron fortificant and placebo (placebo); and V) iron supplementation and mebendazole (Fe tablet+MEB) **(Figure 1).** Sample size was assessed to achieve a statistical power of 95%, based on the expectation that the treatment groups would have a hemoglobin concentration at the end of the study of 5 g/L higher than the control group, assuming a standard deviation of 11 g/L [2]. Furthermore, it was anticipated that some children would drop out during the intervention; therefore, the sample size was increased by 10% at the beginning of the study. In this article, we only concentrate on the effect of the iron fortified noodles (Fe+MEB) compared to that of the standard treatment (Fe tablet+MEB). For this reason, three groups were included in this analysis: (Fe+MEB), (Fe tablet+MEB), and (MEB) as the control group. The effect of iron-fortification and de-worming on iron and anemia status of school children is discussed in another paper [20]. Children were invited to participate in the study, and their parents signed written consent forms. The study was approved by the Scientific Committee of the National Institute of Nutrition and the Ethics Committee of Hanoi Medical University - Ministry of Health.

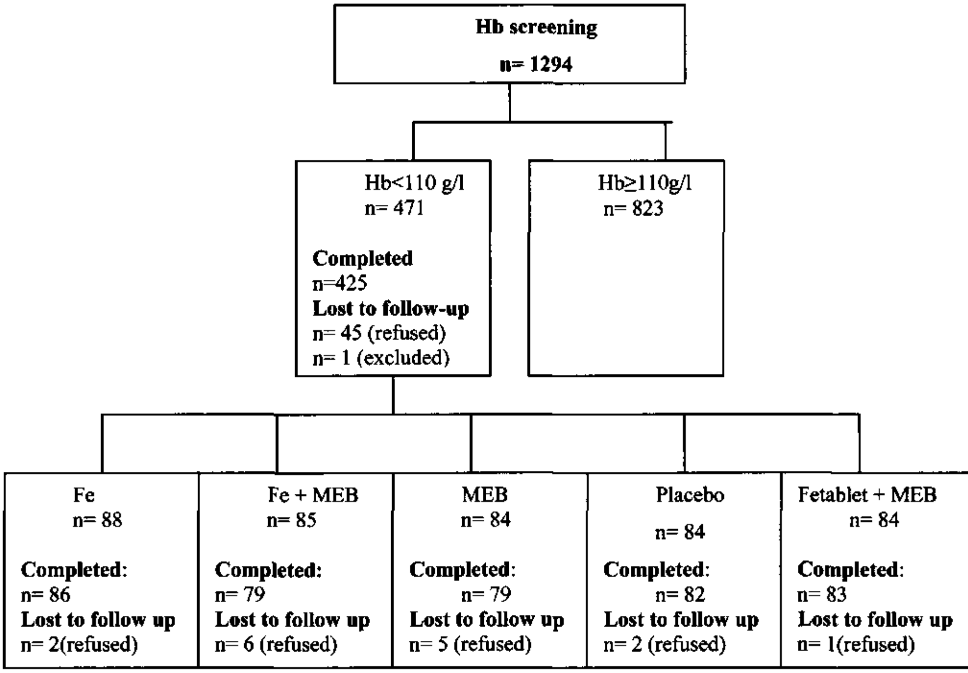

Figure 1: Study profile: initial screening to enroll anemic children in the study, followed by a 6 month intervention.

PRODUCTS AND FIELD PROCEDURES

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

Noodles were prepared in schools by caretakers trained by the field staff and given to children at break time (9:00 am) five days per week during six months under the supervision of teachers and field staff. Children were encouraged to consume all of the noodles and liquid soup. Mebendazole 500 mg was given to children at the beginning of the intervention as well as three months following the intervention. Children, caretakers, teachers, and researchers were not aware of the type of treatment which was given. Iron supplementation in the form of ferrous fumarate 200 mg (equal to 65 mg elemental iron) was taken with a glass of water at break time every school day (five days a week). Ingestion of the supplements was supervised by a teacher and field staff and then recorded in a monitoring book.

DATA COLLECTION

Capillary blood samples were taken from the children's fingers during screening for hemoglobin measurement by cyanmethemoglobin method. Venous blood (5 ml) was collected in the morning at baseline (T0) and after intervention (T6); 20μl whole blood was pipetted immediately before coagulating into a tube containing 5.0 ml of Drabkin's reagent with a Sali pipette for hemoglobin measurement. An aliquot of whole blood was taken for analyzing hemoglobinopathies. The remaining blood was allowed to clot for 30 minutes at room temperature, centrifuged at 3000 x g for 15 minutes, and transferred to five plastic labeled vials (Eppendorf tubes 0.5 ml). The vials were stored in a box protected from sunlight, put into an icebox for transfer to the laboratories, and kept at -80°C for serum ferritin (SF), serum transferrin receptor (TfR), and C-reactive protein (CRP) analysis at the end of the intervention.

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

LABORATORY ANALYSIS

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

DATA ANALYSIS

Anemia was defined as hemoglobin concentrations <115 g/L [24]. Iron deficiency was defined as SF concentrations <12 μg/L [24], and tissue iron deficiency was defined as TfR concentration >8.5 mg/L [25]. Body iron content was calculated using the following formula: body iron (mg/kg) = -(log (TfR/SF ratio)- 2.8229)/0.1207 [26]. CRP concentration was considered to be elevated when ≥ 8 mg/L [27]. Hemoglobin type was determined in each subject on the basis of haematological indexes: HbAA (normal hemoglobin type), HbF, HbA2 (Beta thalassemia), Hb AE (trait for hemoglobin E disease) or HbEE (hemoglobin E disease). The severity of intestinal worm infections was expressed by the number of eggs/g faeces using the WHO classification system [28]. We excluded all children with thalassemia and hemoglobin E (HbF, HbA2, HbAE) (n= 15) and CRP elevated (n= 5) from the analysis to prevent confounding.

Data was entered into the computer, cleaned and managed using Epi Info version 6, [29] and analyzed using SPSS 11.0 for windows (SPSS Inc., Chicago IL, USA) [30]. Data was checked for normality by visual observation. One-way ANOVA was used to determine differences in hemoglobin concentration and other biochemical indicators between groups. Paired *t*-tests were used to assess the difference in hemoglobin and other biomarkers within the group before and after intervention. Chi-square tests and Wilcoxon tests were used to assess the differences between and within groups according to the proportion of anemia and other indicators.

To assess the association between iron fortification, iron supplementation, and indicators of iron status, we compared children with iron fortification only, children with iron supplementation only, and a control group without fortification or supplementation with respect to their change in hemoglobin concentration, SF, TfR, and body iron, respectively. This was done by using multiple linear regression analysis, including two dummy variables for the intervention groups. Logistic regression was used to compare the effect of iron fortification with iron supplementation on anemia prevalence by including two dummy variables, one for iron fortification and another one for the control group, contrasting both with the supplementation group.

RESULTS

At the baseline, the mean age of children was 87.3 ± 10.3 months. The three groups did not significantly differ in age, hemoglobin concentration, iron status (SF, TfR, and body iron) (**Table 1**) or parasite infection (**Table 2**). The prevalence of iron deficiency was very low as 0.9% of children showed SF concentration below 12 μg/L, and 3.2% of children showed TfR above 8.5 mg/L. Mean body iron was around 6.3 mg/kg body weight **(Table 1).** 66%, 71% and 9% of children were infected with *Ascaris, Trichuris*, and hookworm, respectively (**Table 2**).

Hemoglobin concentration increased in all three groups; however, a larger significant increase was seen in the group receiving iron supplementation: 21.2 ± 10.7 g/L compared to 17.8 ± 7.6 g/L and 14.5 ± 8.5 g/L in iron fortified and control groups **(Table 2**). Prevalence of anemia significantly decreased in all three groups with a larger reduction observed in the two groups receiving iron fortified noodles and iron supplementation (73.6% and 77.6% reduction respectively) compared to the control group (68.5% reduction); however, no significant difference was found between groups (**Table 2**).

SF concentration increased significantly in the two groups receiving iron fortification and iron supplementation (18.5 ± 30.9 μg/L and 111.7 ± 76.5 μg/L respectively) compared to the control group where SF concentration even decreased (-6.5 ± 27.1 μg/L) (**Table 2**). TfR concentration was very limited improved after six months of intervention in all three groups; however, the group receiving iron supplementation showed largest improvement (-0.8 ± 0.9 mg/L) compared to iron fortification and control groups (-0.4 ± 0.9 mg/L and -0.4 ± 0.9 mg/L). There were no significant differences between groups (**Table 2**). Body iron significantly increased in the two groups receiving iron fortification and iron supplementation (1.5 ± 1.9 mg/kg and 4.2 ± 1.9 mg/kg respectively) compared to the control group (-0.1 ± 1.6 mg/kg). Prevalence of *Ascaris* and *Trichuris and* hookworm infection fell significantly in all three groups (**Table 2**).

Table 1: Baseline characteristics of Vietnamese school children by group after random assignment (n=221)

	Fe + MEB (n= 72)	Fe tablet + MEB (n= 76)	MEB (Control) (n= 73)
Male (%)	48.6	51.3	46.6
Age in month[1]	87.3 ± 11.6	86.4 ± 9.8	87.9 ± 10.2
Hemoglobin (g/L)[1,3]	107.6 ± 6.9	108.4 ± 6.7	108.9 ± 6.5
SF (μg/L)[2,3]	46.8 (33.3- 66.4)	54.2 (39.7 -72.4)	54.5 (37.8-79.7)
TfR (mg/L)[1,3]	6.0 ± 1.3	5.9 ± 1.1	6.2 ± 1.4
Body iron (mg/kg)[3]	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
Fe + MEB: Iron-fortified noodles and mebendazole
Fe tablet + MEB: Iron supplementation and mebendazole
MEB: Mebendazole

The additional change in hemoglobin in the intervention groups as compared to the control group was estimated by taking into account the baseline Hb value in the regression model, in addition to accounting for age and sex (**Table 3**). Similar differential changes were calculated for SF, TfR, and body iron. The additional improvement of hemoglobin, SF, and body iron level in the group receiving iron fortification was 42% (2.6 g/L compared to 6.2 g/L); 20% (23.5 μg/L compared to 117.3 μg/L) and 31.3% (1.4 mg/kg compared to 4.4 mg/kg) of that in the iron supplementation group (**Table 3**). Compared to the control group, iron fortification and iron supplementation reduced anemia 5.1% and 9.1% respectively (**Table 2**). Relative risk of anemia of fortification compared to supplementation was 1.26 (95% CI= 0.34 - 4.63, p=0.73) after adjustment for hemoglobin at baseline, sex, and age (**Table 4**).

Table 2: Change in hemoglobin, iron status indicators, and worm infection before and after intervention among Vietnamese school children

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

[1]Significant difference between group (one way Anova): [1]$p<0.001$;
[2]Significant difference within group before and after intervention (T test); [2]$p<0.001$;
[3]Significant difference within group after and before intervention (Mc Nemar); [3]$p<0.001$; [4]$p<0.01$; [5]$p<0.05$;
Fe + MEB: Iron-fortified noodles and mebendazole
Fe tablet + MEB: Iron supplementation and mebendazole
MEB: Mebendazole

Table 3: Differential change in hemoglobin, SF, TfR, and body iron at the end of intervention period in two intervention groups compared to the control group, from 4 multiple linear regression models

Outcome variables at end of period	Intervention group			
	Iron fortification*	p	Iron supplementation*	p
Hemoglobin (g/L)[1]	2.59 (-0.22 – 5.40)	0.07	6.19 (3.42 – 8.96)	0.001
SF (µg/L)[2]	23.53 (6.82 – 40.25)	0.006	117.3 (100.86 – 133.64)	0.001
TfR (mg/L)[3]	- 0.04 (-0.32 – 0.23)	0.76	-0.51 (- 0.78 – -0.24)	0.001
Body iron (mg/kg)[4]	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 anemia status at the end of intervention of iron fortification compared to the iron supplementation group

Logistic regression Outcome variable: Anemia status at the end of intervention		
Variable	Prevalence Odds Ratio[1]	p
Iron fortification	1.26 (0.34 – 4.63)	0.73
Control group	2.74 (0.79 – 9.45)	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)*

DISCUSSION

Results from the present study show that in anemic school children, iron fortification was 58% (based on change in hemoglobin level), 80% (based on SF level), and 68.7% (based on body iron) less effective than iron supplementation. However, the risk of being anemic in iron fortification relative to supplementation was only slightly and not significantly increased (OR =1.26, p=0.73).

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

In the present study, the control group also improved hemoglobin and anemia status after 6 months of the intervention which might be explained by the effect of de-worming. However, although de-worming reduced worm infection prevalence, no effect of de-worming on the anemia and iron status could be established [20]. Moreover, a previous analysis suggested that in the absence of other major causes of anemia (like vitamin A deficiency, malaria, and hemoglobinopathies), probably chronic inflammation could have played a role, but this needs to be further addressed [20].

A large part of the selected school children was anemic at baseline (84%) but showed surprisingly few iron deficiency as indicated by the low prevalence of elevated SF and TfR indicators (0.9% and 3.2%). However, an improvement of hemoglobin levels in an anemic population through iron supplementation is commonly seen as an indicator of the presence of iron deficiency [31], and the improvement of hemoglobin levels in our anemic population still indicates possibly mild iron deficiency although not confirmed by the SF and TfR levels.

Compared to the control group, the improvement of hemoglobin concentration was 2.6 g/L and 6.2 g/L, and reduction of anemia was 5.1% and 9.1% in fortification and supplementation, respectively. The improvement of hemoglobin and reduction of anemia in our study population is lower compared to results from other studies. In a study among anemic Vietnamese women consuming daily 10 mL fish sauce containing 10 mg elemental iron from NaFeEDTA during 6 months, hemoglobin changed with 5.7 ± 10.3 g/L and -2.8 ± 8.7 g/L in the intervention and control group respectively [17]. A study in children 12-17 years with mild or moderate anemia in Malaysia reports that after 22 weeks receiving weekly iron supplementation of 60 mg elemental iron (as ferrous sulfate) and 3.5 mg folate, there was an improvement of hemoglobin concentration of 21.4 g/L compared to 9.3 g/L in the control group receiving only 3.5 mg folate [32]. A review of fortification and supplementation studies in Indonesia demonstrates that iron supplementation can reduce anemia prevalence in pregnant women by 20 to 25 % and iron fortification (adding 10 mg of elemental iron) can reduce anemia by 20% among those consuming the fortified foods [33]. As the amount of iron absorbed, and hence the magnitude of improvement of hemoglobin concentration and reduction of anemia, depends on the iron and anemia status of the individual [32], the lower improvement in our study may indicate a mild iron deficiency compared to the study in Vietnamese women (69.9% with iron deficiency anemia) [17].

On the basis of the change in hemoglobin in this population with anemia but mild iron deficiency, iron fortification is 58% less effective than iron supplementation. This reduced efficacy can be explained by the difference in the given amount of iron being lower in the fortification than in the supplementation group. Most of the supplementation programs for women, school-age children or adolescents usually use 60 mg iron/day [34]. However, although in our study the daily amount of iron received from iron-fortified noodles (10.7 mg/day) is 6 times less than from iron supplementation (65 mg/day), the improvement of hemoglobin level in the group receiving iron fortification reaches almost half of the improvement seen in the iron supplementation group (2.6 g/L compared to 6.2 g/L) **(Table 4)**. Also, the risk of being anemic in iron fortification relative to supplementation was only slightly and not significantly increased. Iron stored, as indicated by SF, was 5 times higher in the group receiving supplementation than in the group receiving iron fortification (117.3 μg/L and 23.5 μg/L respectively). However, our study population were anemic children with low prevalence of iron deficiency; therefore, the effect of iron fortification relative to iron supplementation may differ from a population with a high prevalence of iron deficiency.

Food fortification is often suggested as one of the most cost-effective and sustainable strategies for increasing iron intake in the general population [4, 35]. We used NaFeEDTA as iron fortificant in our study. Besides the advantages of NaFeEDTA with regard to iron absorption and stability, the main disadavantage is its relatively high price compared with other fortificants like ferrous sulphate. The price of imported NaFeEDTA was $6/kg. In our study, the additional cost has been estimated to be $ 0.01/kg of instant noodles. This is affordable for people in the rural areas and is comparable to the fishsauce fortification program in Vietnam in which the additional cost of NaFeEDTA fortified fishsauces was $0.02/L [17, 18].

In conclusion, the efficacy of iron fortification based on the change in hemoglobin level is 58% less than that of supplementation due to the lower dose of iron used in fortification compared to iron supplementation. However, in a population of anemic children with mild iron deficiency, iron fortification should be the preferred strategy to combat anemia.

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Chapter 7: General discussion

The present thesis aimed to determine the efficacy of a school-based food fortification program to improve hemoglobin concentrations and iron stores of school children prone to intestinal parasites. Furthermore, this thesis also compared the effect of iron fortification and iron supplementation on changes in hemoglobin and iron status in order to assist public health nutritionists in making an informed choice for an appropriate strategy to combat iron deficiency and anemia among school children in rural Vietnam.

MAIN FINDINGS

A cross-sectional study **(Phase one)** among primary school children (6-8 years old) in Tam Nong district of Phu Tho province showed a high prevalence of anemia with low iron deficiency as indicated by SF (0.5%) and TfR (2%). More than 90% of children were infected by intestinal parasites, and especially *Trichuris* infection was associated with a doubled risk of anemia. Subsequent studies were carried out to evaluate the acceptability and efficacy of iron fortification on anemia in an infection-prone population.

The acceptability study **(Phase two)** showed that instant noodles were an appropriate vehicle for iron fortification; noodles were consumed by half of the children at least once a week and were mainly associated with positive attributes. Although children and adults were able to differentiate between iron fortified and non-fortified noodles, the difference was very subtle, and there was no preference for either one. Although boredom for noodles increased slightly over time, the acceptance ratings remained high.

In the next phase **(Phase three),** we used a randomized placebo controlled double blind trial with iron fortified noodles and de-worming treatment plus standard treatment (iron supplementation and de-worming) to test our hypothesis that de-worming is more effective than iron fortification in an anaemic – infection prone – population that was not considered to be iron deficient. However, results did not confirm the above hypothesis. De-worming did not show an effect on hemoglobin level and iron status, while the consumption of iron fortified noodles slightly but significantly improved hemoglobin concentration, SF, and body iron, with no effect on TfR levels. Remarkably, a seasonal improvement in hemoglobin levels and anemia status occurred in all groups, including the placebo group. Furthermore, the efficacy of iron fortification based on hemoglobin change was 58% less than that of supplementation. ***Table 1*** describes the main findings of this thesis.

Table 1: **Main findings of the research conducted in primary school children aged 6-8 years in Tam Nong district, Phu Tho province, Vietnam from 2003-2005**

Phase 1 ***Cross sectional study***	• Anemia (Hb<115g/L): 25% • Iron deficiency: SF< 12μg /L: 0.5%; TfR>8.5 mg/L: 2% • Worm infection: 92% (Ascaris: 71%; Trichuris: 76%; Hookworm: 6%) • Energy intake: 5.1 $\pm$ 1.7 MJ (67% RDI); Iron intake: 7.5 $\pm$ 4 mg (46% RDI) • Prevalence odds ratio of *Trichuris* infection for anemia was 2.02 (95% CI: 1.11- 3.68)
Phase 2 ***Acceptability study***	• 50% consumed instant noodles at least once a week • Mainly positive attributes for instant noodles • Non-fortified instant noodles were not preferred above fortified instant noodles when seasoning is added • Slight increase in boredom • Acceptance ratings remained high; boredom ratings remained low
Phase 3 ***Efficacy study***	• Hb and anemia status improved in all groups, including the placebo group • SF and body iron improved in the fortification and supplementation groups, not in de-worming only and placebo groups • Improvement in iron fortification compared to placebo group of Hb: +2.6 g/L(2.4%), SF: +16.2 μg /L(32.3%); body iron: +1 mg/kg (16.1%) • De-worming showed no effect on Hb • Fortification 58% less effective in Hb improvement compared to supplementation

METHODOLOGICAL CONSIDERATIONS

In order to judge the present study on its scientific relevance, we first evaluate the methodology. We made several decisions about our selection of subjects, our study design, our measurements and our data analysis. In the following paragraphs, we discuss each topic and hypothesize how the decision we made could have influenced the validity of our results.

Selection of study population

The research described in this thesis was carried out in a poor, rural, agricultural-based district in northern Vietnam **(Chapter 1)**. This area was selected because a previous study showed high prevalence of anemia among pregnant women (24.5%) and young children from 6-23 months (68%) [1] and because there was no anemia intervention program for school children. We think that the study population is appropriate for the purpose of our research; however, the study area is located in northern Vietnam and may not be representative of the situation of primary school children in the whole country because of different environmental and socio-economical regional contexts.

In our study, we included all 20 primary schools in the district. For the cross sectional study, 400 primary school children were selected from the school lists using systematic sampling where all children had equal chance to be included in the study. As 92% of Vietnamese children attend primary school [2], the selected sample in our studies represents the school children in the district.

Based on the findings of the cross sectional study, 6 primary schools were selected for the intervention study. The criteria for selection was high prevalence of anemia (above 20%), high commitment of school staff, and representative for low, medium and high economic status in the area. All 1294 children from grade one to grade three in these six schools were screened for hemoglobin concentration using finger punctured blood. The screening showed 849/1294 children having Hb <115 g/L (65.6%); this percentage was much higher compared to the prevalence of anemia found in the cross sectional study (25%) [3].

We suspect that the difference of results could have been caused by the technique of obtaining finger punctured blood. Strong squeezing can cause tissue fluid to be expressed, compromising specimen integrity which may produce inaccurate results [4]. A previous study showed a mean difference between duplicate skin puncture hemoglobin measurements of 5 g/L, with 10% of paired values differing by $\geq$ 10 g/L, and it was concluded that analysis of single drops of blood, even by experienced personnel, can give rise to misleading results [5]. Therefore, we decided to include children with an Hb below 110 g/L (471 children) in the trial. We do not think that we missed any anemic children, and hemoglobin levels were checked with venous blood at the beginning of the intervention.

425/471 children were involved in the baseline. 46 (9.7%) children did not participate in the baseline because 4 of them moved to another district, 3 were sick (1 was seriously thalassemic), and 40 others refused to give blood due to fear. We do not think that the absence of these children affected our study results because the mean Hb at the screening did not change when excluding these children (103.2 $\pm$ 5.8 g/L and 103.5 $\pm$ 5.4 g/L for 471 and 425 children respectively). 425 children were then randomly allocated into 5 groups in the study **(Chapter 5).**

Study design and potential confounders

The study concerns a randomized, placebo-controlled double blind parallel trial with a 2×2 factorial design plus standard treatment (iron supplementation and de-worming). We selected a 2x2 factorial design because we were interested in the efficacy of two different treatments separately (de-worming and iron fortification) as well as their interaction. A 2x2 design also includes a placebo group (absence of both treatments) which enables us to correct any unexpected factors influencing the outcome variables during the study period. At the start of the study, we elaborated on potential confounders (sex, age, CRP elevated) to be included in the baseline and endline measurements. We minimized the effect of (un)measured confounders by the stratified randomization procedure based on Hb levels and age. Therefore, we assume that all the expected (sex, age, CRP) and non-expected potential confounders are comparably distributed in the treatment groups by the randomization process. Furthermore, during analyses we adjusted for other measured factors (like CRP elevated) that may have affected the relationship between the outcome variable and main factors.

All the steps in our research- from selecting study population, designing the study, and collecting data- were conducted in an appropriate way; therefore, we believe our results reflect the real conditions of the study population. We questioned ourselves whether or not there could have been a systematic error in hemoglobin measurement, as Hb was measured directly after drawing blood, while analysis of other blood parameters was done at the end of the intervention. However, Hb was measured in vein blood by experienced staff from National Institute of Nutrition. Data from the cross sectional study and the baseline study were both based on vein blood samples in the same period (October 2003 and October 2004) and showed a similar anemia prevalence in this population. Therefore, we do not believe there was a systematic error in Hb determination.

Randomization and blinding of the study

Randomization was carried out by a researcher from the Division of Human Nutrition, Wageningen University, The Netherlands, who did not know the children and could not introduce bias in the randomization. Stratified randomization was applied based on the Hb levels and age of the children to ensure equal distribution of Hb and age across groups. Randomization was successful, as at the baseline of intervention the groups were comparable for main outcome variables such as Hb, SF, TfR, and body iron as well as for main potential confounders such as age and sex (**Chapter 5**).

To avoid information bias, subjects, project staff and data analysis were kept unaware of the treatment allocation. Noodles were produced and labelled under the supervision of a researcher from the Nutrition Department, Hanoi Medical University. Labelling was done for each package of noodles as well as for the whole box of noodles (which contains a total of 30 packages). The color of noodles differed slightly between iron fortified and non fortified **(Chapter 4).** In order to ensure that the children could not recognize the difference between iron fortified and non fortified noodles, they were assigned to a specific chair and table from the beginning of the intervention; each group had its own table to make sure there was no mixing between the groups and to verify that the children could not exchange or compare the noodles with each other. Mebendazole and placebo were labelled by the same researcher from the Nutrition Department, Hanoi Medical University. Children, teachers, caretakers, researchers, and field staff were blinded for the treatments.

However, after taking mebendazole, some children discovered that they did not see worms in their faeces like children in other groups. This may have caused that blinding of de-worming was not complete. The treatment groups were un-blinded after data collection, measurement, and analysis was carried out. The results, therefore, correctly reflect the effect of each treatment in our study.

Study duration and compliance

Our intervention study was carried out during a six month period. This duration should be enough to observe the effects of the treatments as shown by previous studies. Iron deficiency decreased from 70% to 25% in Vietnamese women who consumed 10mg iron per day from NaEDTA fortified fish sauces over a period of 6 months [6]. A study using NaFeEDTA fortified soy sauce in China among women showed high significant improvement of Hb and anemia status after 6 months of intervention [7]. A series of studies on fortification of foods and beverages with iron in Brazilian children also showed effects after 6 months of intervention [8].

During the intervention, 100% of our study population consumed the given noodles or iron tablet. More than 95% of children were present every schoolday (5 days a week) and more than 95% finished the whole portion of the noodles given to them daily under the supervision of caretakers and field staff (based on notes taken by caretakes in a monitoring book). The duration of study and food compliance therefore did not affect the validity of the research.

Measurement

Defining iron status in developing countries is a challenge. Hemoglobin is often used to screen for anemia as a proxy for iron deficiency because of its simplicity and low cost. However, hemoglobin detects only the late stages of iron deficiency and its specificity is poor, particularly in developing countries. Biochemical tests for serum iron, total-iron-binding capacity (TIBC), transferrin saturation, serum ferritin (SF), serum transferrin receptor (TfR), and erythrocyte zinc protoporphyrin (ZnPP) are more specific than hemoglobin measurement. However, their interpretation in developing countries is limited by the confounding effects of infection, inflammation, and malnutrition [9]. SF, ZnPP, and TfR are recommended for the assessment of early stages of iron deficiency [10]. To improve specificity, SF is often combined with TfR or ZnPP, or both [9]. Based on these recommendations, we decided to use Hb, SF and TfR to assess the anemia and iron status of our study population.

Blood samples were collected by well-trained staff from the Pediatrics Hospital and National Institute of Nutrition Vietnam. Hemoglobin concentration was measured in whole vein blood within 12 hours of sampling. Serum samples were transported and stored under standard conditions. Serum samples before and after intervention were analyzed at the same time after intervention for SF, TFR, CRP, and IgE to avoid variation between different measurements. Re-analysis of sub samples for SF and TfR were carried out at SHO (Stichting Huisartsenlaboratorium Oost, Velp, The Netherlands) and gave similar results.

Assessment of worm infection was based on the eggs count by the Kato-Katz method. All the parasitological analysis was carried out on the same day the samples were collected at the District Health Center by parasitologists from Institute of Parasitology, Vietnam. A 10% sub sample of smears was re-examined for quality control.

Iron contents in the fortified and non-fortified noodles was double checked in the laboratories of the National Institute of Nutrition and Wageningen University and showed similar results.

Iron intake has a large day-to-day variation [11]. A single 24-hour recall is therefore not sufficient to describe an individual's usual iron intake [12]. Records of 12 days are required to be within 10% of the mean iron intake of an individual [11]. However, fewer days are required to estimate the intake of a group of individuals with a specified degree of error [13]. In the present study, the repeated 24-hour recall [11] on two non-consecutive days was used to measure the food and nutrient intake of children in rural North Vietnam **(Chapter 3)**. Because the interest of the study was in the average intake of a group, this seems to be enough to estimate the usual energy and macronutrient intake. For iron, however, the measurement of two days might not be enough to estimate the usual iron intake because of the large within person day-to-day variation.

DISCUSSION OF MAIN FINDINGS

THERE IS A HIGH PREVALENCE OF ANEMIA BUT LOW IRON DEFICIENCY AMONG PRIMARY SCHOOL CHILDREN IN RURAL NORTH VIETNAM.

The prevalence of anemia in our study population was 25%, which is in line with other local studies (around 30% anemia in primary school children) [14] [15]. However, the prevalence of iron deficiency was very low. A study among school children in northeastern Thailand showed a high prevalence of anemia without iron deficiency, with hemoglobinopathies, suboptimal vitamin A status and age as the major predictors of hemoglobin concentration [16]. A study in school-age children in Alaska showed 15% anemia with 8% being iron deficient, with bacterial infection (helicobacter pylori) as a contributor of anemia [17]. Another study in school children in the Aral Sea Region of Kazakhstan, showed the prevalence of anemia to be 49.8% although levels were mostly mild. Of the anemic children, 32.4 % were found to have iron deficiency and nearly two-thirds of the anemia cases could not be explained by iron [18]. Data from our study indicates that the prevalence of thalassemia was very low (7%) and therefore could not explain the high prevalence of anemia in our study population. Vitamin A status is considered as a major determinant of anemia [19], but sub samples analyzed for serum retinol levels showed a very low prevalence of suboptimal Vitamin A deficiency (8%) in our population **(Chapter 5)**. Other nutrient deficiencies associated with anemia include deficiencies of vitamins B-6, B-12, riboflavin, and folic acid [20] although not all of the causal pathways are yet clearly understood. Vitamin B12 and folic acid deficiency are associated with an increased TfR [12], but we did not observe elevated TfR levels in our study population. The role of other nutrients could not be verified in the present study. Malaria remains another important cause of anemia in tropical countries [21, 22]; however, malaria was not present in our study area.

To define iron deficiency, we applied the following cut-off points: 12 μg/L for SF and 8.5 mg/L for TfR. SF is an indicator of body storage iron. Iron deficiency leads to lower iron stores: SF values fall progressively as the stores decline, before the characteristic changes in serum iron and total iron–binding capacity (TIBC) [12]. SF identifies absent iron stores when the level is below 12 μg/L [23]. However, it is well known that SF, being an acute phase protein, is elevated during infection and inflammation, masking iron deficiency during infection. Malope et al supposed that SF values between 12-100 μg/L can still indicate iron deficiency especially in the presence of infection and inflammation [23]. In our study, CRP elevated was very low, so the high SF levels found in our study could not be due to acute infection.

Circulating transferin receptor (TfR) has an advantage over SF as it is unaffected by the acute phase response [24] and is therefore considered as a sensitive indicator of iron status in children [10]. Defining normal value for TfR in healthy subjects is difficult because TfR values and the units of expression vary with the assay; even different kits based on the ELISA technique are associated with a different normal range. This makes comparison of results across studies difficult [25]. The diagnostic cut-offs for TfR used to identify iron deficiency were derived from studies in adults [26]. These cut-offs may not apply to children due to their increased erythropoeisis during growth [27]. Based on a study conducted in Morocco and Cote d'Ivoire, Zimmerman suggests a lower cut-off point for white compared to black Africans [9] although the differences between suggested cut-off points are small. In addition, research indicates modest sensitivity and specificity of TfR in identifying iron deficiency in children regardless of diagnostic cut-offs [9, 24]. The cut-off value of TfR used in our study follows the recommendation for the analysis kit based on the ELISA technique. However, we are not sure whether the recommended cut-off value is appropriate for a high risk infection population as in our study population.

The TfR and SF data indicated very low iron deficiency, but the increase in ferritin (32.3%) and hemoglobin (2.4%) with iron fortification treatment suggests that mild iron deficiency is still present in our population. After 6 months of intervention, both groups receiving iron fortification and supplementation showed improvement of Hb and iron status **(Chapter 5 and 6).** A positive response of hemoglobin levels to iron treatment in anemic subjects is considered to be a clear indicator of pre-existing iron deficiency [23]. Moreover, results of the cross sectional study indicated a low energy intake of children covering only 67% RDI and low iron intake covering 46 % RDI **(Chapter 3).** In addition, data from a previous study in the same area also found that iron supplementation (daily for pregnant women and weekly for young children) improved anemia status of pregnant women (from 25.4% to 12.5%) and children (from 68% to 31.7%) after 7 months [1]. Therefore, we believe that our population is still mildly iron deficient and that anemia is partly caused by iron deficiency.

INTESTINAL PARASITE INFECTION DOES NOT PLAY A ROLE IN ANEMIA IN OUR STUDY POPULATION.

High prevalence of intestinal parasite infection in our population was also shown in other studies among school children in Vietnam [15] [14] [28]. Although *Trichuris* doubled the risk of anemia in the cross sectional study, de-worming reduced worm infection in the intervention study but did not show an effect on anemia status. This finding indicated that intestinal parasite infection does not play a role in anemia in school children in Vietnam. Previous studies also found only an association of hookworm infection with anemia not of *Trichuris* nor *Ascaris* infection [29-32]. This may indicate that the association between *Trichuris* infection and anemia found in the cross sectional study might be due to another un-identified factor associated both with *Trichuris* and with anemia. This un-identified factor may be related to IgE, as in the cross sectional study *Trichuris* infection was associated with IgE, and the IgE elevated group showed lower hemoglobin concentration than the non IgE elevated group **(Chapter 2).** In the intervention study, the improvement of hemoglobin concentration was concurrent with the reduction of elevated IgE, even in the placebo group **(Chapter 5)**. However, in our study we could not further confirm this speculation.

Mebendazole treatment was given directly to children at school by researchers and field staff, and the two groups receiving Mebendazole showed a larger reduction in prevalence of all three types of worms **(Chapter 5)**. Surprisingly, the effects on *Trichuris* in both groups receiving iron were remarkable and indicate interplay between certain helminth infections and iron. More data are becoming available that suggest that iron (and other micronutrients) has important effects on the natural resistance to parasites and that iron supplementation may reduce parasite infection. Pederson et al [33] found that iron deficiency increased the severity of *Trichuris suis* in pigs. It has recently been documented that adults given supplemental iron have significantly lower reinfection rates of *Trichuris trichiura*, *Ascaris lumbricoides* and *Schistosoma mansoni* infection [34]. The effect was suggested to be due to reduced risk behavior, to improved immune function or to unfavorable host gut conditions caused by an increased oxidative stress.

In conclusion, the association between *Trichuris* and anemia found in the cross sectional study may be an artifact, and an un-identified factor may be the relevant determinant of anemia.

HEMOGLOBIN IMPROVED REMARKABLY IN ALL TREATMENT GROUPS, INCLUDING THE PLACEBO GROUP.

After the 6 month intervention, the improvement of hemoglobin in all groups, including the placebo group, surprised us. We considered different possibilities which may explain this observation.

Was the improvement of the placebo group due to the seasonal increase in nutrient intake?

During the intervention study, the baseline measurements were conducted at winter time (October), and the endline survey was conducted during summer time (May). The nutrient intake could have increased during the 6 months of intervention. A food consumption study among a sub sample of 59 children (data not shown) showed that in the group not receiving noodles, an increased intake of all nutrients from October to January, with a significant increase for energy (Δ129 kcal ± 312 kcal), fat (Δ10.8 gram ± 12.3 gram) and protein (Δ 4.8 gram ± 11.2 gram) (data not shown). However, no change in iron intake was found between October and January. Although we did not measure iron intake at the end of the intervention in May, we do not have reason to believe that there was a seasonal increase in iron intake. The fat intake in January was almost doubled compared to the intake in October, probably caused by the change in weather from October with a mild climate to January with a cold climate in Vietnam. Lard, as the main fat source in the diet of our study population, changed from liquid in October to solid in January. The population continued to use the same portion size for lard during cooking in both seasons, and the portion size of solid lard provided almost a double amount of fat compared to the same portion size of liquid lard. The increased energy intake was also reflected by the improvement of nutritional status as indicated by a reduction in prevalence of underweight of children from 47.2% at the baseline to 38.3% at the endline **(Chapter 5).** However, the results from the intervention did not show an association of nutritional status with anemia or hemoglobin. Therefore, the intake of nutrients and improvement of nutritional status may not have a role in the improvement of anemia.

Did instant noodles play a role in the improvement of hemoglobin?

We questioned whether the extra energy and nutrients supplemented in the noodles could have caused the improvement of hemoglobin. Data from a sub-study comparing intake of children receiving noodles with those not receiving noodles showed that the intake of energy and fat of the noodle group are significantly higher while the carbohydrate and protein intake are equal to the intake in the non noodle group after two months of intervention. Although the noodles intervention did increase energy and fat intake in the sub-sample, we do not think this caused the improvement of hemoglobin levels, as the iron supplementation with the de-worming group (the "standard" treatment who did not receive noodles) also showed a hemoglobin improvement.

Do the high levels of SF and IgE suggest anemia of chronic infection in this population?

In the absence of aforementioned causes of anemia, our data may suggest the presence of anemia of inflammation. Acute-phase proteins like CRP are often used as markers for infection or inflammation. However, in our population, the proportion of children with elevated CRP is very low as indicated by the data at the baseline **(Chapter 5).** As CRP is a good measure of acute infection or inflammation (but less appropriate when conditions are chronic) [35], this may indicate the inflammation in our population is more chronic in nature. Also SF in our population was much higher compared to the 50th percentile SF value (28.7 μg/L) for the age group 6-9 years in NHANES III [12] being close to the 90th percentile level of 55.9 μg/L. Malope et al (2001) suggests the use of log TfR:SF ratio to differentiate between iron deficiency anemia (ratio > 2.55) and anemia of inflammation (ratio < 2.55) although the latter ratio was not able to exclude iron deficiency [23]. In our study, the ratio of log TfR:SF was 2.06, indicating the existence of anemia of inflammation but being unable to exclude iron deficiency. Children from the placebo group not receiving iron showed increased Hb levels, having a log TfR:SF ratio of 2.07, accompanied by absence of increase (in placebo) or even decrease (in de-worming only) in SF. This indicates a shift of iron from storage to hemoglobin, and according to Malope et al [23], this suggests that besides iron deficiency another likely cause of anemia in our children was infection, which improved during the supplementation trial. In our study, elevated Immunoglobulin E (IgE) was associated with higher SF concentration (mean SF = 33.4 ± 18.2 μg/L in non IgE elevated and 61.4 ± 37.5 μg/L in IgE elevated group at baseline). IgE is one of the body's 5 classes (isotopes) of immunoglobulins (antibodies). Like other immunoglobulins, IgE is produced by B cells and plasma cells [36]. Elevated IgE levels occur in several helminthic infection (Schistosoma, Ascaris lumbricoides, etc), allergic disease (eczema, Asthma, allergic rhinitis, etc), inflammatory diseases (Kawasaki disease, Periarteritis nodosa disease), and infectious disease (leprosy, Bronchopulmonary aspergillosis, Aspergilloma) [36]. In our study, the high level of IgE and the reduction of IgE levels concurrently with reduction of anemia independent of treatment suggested that a positive seasonal effect of chronic infection may play a role in inflammation anemia in our school children. Recent studies discovered a liver-made peptide hepcidin [37] as a key mediator of anemia of inflammation [38]. It is suggested that the inflammation cytokine IL-6 induces hepcidin during inflammation, thereby reducing iron absorption and iron recycling leading to anemia of inflammation [39]. However, the role of inflammation, hepcidin and interleukin-6 in Vietnam needs to be further investigated.

INSTANT NOODLES ARE A SUITABLE VEHICLE FOR IRON FORTIFICATION AS THEY DID NOT CHANGE THE PREFERENCE AND WERE ACCEPTED BY CHILDREN IN A LONG TERM TRIAL.

A fortification program can only be successful if consumers accept the fortified product; therefore, the effect of the added iron fortificant on the sensory quality of the food product should be minimal [40, 41]. Also, the fortified product should be accepted for a long period. Besides rice, noodles are a staple cereal food of Vietnamese people. The 2000 National Nutrition Survey showed that on average Vietnamese people consumed 44.7gr/capita/day [42]. Moreover, another study showed that consumption of instant noodles has risen steadily since 1995 in Vietnam and some other Asian countries [43]. Data from our study shows that noodles were consumed by half of the children at least once a week and were mainly associated with positive attributes **(Chapter 3)**. Although children and adults were able to differentiate between iron fortified and non-fortified noodles, the difference was very subtle, and there was no preference for either one. Although boredom for noodles increased slightly over time, the acceptance ratings remained high during the trial **(Chapter 4).**

The stability of iron fortification is also important for the success of a fortification program [40]. Data from our study showed that the production process of instant noodles, including deep frying in oil under high temperatures (140-160^{0}C), did not affect the iron content of instant noodles **(Chapter 3)**. A previous study also mentioned that NaFeEDTA stays stable when it is exposed to heat or humidity [44]. From our findings and information from other studies mentioned above, we believe that noodles fortified with NaFeEDTA are a suitable vehicle to be used in an iron fortification program in Vietnam to combat anemia and iron deficiency.

ALTHOUGH IRON UPTAKE AND STORAGE THROUGH SUPPLEMENTATION IS LARGER THAN THROUGH IRON FORTIFICATION, IRON FORTIFICATION SHOULD BE THE PREFERRED STRATEGY TO COMBAT ANEMIA IN A POPULATION OF ANAEMIC CHILDREN WITH MILD IRON DEFICIENCY.

It is generally accepted that iron supplementation in itself is highly effective in reducing iron deficiency anemia, but low coverage and compliance reduces effectiveness of iron supplementation. Food fortification is often suggested as one of the most effective and sustainable strategies for increasing iron intake in the general population [45]. In making a choice for an intervention strategy, it should be kept in mind that the allowed iron level in fortification is always lower than that in supplementation. We used the maximum acceptable iron level for fortification using NaFeEDTA for school-age children (10.7 mg). The iron dose used in supplementation was 65 mg **(Chapter 6)**. Although the iron level in fortification was only 17.6% that of supplementation, we found that after 6 months of intervention the improvement of hemoglobin was 42% (2.6 g/L versus 6.2 g/L), SF was 20% (23.5 μg/L versus 117.2 μg/L), and body iron was 31.3% (1.4 g/kg versus 4.4

g/kg) in the group receiving an iron fortification compared to the iron supplementation group **(Chapter 6)**. NaFeEDTA was used as iron fortificant in the present study.

In addition to the advantages of NaFeEDTA concerning iron absorption and stability, the main disadavantage is the relative high price compared to other fortificants like ferrous sulphate. However, the additional cost has been estimated to be $0.01/kg instant noodles in our study. With the price of noodles at $1.25/kg, this corresponds with an increase of 0.8%. The increase in price of iron fortified noodles was comparable to that of iron fortified fish sause in the fish sauce fortification program in Vietnam [6], and as experienced in this fish sauce program, the additional cost was affordable and acceptable for people in rural areas of Vietnam **(Chapter 6)**. In view of the effect of fortification despite the low iron level and the affordability of the additional costs, iron fortification should be the preferred strategy to combat anemia in a population of anemic children with mild iron deficiency who probably do not need iron supplementation.

CONCLUSION

MAIN CONCLUSIONS

Although iron deficiency was considered as the main cause of anemia in developing countries, our study among school children in rural Vietnam showed that anemia is only partly caused by iron deficiency.

Although the prevalence of iron deficiency was low, iron fortification slightly but significantly improved anemia and iron status among primary school children in rural Vietnam.

Parasite infection was highly prevalent in Vietnamese school children, but it does not seem to play a role in anemia.

Instant noodles are a suitable vehicle for fortification with NaFeEDTA as it did not change the preference and acceptance by children.

Although iron level was six times less in fortification compared to supplementation, the effectiveness of iron fortification is 42% that of iron supplementation. However, in a population of anemic children with mild iron deficiency, iron fortification should be the preferred strategy to combat anemia.

PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH

The knowledge gained by the present thesis could be of use for health professionals and those working in public health nutrition.

To combat anemia and iron deficiency, it is necessary to have multiple intervention strategies. Iron supplementation is a main intervention program applied in Vietnam for women and young children. From this study, however, we learned that even with a high prevalence of anemia, our children had mild iron deficiency. Iron supplementation may therefore not be necessary as a public health intervention to reduce anemia in school children in rural Vietnam. De-worming showed no effect on anemia and hemoglobin level in our study population, but a regular school de-worming program should be encouraged to reduce worm infection burden and general discomfort for the children.

Based on the findings of the present study, we recommend to the Vietnamese Government to support a school feeding program with iron fortified staple foods because of the following reasons: Firstly, the children in our study only ate three main meals a day with no snacks between meals. The energy and nutrient intake was low, and prevalence of malnutrition was high **(Chapter 2).** WHO recommends that children from two years and older should have a nutritious snack twice a day in addition to three main meals [46]. Therefore, an extra snack offered at school is recommended to improve the nutritional status for better school performance. Previous studies also show that providing school meals improved cognition, behavior, and school attendance [47-52]. Secondly, the children in our study showed a low prevalence of iron deficiency. However, these children are in their pre-adolescent period during which iron storage is essential, especially for adolescent girls [53]. Therefore, a school feeding program with iron fortified food may contribute to an increase of energy as well as iron and other nutrients for school children. Thirdly, NaFeEDTA fortified instant noodles should be considered as an iron fortified food to be offered at school in order to improve energy and iron intake of school children in rural Vietnam. Both children and parents in our study population prefer to have a staple food fortified with iron rather than biscuit or other products at school. Besides rice, noodles are a common staple food consumed in the local diet. Our data also indicated that instant noodles are a suitable vehicle for fortification with NaFeEDTA as it does not change the preference and acceptance of children and improves hemoglobin and anemia of our children. Moreover, it appears to be easy to prepare and serve instant noodles to children in a school setting.

Communication for nutrition behavior change to improve dietary diversity (including animal foods and other iron-rich food) is an important strategy [53] as dietary diversity is associated with improved nutrient adequacy [54]. In addition to a schoolfeeding program, a health and nutritional education program may help children to perform improved eating practices and hygienic behaviors and may assist in improving nutritional and anemia status.

The present thesis provides answers to some prominent questions; it also raises more questions that need to be answered by other research studies. The high prevalence of anemia among our study population with low iron deficiency not related to hemoglobinopathies, vitamin A deficiency or malaria as well as the low proportion of children with elevated CRP may suggest the existence of chronic infection or inflammation anemia. However, chronic infection/inflammation in school children and its association with anemia has never been studied in Vietnam. In a previous study a relationship between total serum IgE and respiratory disease was found [55]. We do not have any data about the disease pattern of our children, but upper respiratory infection and rheumatic infection are very common among children in Vietnam. These diseases are often related to the change in climate and season. Recent studies have found that a liver-synthesized peptide hepcidin acts as a key mediator of anemia of inflammation [38]. It is suggested that the pro-inflammatory cytokine IL-6 induces hepcidin during inflammation, thereby reducing iron absorption and iron recycling leading to anemia of inflammation [39]. However, the potential role of chronic inflammation in anemia and the interaction between hepcidin and interleukin-6 during inflammation needs to be further assessed in the specific situation in Vietnam.

Our study indicated that NaFeEDTA fortified noodles in a school feeding program improved the iron and anemia status of school children in a period of 6 months. Concerning the disadvantages of NaFeEDTA related to costs and supply, other iron compounds may be cheaper and more available. However, we do not know whether these compounds have effects on hemoglobin status comparable to those of NaFeEDTA when used as iron fortificant in noodles. In their study, Zimmermann et al compared the efficacy of wheat snacks fortified with different compounds and indicated that relative efficacy of the electrolytic and hydrogen-reduced iron compared with ferrous sulfate was 77% and 49% respectively [56]. Future research should investigate the efficacy of different iron fortificants of noodles on anemia and iron status of school children in rural Vietnam.

The present study estimates a slight increase in the cost of NaFeEDTA fortified instant noodles. However, to estimate the cost–effectiveness of a school feeding program, costs related to management, legislation, health education and supervision should be included [57]. Also, the effect of NaFeEDTA was only measured in a relative short period. Future research should investigate the long term cost–effectiveness of iron fortified staple food like noodles to reduce anemia among school children in rural Vietnam.

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Summary

Anemia is a public health problem in school children in Vietnam. As indicated by several local studies the prevalence of anemia among primary school children in rural Vietnam is around 30%. Intestinal parasite infection is also highly prevalent among school children, especially *Trichuris* and *Ascaris* infection. It is generally accepted that iron deficiency is a major cause of anemia in developing countries. However, anemia can also be caused by health factors such as parasite infection or hemoglobinopathies and chronic infection. It is well-known that anemia impairs cognitive function and affects the psychomotor performance of children. An intervention to improve the anemia status for school children in rural Vietnam is necessary.

This thesis aimed to determine the efficacy of a school-based food fortification program to improve hemoglobin concentrations and iron stores of intestinal parasites-prone school children. Furthermore this thesis also compares the effect of iron fortification and iron supplementation on the changes in hemoglobin and iron status in order to assist public health nutritionists in making an informed choice for an appropriate strategy to combat iron deficiency and anemia among school children in rural Vietnam.

The research comprising three phases was carried out in Tam Nong District of the Phu Tho Province from October 2003 till September 2005. The cross sectional study **(Phase one)** was carried out to measure the prevalence of iron deficiency anemia and intestinal parasite infection and their relationship in Vietnamese rural school children. An acceptability study **(Phase two)** was carried out to assess the (short/long term) acceptability of consumption of iron-fortified food by rural school children. A randomized control trial **(Phase three)** was undertaken to measure the efficacy of iron-fortified food to iron and anemia status of rural school children and to compare the effects of consumption of iron-fortified food with the 'golden standard' treatment (iron supplementation + de-worming) on iron and anemia status.

400 primary school children age 6-8 years from 20 schools in the Tam Nong district were included in the cross sectional study **(Chapter 2).** Blood samples were analysed for serum ferritin (SF), transferrin receptor (TfR), C-reactive protein (CRP) and total immonoglobulin E (IgE). Stools samples were examined for Hookworm, *Trichuris*, and *Ascaris* infection. The prevalence of anemia was 25%. Iron deficiency (TfR >8.5mg/L) occurred in 2% of the children. The prevalence of intestinal parasites was 92% with the

highest prevalence for *Trichuris* (76%) and *Ascaris* (71%). More than 30% and 80% of the children showed an elevated CRP ($\geq$ 8 mg/L) and IgE (> 90 IU/ml) concentration respectively. Anemia status was not associated with SF, TfR and CRP. The prevalence odds ratio for *Trichuris* infection was 2.02 (95% CI: 1.11- 3.68). It is concluded that anemia is highly prevalent among school children in Vietnam but not associated with iron deficiency. *Trichuris* infection is associated with a doubled risk of anemia, not mediated through iron deficiency.

Subsequent studies were carried out to evaluate the acceptability and efficacy of iron fortification on anemia in an infection prone population. An acceptability study aimed to identify an appropriate vehicle for iron fortification to be used in a school feeding programme to improve the iron and anemia status of school children in rural Vietnam. Two primary schools in Tam Nong district were included in the study and three possible food vehicles were considered: instant noodles, instant rice soup and biscuits. Data from this study **(Chapter 3)** showed that the average daily meal consumption was 3.2 $\pm$ 0.4 with an average energy and iron intake of 5.1 $\pm$ 1.7 MJ and 7.5 $\pm$ 4.0 mg respectively. Compared to biscuits and instant rice soup, instant noodles were more frequently consumed, with larger portion sizes and are more acceptable as child food in the culture of the local people. The iron-level of the fortified product did not affect the mean consumption of noodles but a higher level of iron was associated with a lower mean consumption of biscuits ($p<0.05$). The production process did not affect the NaFeEDTA level in noodles; however during preparation at least 70% is leaked to the soup. The following study aimed to investigate the effect of NaFeEDTA fortification on taste and long term acceptance of noodles **(Chapter 4).** The results showed that both children and adults could discriminate fortified from non-fortified noodles with and without seasoning added but there was no preference for fortified or non-fortified noodles. A slight but significant increase of boredom over time occured, but acceptance ratings remained high and boredom ratings remained low throughout time. Based on both studies, it is concluded that instant noodle is a suitable vehicle for iron fortification for use in a school based intervention to improve iron deficiency anemia among primary school children in rural Vietnam.

Based on the results of the cross sectional study, our hypothesis for the intervention study was that de-worming is more effective than iron fortification in an anaemic - infection prone- population that was not considered iron deficient. 425 anaemic children aged 6 to 8 years were included in a randomized, placebo-controlled double blind parallel trial with a

2×2 factorial design plus standard treatment (iron supplementation and de-worming) and an intervention period of six months **(Chapter 5 and 6).** Blood samples were collected before and after intervention for hemoglobin, SF, TfR, CRP, IgE and hemoglobinopathies analysis. Stool samples were examined for hookworm, *Trichuris* and *Ascaris* infection. Hemoglobin improved in all groups after 6 months of intervention. Iron fortification significantly improved hemoglobin, SF and body iron (2.6 g/L, 16.2 μg /L and 1 mg/kg respectively) **(Chapter 5)**. The improvement of hemoglobin, SF, and body iron level in the group receiving 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 g/kg versus 4.4 g/kg) of that in the iron supplementation group compare to iron supplementation **(Chapter 6)**. TfR was slightly improved however not significantly different between groups. Prevalence of IgE elevated was very high at baseline (99%) and significantly reduced to about 75% in all groups after intervention. De-worming showed no effect on hemoglobin, iron status and IgE level. It is concluded that the TfR and SF data did not indicate iron deficiency but the increase in SF and Hb with treatment suggest there was iron deficiency, although the positive seasonal effect in all treatment groups may be associated with chronic infection or other un-identified factors. The efficacy of iron fortification based on the change in hemoglobin level is 58% less than that of supplementation.

In conclusion, anemia in school children in rural Vietnam is partly caused by iron deficiency although not reflected by SF and TfR. Parasite infection does not play a role in anemia in primary school children in rural Vietnam. Chronic infection may be a cause of anemia among primary children in rural Vietnam. Iron fortification was less effective compared to iron supplementation on the improvement of Hb levels and iron status. However, in a population of anaemic children with mild iron deficiency, for sustainability reasons, iron fortification should be the preferred strategy to combat anemia.

Samenvatting

Bloedarmoede vormt een problem voor de volksgezondheid van schoolkinderen in Vietnam. Uit verschillende lokale studies blijkt, dat de prevalentie van bloedarmoede bij basis school kinderen op het platteland van Vietnam rond 30% is. Parasitaire infecties van de darmen komen ook vaak voor bij schoolkinderen, in het bijzonder infecties door *Trichuris* en *Ascaris*. Het wordt algemeen aangenomen dat ijzergebrek de belangrijkste oorzaak is van bloedarmoede in ontwikkelingslanden. Maar bloedarmoede kan ook veroorzaakt worden door andere gezondheidsfactoren zoals parasitaire infecties, (genetische) afwijkingen aan rode bloedlichamen en chronische infecties. Bloedarmoede schaadt cognitieve funkties and vertraagd psychomotorische ontwikkeling van kinderen. Interventies ter bestrijding van bloedarmoede zijn daarom noodzakelijk in Vietnam.

Het onderzoek dat in dit proefschrift wordt beschreven, was er op gericht de werkzaamheid te bepalen van een voedselverrijkings programma uitgevoerd op basis scholen ter verbetering van de hemoglobine concentraties en ijzer opslag van schoolkinderen die gevoelig zijn voor parasitaire infecties van de darmen. Daarnaast vergelijkt dit proefschrift het effect van voedselverrijking met ijzer, met dat van ijzer supplementatie op veranderingen in hemoglobine en ijzer status met als doel voedingskundigen werkzaam in volksgezondheid te ondersteunen in het maken van een 'evidence-based' keuze voor een geschikte strategie ter bestrijding van ijzer tekorten en bloedarmoede bij schoolkinderen in Vietnam.

Het onderzoek omvatte drie fases en werd uitgevoerd in Tam Nong District, Phu Tho Province van oktober 2003 tot september 2004. In dwarsdoorsnede onderzoek **(Fase één)** werd de prevalentie van bloedarmoede en ijzer tekort, en van parasitaire darminfecties en hun onderlinge samenhang vastgesteld bij Vietnamese schoolkinderen afkomstig van het platteland. Een acceptatie studie **(Fase twee)** werd uitgevoerd om de korte en lange termijn acceptatie van met ijzer verrijkt voedsel vast te stellen bij de schoolkinderen. Een experimenteel onderzoek op basis van randomisatie **(Fase drie)** werd uitgevoerd om de werkzaamheid te bepalen van ijzerverrijkt voedsel op ijzer status en bloedarmoede van de schoolkinderen en om de effecten van de consumptie van ijzerverrijkt voedsel te vergelijken met die van een zogenaamde standard behandeling (ijzer supplementatie met ontworming).

400 basischoolkinderen in de leeftijd van 6 tot 8 jaar afkomstig van 20 scholen in the Tam Nong district deden mee in het dwarsdoorsnede onderzoek **(Hoofdstuk 2).** Bloedmonsters werden geanalyseerd op gehalte van serum ferritine (SF), transferrin receptor (TfR), C-reactive protein (CRP) and totaal immonuglobuline E (IgE). In faeces monsters werd infectie van mijnworm, *Trichuris* en *Ascaris* vastgesteld. De prevalentie van bloedarmoede was 25% en ijzer deficientie (TfR >8.5mg/L) kwam voor bij 2% van de kinderen. De prevalentie van darmparasiet was 92% met de hoogste prevalentie voor *Trichuris* (76%) en *Ascaris* (71%). Meer dan 30% en 80% van de kinderen hadden een verhoogde CRP ($\geq$ 8 mg/L) en IgE (> 90 IU/ml) gehalte. Bloedarmoede was niet gerelateerd met SF, TfR and CRP. De prevalentie odds ratio voor *Trichuris* infectie was 2.02 (95% CI: 1.11- 3.68). We concludeerden dat bloedarmoede zeer prevalent was bij schoolkinderen in Vietnam maar niet geassocieerd met ijzer deficientie. *Trichuris* infectie was geassocieerd met een verdubbelde risiko op bloedarmoede maar niet via ijzer deficiëntie.

Vervolg studies warden uitgevoerd om de acceptatie en werkzaamheid van ijzerverrijking voor bloedarmoede vast te stellen in een populatie gevoelig voor darminfecties. Een acceptatie studie richtte zich op het identificeren van een geschikt voedingsmiddel die, wanneer verrijkt met NaFeEDTA, gebruikt kan worden in een schoolvoedingsprogramma ter verbetering van ijzer status en bloedarmoede van schoolkinderen afkomstig van het platteland van Vietnam. De studie werd uitgevoerd op twee basisscholen in Tam Nong district en drie mogelijk geschikte voedingsmiddelen werden onderzocht: instant noodles, instant rijst saus en biscuitjes. Resultaten van deze studie lieten zien dat de kinderen gemiddeld 3.2 ± 0.4 maaltijden per dag consumeren, met een energie en ijzer inname van 5.1 ± 1.7 MJ en 7.5 ± 4.0 mg respektievelijk. Vergeleken met biscuitjes en instant rijst saus, werd instant noodles vaker gegeten, in grotere porties en werd in de locale cultuur beter gewaardeerd als voedsel voor kinderen. Het ijzer niveau in het verrijkte voedingsmiddel had geen invloed op de gemiddelde hoeveelheid gegeten noodles, maar een hoger ijzer niveau in biscuitjes was gerelateerd aan een kleinere hoeveelheid gegeten (P<0.05). Het productie process had geen effect op het niveau van NaFeEDTA in de noodles, maar gedurende de productie lekte tenminste 70% vanuit de noodles in de soep.

Een volgende studie had tot doel het effect te onderzoeken van verrijking met NAFeEDTA op de smaak en lange termijn acceptatie van noodles **(Hoofdstuk 4).** De resultaten lieten zien dat zowel kinderen als volwassenen onderscheid konden maken tussen ijzer verrijkte en niet-verrijkte noodles met of zonder smaaktoevoeging. Maar beiden hadden geen voorkeur voor ijzerverrijkte of niet-verrijkte noodles. Een kleine maar wel significante toename van verveeldheid trad op na het eten van ijzerverrijkte noodles over een periode van tien weken, maar de mate van verveeldheid bleef laag terwijl de acceptatie hoog bleef. Gebaseerd op beide studies, concludeerden we dat instant noodles een geschikt voedingsmiddel is voor verrijking met NaFeEDTA, dat gebruikt kan worden in een schoolvoedingsprogramma ter verbetering van ijzer status en bloedarmoede bij Vietnamese kinderen.

Gebaseerd op de resultaten van het dwarsdoorsnede onderzoek, veronderstelden we in de interventie studie, dat ontworming effectiever zou zijn dan ijzerverrijking in een populatie met bloedarmoede, gevoelig voor parasitaire infecties maar zonder ijzerdeficiëntie heeft. De interventie studie betrof een dubbel-blind experiment met een 2x2 factoriele proefopzet, waarbij 425 kinderen met bloedarmoede, in de leeftijd van 6 tot 8 jaar, random werden toegewezen aan behandeling met NaFeEDTA verrijkte of niet-verrijkte noodles, en deworming of placebo, gecombineerd met een vijfde groep die een standaard behandeling kreeg (ijzer supplementatie met deworming) over een periode van 6 maanden **(Hoofdstuk 5 en 6)**. Bloedmonsters werden vóór en na de interventie verzameld voor het bepalen van hemoglobine, SF, TfR, CRP, IgE concentraties en op het voorkomen van afwijkingen van de rode bloedlichaampjes. Feces monsters werden onderzocht op het voorkomen van mijnworm, *Trichuris* and *Ascaris* infectie. Hemoglobine concentratie verbeterde in alle groepen na een periode van 6 maanden. Ijzerverrijking verbeterde signifikant het hemoglobine gehalte, SF en gehalte van lichaamsijzer (2.6 g/L, 16.2 µg /L en 1 mg/kg respectievelijk) **(Hoofdstuk 5)**. De verbetering van hemoglobine, SF, and lichaamsijzer concentraties in de groep die ijzerverrijking ontving 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 g/kg versus 4.4 g/kg) van dat van de ijzer supplementatie groep **(Hoofdstuk 6)** TfR was licht verbeterd maar er waren geen signifikante verschillen tussen de interventie groepen. De prevalentie van een verhoogde concentratie IgE was erg hoog aan het begin van de studie (99%) en nam signifikant af tot rond 75% in alle groepen na de intervention. Ontworming had geen effect op hemoglobine concentratie, ijzer status en IgE concentratie. We concludeerden dat, hoewel TfR en SF concentraties niet aangaven dat er ijzerdeficiëntie was, de toename in SF en hemoglobine na de interventie toch suggereerden dat er (milde) ijzer deficiëntie aanwezig is, maar dat het positieve seizoensmatige effect gevonden in alle groepen wel eens geassocieerd kon zijn met chronische infectie of een ander (niet geidentificeerde) factor. De effectiviteit van ijzerverrijking op hemoglobine concentratie was 58% van dat van ijzer supplementatie.

Gebaseerd op bovenstaande studies concluderen we, dat bloedarmoede bij schoolkinderen in Vietnam gedeeltelijk door ijzerdeficientie wordt veroorzaakt, hoewel niet aangetoond door de gevonden SF en TfR concentraties. Parasitaire infecties blijken geen rol te spelen bij bloedarmoede van basisschool kinderen. Chronische infectie zou een mogelijke oorzaak van bloedarmoede in deze populatie kunnen zijn. Ijzerverrijking was minder effectief dan ijzer supplementatie op hemoglobine concentratie en ijzer status. Maar in een popultaie van kinderen met bloedarmoede en milde ijzer deficientie zou, ijzerfortificatie de voorkeur hebben om bloedarmoede te bestrijden.

TÓM TẮT

Thiếu máu ở trẻ em học đường là vấn đề sức khỏe cộng đồng tại Việt Nam. Một vài nghiên cứu cho thấy tỷ lệ thiếu máu ở trẻ em tiểu học vào khoảng 30%. Nhiễm giun cũng là một vấn đề rất phổ biến và có tỷ mắc rất cao ở trẻ em học đường, đặc biệt là nhiễm giun tóc và giun đũa.

Thiếu sắt thường được coi là nguyên nhân chính gây nên tình trạng thiếu máu ở các nước đang phát triển. Tuy nhiên, thiếu máu cũng có thể gây ra bởi các nguyên nhân khác như là nhiễm ký sinh trùng, các bất thường về máu hoặc các bệnh nhiễm trùng mãn tính.

Các nghiên cứu đã chỉ ra rằng thiếu máu ảnh hưởng đến khả năng phát triển trí tuệ, khả năng học tập và sự phát triển tâm sinh lý của trẻ. Do vậy một chương trìmh can thiệp để cải thiện tình trạng thiếu máu cho trẻ em học đường đặc biệt tại các vùng nông thôn Việt Nam là cần thiết.

Mục đích của đề tài này nhằm xác định hiệu quả của chương trình tăng cường sắt vào thực phẩm tại trường học để cải thiện hàm lượng hemoglobin và dự trữ sắt của trẻ em học đường, nhóm có nguy cơ nhiễm ký sinh trùng đường ruột cao. Bên cạnh đó đề tài cũng so sánh hiệu quả của tăng cường sắt vào thực phẩm với việc bổ sung viên sắt bằng đường uống đến sự thay đổi hàm lượng hemoglobin và sắt nhằm giúp những nhà dinh dưỡng cộng đồng lựa chọn được chiến lược phù hợp để phòng chống thiếu máu thiếu sắt ở trẻ em học đường nông thôn Việt nam.

Nghiên cứu được triển khai tại huyện Tam Nông Tỉnh Phú Thọ, từ tháng 10 năm 2003 đến tháng 9 năm 2005, bao gồm ba giai đoạn. Nghiên cứu cắt ngang **(Giai đoạn một)** được tiến hành để xác định tỷ lệ thiếu máu, thiếu sắt, tỷ lệ nhiễm ký sinh trùng đường ruột và mối liên quan của thiếu máu với nhiễm giun trên trẻ em tiểu học tại một vùng nông thôn Việt Nam. Nghiên cứu khả năng chấp nhận **(Giai đoạn hai)** được tiến hành để đánh giá khả năng chấp nhận thực phẩm có tăng cường sắt của trẻ em tiểu học trong thời gian ngắn hạn và dài hạn. Nghiên cứu can thiệp **(Giai đoạn ba)** được tiến hành nhằm xác định hiệu quả của việc tiêu thụ thực phẩm có tăng cường sắt đến sự thay đổi hàm lượng hemoglobin và dự trữ sắt của trẻ em tiểu học. Nghiên cứu này cũng nhằm so sánh hiệu quả của tăng cường sắt vào thực

phẩm với phương pháp can thiệp chuẩn (uống viên sắt + tẩy giun) đến tình trạng thiếu máu và thiếu sắt của học sinh.

400 trẻ em tiểu học từ 6-8 tuổi từ 20 trường tiểu học của huyện Tam Nông tỉnh Phú Thọ đã được tham gia vào nghiên cứu cắt ngang ***(Chương 2)***. Xét nghiệm máu được tiến hành để đo lường các chỉ số serum ferritin (SF), transferrin receptor (TfR), C-reactive protein (CRP) và immonoglobulin E (IgE). Mẫu phân được xét nghiệm để tìm trứng giun móc, giun tóc và giun đũa. Kết quả nghiên cứu này chỉ ra rằng tỷ lệ thiếu máu là 25%. Thiếu sắt với (TfR >8.5mg/L) là 2%. Tỷ lệ nhiễm ký sinh trùng đường ruột là 92% trong đó giun tóc có tỷ lệ mắc cao nhất (76%) và giun đũa là (71%). Trên 30% trẻ có nồng độ CRP tăng cao ($\geq$ 8 mg/L) và 80% trẻ có nồng độ IgE tăng cao (> 90 IU/ml). Tỷ lệ Odd ratio của nhiễm giun tóc là 2.02 (95% CI: 1.11- 3.68). Nghiên cứu kết luận rằng tuy tỷ lệ thiếu máu ở trẻ em nông thôn Việt Nam cao nhưng dường như không liên quan đến thiếu sắt. Nhiễm giun tóc làm tăng nguy cơ thiếu máu gấp hai lần nhưng cơ chế không thông qua thiếu sắt.

Các nghiên cứu tiếp theo được tiến hành để đánh giá khả năng chấp nhận và hiệu quả của thực phẩm tăng cường sắt đến tình trạng thiếu máu của quần thể trẻ em có nguy cơ nhiễm trùng. Nghiên cứu khả năng chấp nhận, với mục đích xác định chất mang phù hợp dùng để tăng cường sắt sử dụng trong bữa ăn học đường tại trường học để cải thiện tình trạng thiếu máu, thiếu sắt của trẻ em học đường tại nông thôn Việt Nam. Hai trường tiểu học tại huyện Tam Nông đã được lựa chọn tham gia vào nghiên cứu; 3 chất mang được xem xét đó là: mì tôm, cháo ăn liền và bánh bích qui. Kết quả từ nghiên cứu này **(Chương 3)** cho thấy rằng, số bữa ăn trung bình trong ngày của trẻ là 3.2 ± 0.4 bữa, với năng lượng và sắt khẩu phần trung bình là 5.1 ± 1.7 MJ và 7.5 ± 4.0 mg. So với cháo ăn liền và bánh bích qui, mì ăn liền được tiêu thụ thường xuyên hơn, khối lượng tiêu thụ mỗi lần cũng nhiều hơn và được trẻ em cũng như cha mẹ đánh giá là thức ăn phù hợp với trẻ. Nồng độ sắt tăng cường vào mì tôm không ảnh hưởng đến lượng mì trẻ tiêu thụ nhưng nồng độ sắt tăng cường ở mức cao hơn thì mức tiêu thụ bánh bích qui trung bình của trẻ giảm xuống ($p<0.05$). Qui trình sản xuất không ảnh hưởng đế hàm lượng sắt tăng cường vào mì tôm, tuy nhiên trong quá trình chế biến mì tôm có tới 70% lượng sắt hòa tan vào nước. Nghiên cứu tiếp theo với mục đích nhằm xác định ảnh hưởng của NaFeEDTA đến khẩu vị và khả năng chấp nhận lâu dài sản phẩm mì tôm **(Chương 4).** Kết quả nghiên cứu cho thấy rằng cả trẻ em và người lớn đều có thể phân biệt được mì tôm có tăng cường sắt với mì tôm không tăng cường sắt khi có hoặc không có gia vị thêm vào. Cả trẻ em và người lớn đều không có sự lựa chọn cao hơn cho mì không tăng cường sắt hoặc mì tôm có tăng cường sắt. Sự chán ăn mì có tăng nhẹ theo thời gian, nhưng khả năng chấp nhận sản phẩm của trẻ vẫn duy trì ở mức độ cao trong suốt thời gian nghiên cứu. Dựa trên kết quả của cả hai nghiên cứu này, có thể kết luận rằng mì tôm là chất mang phù hợp để tăng cường sắt sử dụng cho chương trình can thiệp ăn bổ sung tại trường học để cải thiện tình trạng thiếu máu thiếu sắt của trẻ em tiểu học tại nông thôn Việt Nam

Dựa trên kết qủa của nghiên cứu cắt ngang, giả thuyết cho nghiên cứu can thiệp là tẩy giun sẽ có hiệu quả cao hơn tăng cường sắt đối với việc cải thiện tình trạng thiếu máu ở quần thể trẻ được coi là không có thiếu sắt nhưng có nguy cơ nhiễm trùng. 425 trẻ từ 6-8 tuổi đã được tham gia vào nghiên cứu can thiệp, thiết kế can thiệp ngẫu nhiên đối chứng mù kép, kết hợp *thêm nhóm điều trị chuẩn (viên sắt + tẩy giun) với thời gian kéo dài trong 6 tháng* **(Chương 5, 6).** Mẫu máu được lấy trước và sau can thiệp để phân tích hàm lượng hemoglobin, SF, TfR, CRP, IgE và hemoglobinopathies. Mẫu phân được xét nghiệm tìm trứng giun móc, giun tóc và giun đũa. Sau 6 tháng can thiệp nồng độ hemoglobin tăng ở tất cả các nhóm. Mức cải thiện Hemoglobin, SF và dự trữ sắt ở nhóm tăng cường sắt lần lượt là (2.6 g/L, 16.2 g /L and 1 mg/kg) **(Chương 5).** So sánh với nhóm uống bổ sung viên sắt, mức cải thiện của nhóm tăng cường sắt ở các chỉ số hemoglobin là 42% (2.6 g/l so với 6.2 g/l); SF là 20% (23.5 µg/L so với 117.2 µg/L) và dự trữ sắt là 31.3% (1.4 g/kg so với 4.4 g/kg) **(Chương 6).** TfR có cải thiện rất ít và sự khác biệt giữa các nhóm không có ý nghĩa thống kê. Tại thời điểm điều tra ban đầu, số trẻ có IgE tăng cao là rất nhiều (99%), nhưng con số này giảm xuống còn khoảng 75% ở tất cả các nhóm sau can thiệp. Tẩy giun không có tác động đến nồng độ hemoglobin, sắt và IgE. Nghiên cứu kết luận rằng mặc dù TfR và SF không nói lên tình trạng thiếu sắt nhưng việc tăng nồng SF và hemoglobin qua can thiệp chứng tỏ rằng quần thể nghiên cứu vẫn có thiếu sắt. Tình trạng thiếu máu được cải thiện ở tất cả các nhóm sau can thiệp có thể có liên quan đến bệnh nhiễm khuẩn mãn tính theo mùa hoặc yếu tố nào đó chưa được làm rõ. Hiệu quả can thiệp bằng tăng cường sắt vào thực phẩm đến hàm lượng hemoglobin kém hơn 58% so với uống bổ sung viên sắt.

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About the author

Le Thi Huong was born on the 6th of August 1968 in Thanh Hoa Province in Vietnam. In 1985, after graduation from high school in Thanh Hoa, she joined the Hanoi Medical University where she got her Medical Doctor degree in 1991. From 1992 to 1997, she worked in the Community Health Research Unit at Hanoi Medical University. She involved in different research and training activities related to maternal and child health, partterns of pesticide use in Vietnam. She started her study Community Nutrition in 1997 and she received her master degree in 1999 at Hanoi Medical University. In the same year, she started working as a lecturer at the Nutrition Department of Hanoi Medical University. In 2002, she obtained a scholarship from the Vietnamese Government for a PhD program and in December the same year, she started her PhD program at the Division of Human Nutrition, Wageningen University, The Netherlands. From December 2002 to September 2006 she carried out the research presented in this thesis alternately in Vietnam and at Wageningen University. She also attended several congresses and international courses within the education program of the VLAG Graduate School (Food Technology, Agrobiotechnology, Nutrition and Health Science) of the Wageningen University.

Educational program

DISCIPLINE SPECIFIC ACTIVITIES

Nutrigenomics Symposium Wageningen, VLAG, April. 2003

Consumer oriented food product design Wageningen, VLAG, May. 2003

Nutrition communication Wageningen, VLAG, October. 2005

Use of software package KOMEET for food consumption surveys. Division of Human Nutrition, April-May. 2003

Training on specific laboratory blood tests; Larenstein, Velp, May. 2003

Seventh International Course on the Production and Use of Food Composition Data in Nutrition, November. 2005

Nutrition and lifestyle epidemiology Wageningen, January-March. 2003

Meetings

Neys-van Hoogstraten Foundation. Workshop on Household food and nutrition security in changing socio-economic conditions. Hanoi Vietnam, May. 2004

International Congress of Nutrition. Durban, South Africa, Sep. 2005

Bioavailability Conference in Chiangmai Thailand, March. 2006

GENERAL COURSE

VLAG PhD week, 2002

Scientific writing and presentation, May. 2003

OPTIONAL COURSES

Sensory evaluation course; January- March. 2006

Preparation PhD research proposal

Literature study program, Wageningen 2002-2006

International PhD- excursion; October. 2005

Staff seminars