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Research Article

Compliance with WHO's Guidelines for Multiple Micronutrient Powder Fortification and Vietnam Recommended Dietary Intakes to Determine Micronutrient Levels of Milk Fortification and Effectiveness Study on School Children Aged 7-10 Years

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Abstract

Micronutrients deficiency including vitamin A, iron, zinc and iodine deficiencies are public health problems in Vietnam, causing high prevalence of stunting. A School milk program has been applied to improve the quality of school meals and promote growth and development of school children. Types and doses of vitamins and minerals fortified in milk were determined adhering to WHO guidelines for use of multi-micronutrient powders for point-of-use fortification of foods consumed by infants 6-23 months and young children 2-12 years and from Vietnam Recommended Dietary Allowance (RDA). Although Vitamin A, iron and zinc were the main outcome indicators, 17 additional micronutrients were added in the milk for comprehensive support of children development because the monotonous and poor diet causes multi-micronutrient rather than single vitamin/mineral deficiencies. In addition, multi-micronutrients supplementation is more effective in improving iron and vitamin A status than that of single fortification. School children with a Z-score of -1SD (n=501) were divided into two groups: those receiving micronutrient fortified milk (group M) and those with normal diet (group C). After 6 months of intervention, all nutritional and micronutrient status were improved in group M compared to those in group C. In conclusion, multi-micronutrient fortified milk is effective in improving nutritional status including vitamin A, iron, zinc status in school children.

Keywords: Milk fortification, micronutrients

Introduction

Vietnam is one of the countries that has reduced the rate of weight malnutrition by age to nearly the millennium target of 12% in 2015. However, stunting malnutrition remains high at 26.4%. In 2017, compared with other countries in the region and the world, micronutrients deficiency (MD) (vitamins A, iron, zinc, iodine ...) remains high and is one of the main causes of stunting malnutrition [1]. SEANUT 2011 survey shows that in primary school children the rate of anemia is 11.8%, children with depleted iron reserves (ferritin <15mcg/l) is 6%, children with low iron reserves (ferritin <30mcg/l) is 28.8% and both iron deficiency and anemia is 23.9% [2].

The main cause of micronutrient deficiency is the lack of micronutrients required for the body's daily needs [3,4]. The diet of the Vietnamese people usually contains 6.5mg iron per day, reaches only 73% of the RDA [2,5], and only 65% of the recommended amount for Vitamin A. In the northern mountainous region, Central Highlands and central Vietnam, vitamin A retinol in the diet achieved 34%, 36% and 42% of the recommended amount, respectively. Survey of diets of children 6-11 years old in 2011 in 6 provinces showed that the energy ration reached approximately 76% of the recommended amount while the micronutrient portion did not. Diets for children aged 6-9 and 9-11 contained 18% and 13% of vitamin D, 54% and 43% of Vitamin A, 61% and 49% of vitamin C, 59% and 45% of calcium, 68% and 54% of iron, respectively, compared to the recommended amount [2].

Research in low and middle-income countries around the world also shows that school meals are often poor in fruits, vegetables and animal foods, leading to an inadequate supply of protein and micronutrients [3]. When that diet is monotonous, in the same study group there is often a lack of multiple micronutrients, in other words, a lack of more than one vitamins or minerals [6-8]. Good health and nutrition during schooling years will make an important contribution to the children's academic achievement, growth and development, and ensure the quality of their lives later on as well as the development of the whole society [9].

Fortifying micronutrients in food is an initiative adopted by many countries and was evaluated in the 2012 Copenhagen consensus as an effective solution to improving nutritional status in developing countries [10]; furthermore, it was proved to be effective in improving the micronutrient status in school children [11]. Fortifying multi-micronutrients in food has multiple effects including improving both the anemia and vitamin A deficiency status [7]. Vietnam has a nationwide micronutrient fortification program which has been successfully deployed to a wide range of food vehicles such as salt, cooking oil, fish sauce, spices, etc. For school-age children, milk is a nutrient-rich food with calcium, vitamins and minerals, not to mention easily digested and absorbed. However, one disadvantage of milk is that the content of micronutrients is not high. Therefore, fortifying milk with micronutrients can overcome this issue and meet the nutritional requirements of the child's body during important development periods. Following the guidelines for implementation and monitoring those programs closely will help to prevent excessive intake of micronutrients [12]. Each micronutrient plays a different role and need for the body growth, so the amount of fortification also varies. Therefore, this study aims to determine the type and amount of micronutrient, the amount of fortified milk and evaluate the effect of fortified milk on micronutrient status in school-aged children ranging from 7 to10 years of age.

Subjects and Methods

Fresh milk is produced by Vinamilk Corp. Following Vietnamese standards, then fortified with micronutrient at the company's factory. The micronutrients recommended by the HF-HTAG group to be used in Vietnam in the composition of multi-micronutrient supplement [13,14] have been selected to enhance that substance. The National Institute of Nutrition determined the levels of micronutrients to be fortified in milk based on guidelines by WHO on the use of multi-micronutrient supplements for children aged 6-23 months and 2-12 years old [15] and RDA [16] for Vietnamese people.

A controlled clinical study to evaluate the effectiveness of fresh milk enhancing micronutrients for nutritional and micronutrient status of children aged 7 to 10 years was conducted.

Research subjects

The Study was conducted in 5 communes in Phu Binh District, Thai Nguyen province, located in the northern area of Vietnam,

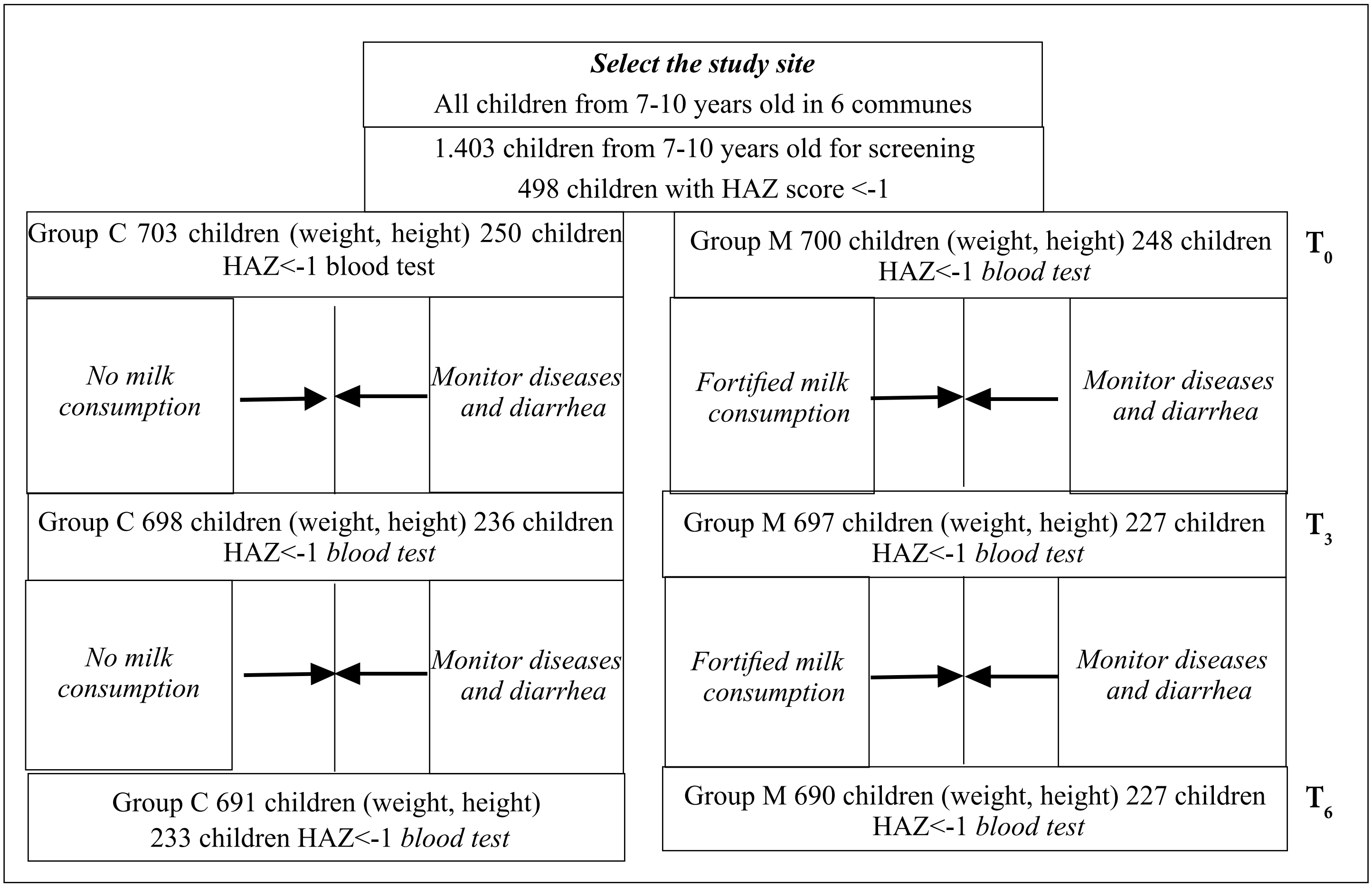

Figure 1: Sample Size

where majority of the population has a low income.

Sample size calculation indicated that 196 children are needed for each group based on the improvement of hemoglobin, vitamin A and iron, zinc serum before and after intervention. With an anticipated drop-out rate of 20%, 250 children were randomly selected for each research group for biochemical indicators evaluation. 1403 subjects from 6 primary schools have been screened to select 498 children who are stunted or at risk of stunting (HAZ Score<-1.0) to participate in the research for blood test analysis. The remaining 815 children were also randomly divided into two groups, one of which consumed milk during research time with the intervened group and the other consumed milk with the control group after 6 months of intervention. All children who consumed milk were evaluated for nutritional status. Exclusion criteria included lactose intolerance, severe or stunting malnutrition (HAZ Score <= -3), underweight (WAZ <= - 3 SD), chronic non-communicable diseases, severe anemia (hemoglobin <80g/l) or severe vitamin A deficiency (serum retinol concentration <0.35 μmol/l) (Figure 1). Among the 498 children (HAZ Score <-1.0), 250 children were randomly selected for the intervention group (Group M) and 248 children for the control group (Group C), assessed at three time frames: before the intervention (T0), after 3 months (T3) and after 6 months of intervention (T6). During the intervention, some children gave up or moved to another locality, so the intervention group evaluated 250 children than 233 children, the control group 250 children, then 227 children.

Research design

The study was conducted for 6 months (from March to August 2017) in 6 communes of Phu Binh district, Thai Nguyen Province located in the Northern part of Vietnam, where the population is mostly poor or low income. The research group (group M), besides the daily-usual diet, 2 servings of micronutrient fortified milk, each serving of 180 ml, were served. The time of consumption was between 9:00 a.m. and 3:00 p.m. Teachers distributed the milk and checked whether it was fully consumed, recorded the amount of milk consumed and medical record (if present) of the children in the process of research. On Friday, students were given milk to drink on Saturday and Sunday. During the summer holidays, they consumed milk at home and the caretakers received the milk, recorded the amount of milk consumed and their health condition. The control group (group C) only ate a normal diet, after 6 months and complete final data collection each child would also be given 2 servings of milk in the next 6 months.

Anthropometric evaluation indicators were evaluated including height (microtoise, precision 0.1cm) and weight (Tanita SC330, precision 0.1kg). Dietary intake was assessed by 24 hours recall method.

Blood samples were taken (5 ml of venous blood). Afterwards, 0.5 ml of whole blood was separated for Hb test. The rest was put in another test tube, immediately stored in cold thermos, centrifuged within 4 hours at 3000 rounds/minute for serum separation which were transferred to the National Institute of Nutrition to be kept at about -80°C until analysis. Hemoglobin (Hb) was evaluated by cyanmethemoglobin method. Assessment of anemia is based on WHO guidelines: children are considered anemic when Hb concentration is < 115 g/l; The Hb levels of severe, moderate and minor anemia were <70 g/l; 100> Hb > 70 g/l and 115 > Hb > 100 g/l, respectively [17].

Serum retinol was analyzed based on high-pressure liquid chromatography (HPLC) method. Assessment of serum Vitamin A deficiency is based on WHO guidelines [18,19]. Levels of serum retinol for sub-clinical vitamin A deficiency, severe deficiency and risk of sub-clinical vitamin A deficiency are <0.7 μmol/L and ≥0.35 μmol/l; <0.35 μmol/L; and <1.05 μmol/l) respectively.

Serum zinc is quantified by the method of atomic adsorption spectroscopy (AAS). Assessment of zinc deficiency is based on WHO guidelines and international zinc consulting organization. Zinc deficiency is defined as blood zinc levels (morning) at <9.9 μmol/L (i.e. <65 μg/dL) [19].

Serum ferritin is quantified according to the method of determining specific antibody nature using ELISA kit. Assessment of the depletion of iron reserves when serum ferritin content <15 mg/L is under the guidance of WHO, 2001 [20].

Data analysis

Data were entered using Epidata software. Anthropometric data were analyzed by WHO Anthro Plus 2006 software. Biochemical and hematological indicators were presented as mean and 95% CI. The difference of the indicators among baseline surveys, after 3 months and after 6 months was assessed using statistical tests including T-test, ANOVA, $\chi 2$-test, Krusskal – Wallirest. Data was analyzed using PASW statistics software 20.0 (SPSS Inc., U.S.A.)

Results

Vietnamese children's diets are relatively monotonous, and often lack many micronutrients. Baseline survey showed that the diet of the two groups was similar (data not shown). In both groups, micronutrients do not meet the nutritional needs of children. The level of meeting the nutritional needs of the diet is iron 67.3%, Vitamin A 56.7%, zinc 64.3%, vitamin B1 15%, Vitamin B2 13.4%, respectively. There is no malaria circumventing in this area.

The micronutrient fortified milk is fortified with most vitamins and minerals for the child's developmental needs. Using WHO guidelines on the use of multi-micronutrient supplement powder for children 6-23 months and children 2-12 years and Vietnamese RDA [16] the amount of each micronutrient fortified into milk was determined at the following levels (Table 1).

Micronutrient fortified milk have labels with the formula imprinted and legalized on the market based on standards of Vietnam Food Administration and the Ministry of Health**.** Thus, the daily serving of 180ml milk cartons provides additional nutritional needs depending on the age group of children, the amount of energy from 13% to 21%, protein from 20% to 43%, vitamin A from 38% to 50%, vitamin D approximately 25%, vitamins in water from 26% to 70%, and minerals including iron from 30% to 62%, zinc from 33% to 60%, respectively [21]. The upper tolerance level for different micronutrients in children are set up much higher than the RDA [16].

Table 1: Nutritional value and list of micronutrients in 180 ml of milk after fortification.

Components	Nutritional values in 1 carton of 180 ml milk	Components	Nutritional values in 1 carton of 180 ml milk
Vitamin A µg	116.1	Lipid g	6.30
Vitamin D µg	2.02	Protein g	5.40
Vitamin E mg	0.72	Carbohydrate g	15.84
Vitamin K µg	4.68	Calcium mg	216.00
Vitamin C mg	12.0	Iron mg	2.70
Vitamin B1 µg	180.0	Zinc mg	2.16
Vitamin B2 µg	234.0	Copper µg	111.60
Vitamin B3 mg	2.16	Selenium µg	5.94
Pantothenic Acid mg	0.54	Iodine µg	32.40
Vitamin B6 µg	162.0	Magnesium mg	18.0
Folic Acid µg	50.4	Phospho mg	162.0
Vitamin B12 µg	0.34	Lysine mg	450.0
Biotin µg	9.0	Taurine mg	12.6

The average diet of primary school students combined with the use of two micronutrient fortified milk cartons would significantly improve the nutritional needs: vitamin A intake is 774 IU/day, meeting 79% of the recommended amount, iron is 5.4 mg/day, 97.8% of the recommended amount, zinc 4.3 mg, 98.9% of the recommended amount.

The initial anthropometric survey showed that the percentage of underweight in boys were 23.4% and girls were 20.2%, as well as stunting malnutrition were 15.2% and 13.5%, respectively (Table 2).

Difference in average of BMI, WAZ, HAZ, BAZ indicators have statistically significant differences between group M and group C at the time after intervention 3 months and 6 months ($p < 0.001$) (Figure 2-7).

There was an increase in weights by age, height by age, BMI by age before and after intervention as well as between group M and group C but the differences were not significant (Table 3).

There were statistically significant improvements with the difference in mean serum zinc, hemoglobin, vitamin A after intervention compared to before the intervention, with the intervention group being higher than in the control group (Table 4).

Discussion

Children's diets are relatively monotonous, and often lack many micronutrients. Research in several other developing countries in Africa also results in children's diets not providing enough energy, folic acid, calcium, iron, vitamin C, vitamin A and zinc according to the recommended nutritional needs [3,4,8,22]. Research results in Vietnam and some Southeast Asian countries in 2011 show that more than half of Indonesian children have diet energy levels lower than the recommended energy amount, more than 50% of Thai children consume diets that have low levels of calcium, iron, zinc, vitamin A and vitamin C. Inadequate vitamin D and calcium levels in Malaysian children are also worrisome because up to one third of children do not meet the needs of vitamin D and calcium in the diet [6,23-25].

Table 2: Nutritional status of boys and girls at baseline, after 3 and 6 months.

Nutrition status	Time	Boys		Girls	
		N	% (n)	N	% (n)
Underweight	T0	666	23.4% (156)	574	20.2% (116)
	T3	640	25.2% (161)	555	20.2% (112)
	T6	535	24.5% (131)	479	17.5% (94)
Stunting	T0	705	15.2% (107)	608	13.5% (82)
	T3	705	13.8% (97)	606	12.5% (76)
	T6	705	13.9% (98)	608	11.8% (72)

Table 3: Mean height, weight, BMI at baseline and after 3and 6 months (T-test).

		Group C		Group M		
Indicator	Time	N	Mean (95% CI)	N	Mean (95% CI)	P-value
Height, cm	T0	703	123.3 (122.8-123.9)	700	122.4 (121.9-122.9)	0.02
	T3	698	124.9 (124.3-125.4)	697	123.8 (123.3-124.3)	0.004
	T6	691	126.6 (126.1-127.2)	690	125.5 (124.9-126.0)	0.002
Weight, kg	T0	703	22.9 (22.6-23.2)	700	22.2 (21.9-22.5)	0.001
	T3	698	23.6 (23.3-25.1)	697	22.4 (22.1-22.7)	<0.0001
	T6	691	24.7 (24.3-25.1)	690	23.6 (23.3-23.9)	<0.0001
BMI, kg/m^2	T0	703	14.9 (14.8-15.0)	700	14.7 (14.6-14.8)	0.005
	T3	698	15.0 (14.9-15.2)	697	14.5 (14.4-14.6)	<0.0001
	T6	691	15.3 (15.1-15.4)	690	14.9 (14.7-15.0)	<0.0001

Table 4: Micronutrients levels in group C and group M at baseline and after 3, 6 months (T test).

		Group C		Group M		
Indicator	Time	N	Mean (95% CI)	N	Mean (95% CI)	P value
Hb, g/l	T0	250	121.0 (119.92-121.9)	248	120.80 (119.92-121.10)	0.84
	T3	236	122.61 (121.40-123.82)	227	121.0 (119.87-122.18)	0.06
	T6	233	122.44 (121.04-123.84)	227	125.68 (124.10-127.25)	0.003
SF, µg/L	T0	250	63.70 (59.04-68.0)	248	59.02 (54.27-63.80)	0.15
	T3	236	71.3 (66.6-68.11)	227	63.60 (59.10-68.10)	0.02
	T6	233	71.2 (66.4-76.0)	227	76.10 (71.30-80.75)	0.15
Vit A, µmol/L	T0	250	1.13 (1.09-1.17)	248	1.18 (1.12-1.24)	0.19
	T3	236	1.19 (1.13-1.24)	227	1.30 (1.24-1.36)	0.008
	T6	233	1.19 (1.13-1.24)	227	1.30 (1.23-1.36)	0.009
Zinc, µmol/L	T0	250	9.7 (9.43-9.97)	247	9.43 (9.17-9.71)	0.16
	T3	236	9.56 (9.29-9.82)	227	9.73 (9.49-9.97)	0.33
	T6	233	9.69 (9.38-9.99)	227	10.07 (9.73-10.40)	0.1

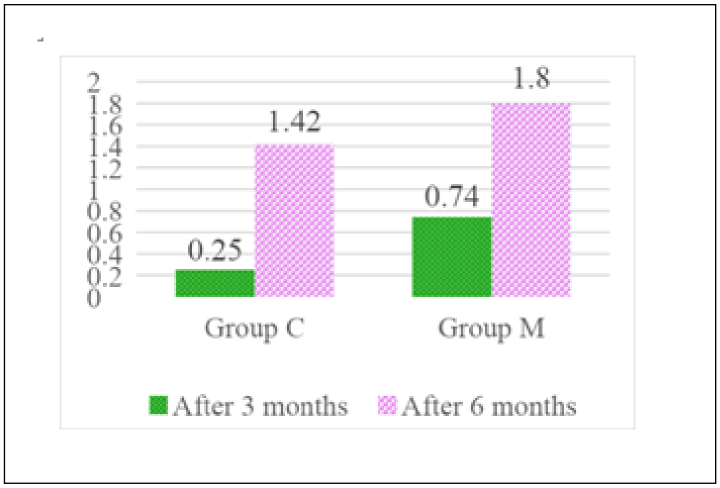

Figure 2: Weight diferences after 3 months and 6 months, Anova test, P<0.001.

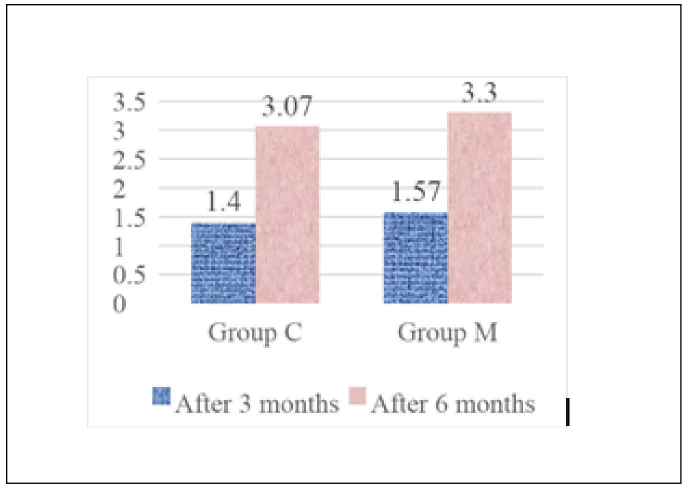

Figure 3: Height diferences after 3 months and 6 months, ANOVA test, P<0.001.

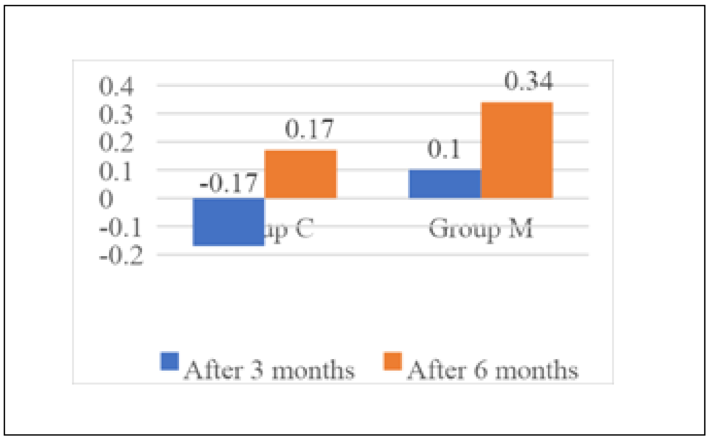

Figure 4: BMI differences after 3 months and 6 months, ANOVA test, P<0.001.

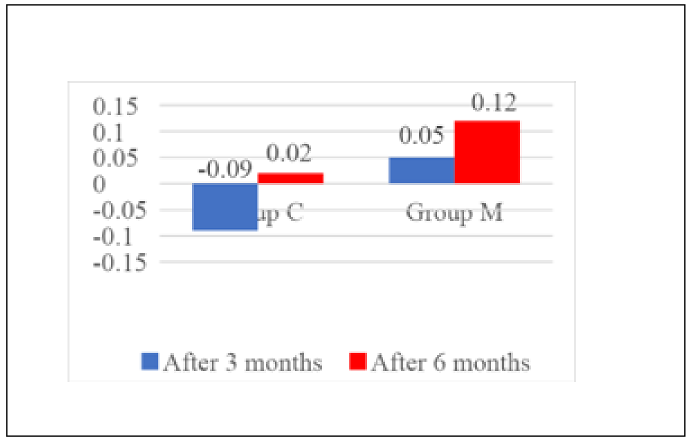

Figure 5: WAZ diferences after 3 months and 6 months, ANOVA test, P<0.001.

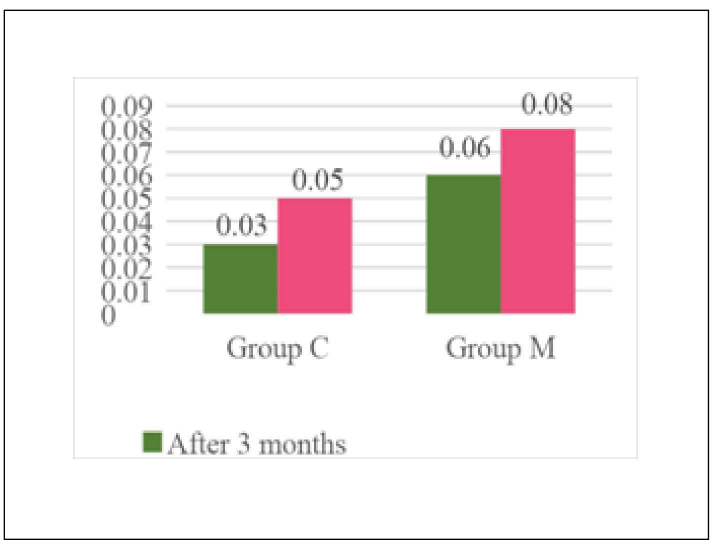

Figure 6: HAZ differences after 3 months and 6 months, ANOVA test, P<0.001.

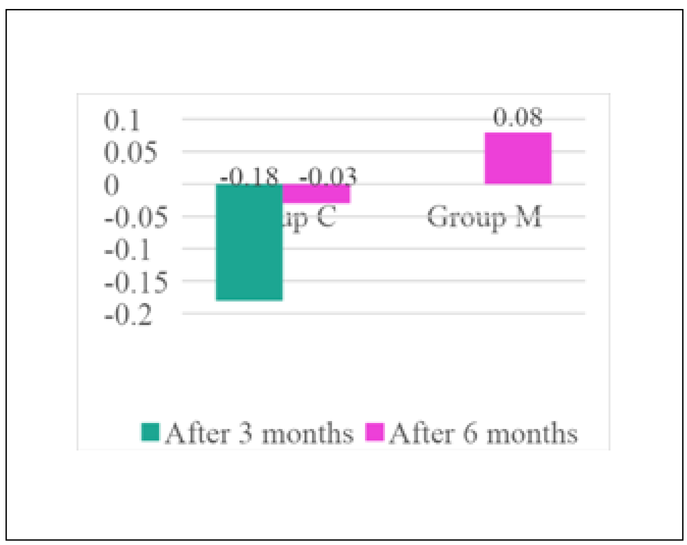

Figure 7: BAZ diferences after 3 months and 6 months, ANOVA test, P<0.001.

Micronutrient fortified milk is fortified with most vitamins and minerals for the child's developmental needs. The improvement of blood and biochemical indicators are similar to other studies which also demonstrated that multi-micronutrient supplementation/fortification is most effective against micronutrient deficiency rather than a single micronutrient supplement [24].

The concentration of serum retinol of children is 1.18 µmol/l before the intervention, began to increase after 3-month and remained at the increased value at the 6-month period (1.30 µmol/l and 1.30 µmol/l, respectively) (Paired t-test, p <0.0001) and this increase was significant compared to the control group (ANOVA test, p <0.01). Most studies of vitamin A fortification showed an improvement in vitamin A status in children, but these studies all used higher levels of vitamin A with a higher level of demand [25-29]. This suggests that the fortified dosage in milk for 3 months is sufficient for subsequent studies. At the same time, the percentage of students with sub-clinical vitamin A deficiency and the risk of sub-clinical vitamin A deficiency in the group M significantly decreased from 44.5% before intervention to 23.3% and 19.8% after 3 and 6 months of intervention respectively. This decrease is also significantly different compared to control group at 3-months and 6-months after the intervention ($\chi 2$ test, p <0.01) (Figure 8 and 9).

Fortifying multi-micronutrients in milk is effective for the body's iron status in children under 5 years old [30-32]. The results of this study confirm and broaden the conclusions about fortification of milk that with a dose of 2.7mg/180ml could effectively increase hemoglobin levels, and improve the iron status of school-aged children in Vietnam. There was a significant improvement in hemoglobin and SF levels in the intervention group.

The mean hemoglobin concentration of children in both study groups increased significantly compared to that before the intervention, but in the control group increased only at 6 months (t-test paired with p <0.01), while the intervention group increased gradually from the period of 3 months and 6 months (t-test paired with p <0.01). After 6 months, the average hemoglobin concentration was 125.7 g/l, significantly different from the control group (122.4 g/l) (ANOVA test, p <0.01) (Table 3).

Both groups also had changes in ferritin content. The SF of the intervened group was 59.02 µg/l at baseline and significantly increase after 3 months (63.6 µg/l, T test P<0.05) and continued the increase after 6 months (76.1 µg/l, T test P<0.0001). In contrast, the SF of the control group increased after 3 months (from 63.7 µg/l to 71.3 µg/l, T-test, P<0.0001) but did not increase after 6 months. The increase was significant different between two groups after 3 months (T-test, P<0.05) but was not after 6 months. The increase in Hb and SF in the control group may be due to the fact that when a nutrition intervention program is available, even if the child is not drinking milk, the family is more aware of the child's meal than before.

Surprisingly, the rate of anemic children decreased from 22.3% before the intervention to 20.5% and 18.5% after 3 and 6 months of intervention, respectively (Figure 8); but this reduction is not statistically significant. The reason for not seeing a significant improvement in the rate of anemia in the study subjects may be due to the average anemia rate of Phu Binh primary school students is high at a level of public health issue [17], the research results are not equivalent to the intervention study of multi-micronutrient sprinkles in Vietnam (providing 17 essential micronutrients) for children with stunting malnutrition from 6 to 36 months of age for 6 months with reduction in rate of anemia the most significant (decreased by 23.2%; p <0.01) in the sprinkles group, while the control group decreased by 10.9%, indicating that the starting point of anemia of this study was very high [33]. Nutritional interventions for areas with high rates of micronutrient deficiencies are often more profound [34], thus there is no proportionality in the study in Phu Binh.

The mean serum zinc content was not significantly different between intervened group and control group after 3 months and 6 months intervention (Table 3). However, difference in mean serum zinc content after 3 and 6 months of intervention was 0.30 µmol/l ± 1.69 and 0.97 µmol/l ± 3.13, respectively compared to that of the control group at -0.14 µmol/l ± 1.75 and 0.13 µmol/l ± 2.99 respectively, which are statistically significant (ANOVA p<0.01 after three months and p <0.05 after 6 months). This difference shows a tendency to improve zinc deficiency and is consistent with several other studies using oral zinc or sprinkles, but with a lower zinc threshold of meeting the RDA (Zinc of these studies ranged from 86% to 100% of the RDA) [35-37].

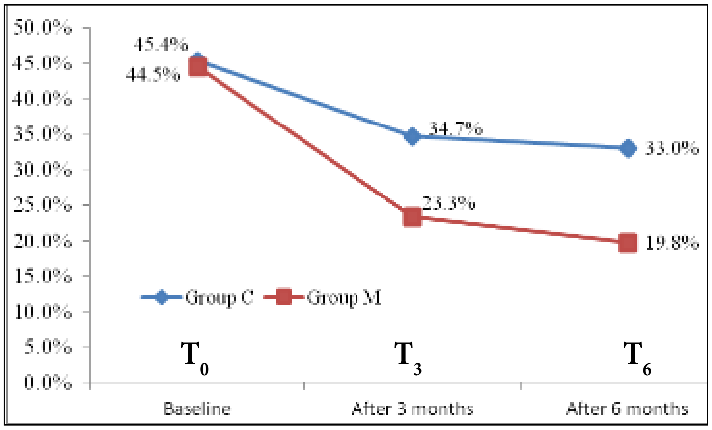

Figure 8: Prevalence of sub-clinical and risk of sub-clinical vitamin A deficiency at T0, T3, T6.

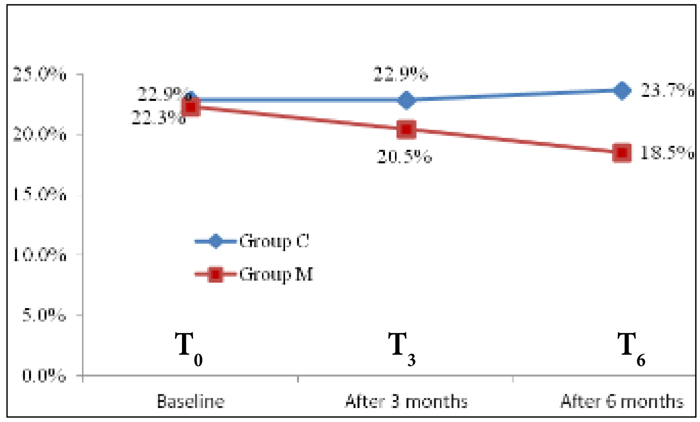

Figure 9: Prevalence of anemia at baseline, after 3 months and after 6 months.

This improvement is similar to the study of using fortified rice in Thailand which resulted in an increase in serum zinc levels in the intervention group statistically significant compared to control group. However, the zinc level in the rice study was higher (9mg Zn/g), and the intervention time was longer (12 months) [38]. A study reported that serum zinc increased within 2 weeks in children receiving supplements but not in children consuming zinc fortified while another study concluded that plasma zinc increased in the zinc supplementation group and did not change significantly in the zinc fortification group over a year of intervention [37] which indicates that zinc serum may not be good indicator for evaluating impact of short time fortification. However, additional studies are needed to understand why the dose of zinc for 72-77% RDA does not have an impact on zinc status. Factors including diseases, parasites infection, inadequacy and absorption, zinc inhibitors in the diet of children may play a role.

This study does not evaluate full effectiveness of school milk program because milk is distributed free to school-aged children rather than with cost sharing from local government and/or parents. However, it provides an objective basis and important information on the feasibility of implementation of a school fortified-milk program. We only measured the amount of micronutrients in the fortified product. During implementation of the school milk program with the contribution of family and social costs, focus should be paid to multi-micronutrients fortification to ensure high effectiveness. In the study, micronutrient fortified milk was well accepted by school-aged children during the 6-month intervention period and compliance with the consumption of two 180ml/day cartons was closely monitored. Although the cost effectiveness has not been evaluated in this study, due to the urgency of the need to improve school nutrition diets and the micronutrients fortified milk was only used for short term, the program has been implemented in Vietnam, the results of this study are an important basis for justifying and planning the implementation of a multi-micronutrient fortification program to improve school meals in Vietnam. Results can also be used to apply in many countries in the ASEAN region where there is a high rate of micronutrient deficiency in school age. In summary, this study shows that fortified milk has a beneficial effect on nutritional status and micronutrient status in school-aged children. Studies to ensure the sustainability of the intervention and the product need to be conducted further.

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Conflict of Interest

The authors declare there is no conflict of interest

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