Paediatrica Indonesiana

VOLUME 49 May • 2009 NUMBER 3

Original Article

Effect of iron-zinc fortified milk on iron status and functional outcomes in underweight children

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Abstract

Background Iron and zinc are essential micronutrients during school-age period. Milk could be an alternative medium for mineral fortification because it is consumed frequently by children.

Objective To evaluate the effect of iron-zinc fortified milk supplementation on iron status and functional outcomes in underweight children.

Methods 442 underweight subjects aged 7-10 years attending 10 primary schools in Jakarta and Solo were recruited in the study. Subjects who had chronic illnesses, severe anemia, thalassemia or cow's milk allergy were excluded. Blood samples were analyzed to determine hemoglobin, serum ferritin and serum zinc. Serum CRP was measured in sub sampled subjects (n= 60). Subjects were randomizedly allocated to receive iron-zinc fortified milk (n= 225) and standard milk (n=217) for six months. The fortified milk will provide additional 6.3 mg iron and 1.5 mg zinc per day (2 feeds). The main outcomes measured were iron status, cognitive function, growth, physical fitness and morbidity.

Results Study groups were comparable at baseline. The fortified milk group had better physical fitness score and exercise duration at baseline. Both milk supplementations reduced the prevalence of anemia and iron deficiency. Fortified milk improved the speed processing score (P< 0.0001), height (P<0.0001) and sitting height (P=0.01) significantly.

Conclusion Supplementation of milk reduces the prevalence of anemia and iron deficiency. Zinc-iron fortified milk gives positive impact on cognitive performance, growth, and physical fitness. **[Paediatr Indones. 2009;49:139-148]**.

Keywords: *Zinc, iron, milk, iron status*

Iron and zinc are essential nutrients for human growth, development and health. Iron deficiency affects cognitive, psychomotor development, physical growth and activity.[1-3] Zinc deficiency affects growth, cognitive and motor performance, immune function and increase the risk of infection.[4-11] Iron and zinc have similar way of absorption chemically and transport mechanisms; both of them compete for absorptive pathways;[12] iron and zinc potentially interact when given together. There is evidence that adequate ratio of those micronutrients may contribute positive outcome of children's growth and development.[13,14] On the other hand, iron supplementation may be pertinent to incidence of infectious diseases; particularly with the morbidity rates i.e. the morbidity of malaria and HIV diseases associates with that of iron supplementation.[15] Nevertheless, the association between iron supplementation and the morbidity of common infectious diseases is still conflicting.[16] Most of Indonesian children have low

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micronutrients intake.[17,18] It is also stated that the Indonesian children consume a lot of phytate that inhibits the absorbtion of micronutrients particularly iron and zinc.[17] Although supplementation with iron and zinc altogether enhances distinct and unique biochemical and functional outcomes,[19] milk fortification is an intriguing medium to increase the iron and zinc intake of the entire population.[20,21] To evaluate the acceptability and the efficacy of delivering micronutrients through fortified milk on iron and zinc status, children's status of growth, cognitive function, morbidity and physical fitness, we conducted a community based, double blind, randomized controlled trial to compare fortified milk with milk without fortification (standard milk).

Methods

Participants

The trial was done at 5 elementary schools in Jakarta and 10 elementary schools in Solo. Data was collected from July 2007 to February 2008. We included children who were underweight (WFA <10th percentile CDC), 7-9 years of age (2nd and 3th class of elementary school), had no chronic illness including renal, congenital thyroid disease, diarrhea, or thalassemia. Signed informed consent was obtained from parents or guardian. In addition, permission was sought from local school boards. Ethical approval of the study protocol was obtained from The Ethics Committee of Faculty of Medicine, University of Indonesia. We excluded those who suffered from severe anemia (Hb <8 g/dl), documented lactose intolerance, documented cow's milk allergy, and concomitant medication during four weeks preceding enrolment. The eligible children were then randomized selected into two groups, the first group received iron fortified milk (n= 225) while the other one received standard milk (n=217) for six months.

Assessment, blood samples and laboratory procedures

After we obtained the informed consent, children were enrolled and underwent several assessments. The assessments were performed by trained observers at baseline, after three months supplementation, and endline of the study. A detailed baseline assessment including physical examination was performed by a physician while socio-demographic information were collected by trained interviewers.

Anthropometric measurement

We measured weight, height, and mid upper arm circumference for anthropometric indicators. Weight was measured to nearest 0.1 kg with an electronic scale (SECA Corporation, Colombia, MD) and height to the nearest 0.1 cm with height scale (*Microtoire*). Mid upper arm circumference was measured to the nearest 1 mm by using a non stretchable plastic measuring tape. All measurements were done in duplicate by trained observers

Blood sample collection

Blood samples were obtained by venipuncture. Hemoglobin concentrations were measured by standard cyanmethemoglobin method. Serum ferritin concentrations were measured using ELISA. Anemia was defined as hemoglobin concentration less than 12 g/dl while iron deficiency was defined as ferritin concentration less than 12 μg/l. Iron deficiency anemia was defined as anemia with ferritin concentration less than 12 μg/l. Plasma CRP was measured by immunoturbidimetric assay for the in vitro quantitative determination of CRP in human serum on Roche automated clinical chemistry analyzers. Plasma CRP levels were considered elevated if more than 5 mg/l.

Dietary evaluation

A three-day food records survey were conducted to investigate dietary nutrition intake. The 24-hour recall method had also been used, and reported nutrition intake with this technique compared with a three-day food records survey. For analysis of daily nutrition intake, NutriSurvey for Windows (Copyright © 2003 Dr. J. Erhadt, Hohenheim University) was used to calculate intake energy and other nutrients.

Cognitive measurement

Cognitive function were measured by using Weschler Intelligence Scale III test, mainly focused on Coding Score (Speed processing), Digit Span Forward Score (Verbal Attention), Digit Span Backward Score (Working Memory), and Visual Search Score (Visual selective attention and visual-motor speed).

Episode of morbidity

The information about episode of morbidity during milk supplementation was collected from the daily medical record which was filled in by trained school teachers. Clinical features reported by the children such as runny nose and/or cough were classified as URTI. Fever was defined if reported to occur. Diarrhea was defined if liquid or loose stool was found more than 3 times in 24 hours. Total episode of illness was defined as the computation of all episode of illness such as URTI, fever and diarrhea found in every visit. The episodes of morbidities were registered in every follow-up visits in week-1, month-1, month-2, month-3, month-4, month-5, month-6.

Physical fitness measurements

Physical fitness was assessed with modified Harvard Step Test which required the subjects to step up and down on a step, 30 times per minute for 5 minutes while wearing a backpack containing bags of sugar equal to 20% of his body weight. The steps were made with following dimensions: height 30 cm, in width 42 cm, and in depth 38 cm. Heart rate were measured at rest, after exercise for 5 min and at 1, 2, 3 and 4 minute after the exercise was completed. A physical fitness score was calculated as follows: fitness score = duration of test x 100 + sum of heart rates per min taken at 1, 2 and 3 min after test completion.[22]

Intervention and compliance

We provided two single serving sachets of 54 g of milk per-day (Nestle Brands, Indonesia). The children of the first group drank the fortified milk containing 6.3 mg of iron and 1.5 mg of zinc (milk-A) while the other one consumed standard milk. At enrollment, teachers were shown how to reconstitute the powdered milk. **Table 1** shows the composition of milk in the two groups. Assistant delivered the milk every month to the schools, the teachers then prepared one serving in the school and provided one sachet to the children to be consumed in the afternoon. Teachers recorded and compiled the returned sachet every week.

Table 1. Composition of milk preparations as nutritive value per serving

Per serving	Unit	27 g	27 g
Nutrient		Fortified milk	Unfortified milk
Energy	Kcal	100.0	100.0
Fat	g	3.7	3.7
Protein	g	4.0	4.0
Carbohydrate	g	12.7	12.7
Sugar	g	6.4	6.4
Sodium	mg	59.0	59.0
Vitamin A	IU	396.0	396.0
Vitamin D 3	IU	51.0	51.0
Vitamin E	Iu	5.0	5.0
Vitamin K	mcg	9.1	9.1
Vitamin C	mg	50.0	50.0
B1	mg	0.09	0.09
B2	mg	0.31	0.31
B3	mg	1.43	1.43
B6	mg	0.14	0.14
B9	mcg	44.0	44.0
B12	mcg	0.4	0.4
Kalium	mg	194.0	194.0
Magnesium	mg	14.0	14.0
Zinc	mg	0.9	0.05
Fe	mg	3.1	0.01
Ca	mg	179.0	179.0
Fosfor	mg	120.0	120.0

Table 2. Baseline characteristics of the two groups. Figures are numbers (percentages) of participants unless stated otherwise.

	Intervention (n= 220)	Control (n=217)
Mean age		
Boys		
Hemoglobin (g/l)		
Mean (SD)	13.01(0.91)	12.92 (0.89)
Mean(SD) serum ferritin (mcg/l)	30.79(22.10)	34.49(22.86)
Mean(SD) serum zinc (mg/l)	13.5 (3.05)	13.46(3.08)
Nutritional status		
Wasted	39(18)	24.4(51)
Stunted	88(40.6)	81(38.8)
Prevalence of anemia	31(14.3)	28(13.4)
Prevalence of iron deficiency	31(14.3)	26(12.4)
Prevalence of iron deficiency anemia	6(2.8)	7(3.3)

Sample size and power

We calculated the sample size by having the objective of increased hemoglobin level by 5 mcg/dl in those consuming fortified milk, with α of 0.05 and 95% power. We estimated 20% drop-out and therefore enrolled 220 children in each group.

Data management and analysis

We used SPSS for WINDOWS software (version 15; SPSS Inc, Chicago). Anthropometric data were shown as mean (SD) *z* scores compared with the CDC 2000 reference population. Conversion to anthropometrical *z* scores was done by using EPI INFO 2005 software (version 3.4; Centers for Disease Control and Prevention, Atlanta) and the 2000 Centers for Disease Control and Prevention reference growth data. Fisher's exact test was used to test associations between categorical variables. Continuous data was analyzed by using independent-t test or Mann-Whitney. Correlation between iron status and episode of illness was analyzed by using linier regression.

Results

Study group were comparable at baseline as shown in **Table 2**. The prevalence of underweight, stunting, and wasting in fortified milk group tended to decrease more than that of standard milk group. The children who consumed fortified milk significantly gained height and sitting height after three months and six months of milk supplementation. Prevalence of iron deficiency anemia among the study population decreased at three months and six months of milk intervention. Even though, there were no significant different between fortified group and standard groups (**Table 3**). Milk compliance was not significantly different between the two groups during the six months of milk supplementation i.e. 78.1% vs 76.3% (P= 0.23).

Cognitive performance

We measured the improvement of cognitive performance by analyzing the proportion of subjects who performed below 50 percentile of the population value. The proportion of subjects who were below 50 percentile of population value for verbal attention, working memory and visual attention is seemed to be less in the milk-fortified group, even though no significant result if compared to standard-milk group. After milk supplementation, subjects with fortified milk had significant decreased of speed processing score below 50 percentile compared to standard-milk group. MANOVA analysis showed that speed processing score in the fortified milk group was constantly higher compared to that in the standard-milk group (**Table 3**).

Physical fitness

The subjects who received iron-zinc fortified milk supplementation tended to have lower number who were able to perform the step-test less than 60 seconds which step test score less than 50 percentile of the baseline data (**Table 3**). However, during milk supplementation, the duration of step test and the step test score increased significantly in both group (**Table 3**).

Ferritin response

Both groups had improved iron status. However, serum ferritin concentrations were already significantly different at baseline (P=0.043). The proportion of children suffered from iron deficiency did not differ significantly between two groups. The serum ferritin concentration increased significantly (P<0.0001) during the milk supplementation in both groups. The prevalence of iron deficiency in both groups decreased significantly after milk supplementation; but but the difference was not significant between the two groups. (Table 3 and 4).

Morbidity

There were no significant difference between the two groups about the effect of intervention on serum CRP level (**Table 3**), total episode of illness and mean disease episode. After three months of milk supplementation, the episode of illness tended to decrease in both groups, but those were not occurring after six months of milk supplementation (**Table 5**). However, we found a weak correlation between morbidity and ferritin level in the last three month period (**Table 6**).

Table 3. Characteristics of the subjects, %(n)

	Baseline		Midline		Endline	
	Milk A	Milk B	Milk A	Milk B	Milk A	Milk B
Nutrition Status						
Prevalence of Underweight	56.2 (122)	59.8 (125)	41.9 (91)	48.8 (102)	49.3 (107)	57.4 (120)
Prevalence of Stunted	40.6 (88)	38.8 (81)	39.2 (85)	41.6 (87)	35.5 (77)	35.4 (74)
Prevalence of Wasted	18 (39)	24.4 (51)	10.1 (22)	11.0 (23)	12 (26)	14.4 (30)
Anemia						
(Hb < 12 g/dl)	14.3 (31)	13.4 (28)	17.5 (38)	13.4 (28)	11.1 (24)	9.1 (19)
Iron Defficiency Anemi	2.8 (6)	3.3 (7)	1.4 (3)	1.4 (3)	0.9 (2)	1.0 (2)
Cognitive Performance < P50						
Speed Processing						
P 50 = 29	48.8 (106)	55 (115)	33.2 (72)	35.9 (75)	19.4 (42)	28.7 (60)1
Verbal Attention						
P 50 = 5	59.9 (130)	59.8 (125)	54.4 (118)	51.7 (108)	49.8 (108)	59.3 (124)
Working Memory						
P 50 = 2	51.6 (112)	48.8 (102)	32.3 (70)	29.2 (61)	24.9 (54)	32.1 (67)
Visual Attention						
P 50 = 6	51.2 (111)	54.5 (114)	31.3 (68)	28.2 (59)	15.2 (33)	16.3 (34)
Physical Fitness						
Duration of Step Test < 60s	34.6 (75)	46.9 (98)2	15.2 (33)	22.0 (46)	6.9 (15)	11.0 (23)
Step Test Score < P50						
P50 = 7082	41.5 (90)	58.9 (123)3	20.3 (44)	27.3 (57)	9.2 (20)	14.4 (30)
Micronutrient Status						
Iron Deficiency						
(Ferritin < 12mcg/dl)	14.3 (31)	12.4 (26)	6.0 (13)	9.6 (20)	6.0 (13)	4.8 (10)
Morbidity						
Elevated CRP Level						
(>5 mg/l)	0.9 (2)	1.0 (2)	1.4 (3)	0.0 (0)	1.4 (3)	0.5 (1)
Dietary Intake < 60% RDA						
Calorie intake	64.5 (140)	66.5 (139)	71.4 (155)6	24.9 (52)	53.5 (116)11	34.4 (72)
Iron intake	60.4 (131)	79.4 (166)4	7.8 (17)	46.4 (97)7	0.5 (1)	28.7 (60)13
Zinc intake	97.7 (212)	98.6 (206)	89.9 (195)	98.6 (206)8	85.7 (186)12	70.8 (148)
Calsium intake	82.5 (179)	90.0 (188)5	21.7 (47)9	10.0 (21)	25.3 (55)	27.8 (58)
Vitamin C intake	71.4 (155)	79.4 (166)	41.0 (89)10	28.7 (60)	45.2 (98)	43.1 (90)

1 Significantly different from milk A group, P value = 0.016, using Fisher's Exact Test
2 Significantly different from milk A group, P value = 0.010, using Fisher's Exact Test
3 Significantly different from milk A group, P value = 0.000, using Fisher's Exact Test
4 Significantly different from milk A group, P value = 0.000, using Fisher's Exact Test
5 Significantly different from milk A group, P value = 0.035, using Fisher's Exact Test
6 Significantly different from milk B group, P value = 0.000, using Fisher's Exact Test
7 Significantly different from milk A group, P value = 0.000, using Fisher's Exact Test
8 Significantly different from milk A group, P value = 0.000, using Fisher's Exact Test
9 Significantly different from milk B group, P value = 0.001, using Fisher's Exact Test
10 Significantly different from milk B group, P value = 0.008, using Fisher's Exact Test
11 Significantly different from milk B group, P value = 0.000, using Fisher's Exact Test
12 Significantly different from milk A group, P value = 0.000, using Fisher's Exact Test
13 Significantly different from milk B group, P value = 0.000, using Fisher's Exact Test

Nutrient intake

The daily nutrient fulfillment had increased on both groups. The subjects who received standard milk had significantly higher energy intake (**Table 5**) than before. Several nutrients intake, such as calcium and vitamin C tended to show a higher fulfillment adequacy during the first three months of supplementation.

Table 4. Response of milk intervention (mean (SD))

	Baseline		Midline		Endline	
	Milk A	Milk B	Milk A	Milk B	Milk A	Milk B
Anthropometry						
Weight (kg) *	19 (SD 1.75)	18.95 (SD 1.72)	20.17 (SD 1.95)	20.02 (SD 1.90)	20.37 (SD 1.98)	20.25 (SD 2.00)
Height (cm) **	117.05 (SD 4.65)	116.9 (SD 4.62)	118.65 (SD 4.77)	118.18 (SD 4.67)	120.09 (SD 4.71)	119.89 (SD 4.74)
MUAC (cm) **	16.63 (SD 0.90)	16.83 (SD 0.86)	16.96 (SD 0.90)	17.25 (SD 0.88)	17.08 (SD 0.83)	17.18 (SD 0.96)
Sitting Height (cm) **	61.85 (SD 3.05)	61.78 (SD 3.02)	63.30 (SD 2.53)	62.85 (SD 2.50)	64.38 (SD 2.49)	64.16 (SD 2.47)
Cognitive performance						
Coding Score **	28.48 (SD 11.07)	27.58 (SD 10.91	34.07 (SD 9.62)	32.66 (SD 10.66)	40.04 (SD 12.66)	35.28 (SD 10.05)
Digit Span Forward Score *	5.71 (SD 3.14)	5.68 (SD 3.21)	6.77 (SD 3.64)	6.85 (SD 3.55)	6.63 (SD 3.48)	6.37 (SD 3.40)
Digit Span Backward Score *	2.60 (SD 1.52)	2.73 (SD 1.95)	3.18 (SD 1.57)	3.19 (SD 1.74)	3.69 (SD 1.98)	3.44 (SD 2.15)
Visual Search Score *	4.99 (SD 7.11)	3.07 (SD 12.72)	7.51 (SD 7.49)	7.42 (SD 9.94)	11.12 (SD 5.97)	10.25 (SD 8.55)
Physical activity						
Step Test Duration (second) *#	89.90 (SD 55.87)	66.41 (SD 33.77)	129.83 (SD 72.36)	98.02 (SD 46.55)	187.92 (SD 96.22)	150.29 (SD 83.82)
Step Test Score *#	9408.81 (SD 5713.02)	7001.73 (SD 3463.13)	13340.03 (SD 7230.79)	10164.17 (SD 4677.46)	19120.94 (SD 9643.01)	15311.54 (SD 8341.36)
Laboratory examination						
Hemoglobin (g/dl) **	13.01 (SD 0.91)	12.92 (SD 0.89)	12.81 (SD 0.85)	12.95 (SD 0.87)	13.07 (SD 0.77)	13.08 (SD 0.83)
Ferritin serum (mcg/dl) *#	30.79 (SD 22.10)	34.49 (SD 22.86)	35.02 (SD 23.96)	36.76 (SD 22.50)	49.16 (SD 48.93)	52.12 (SD 36.39)
CRP level (mg/l) *	1.08 (SD 1.66)	1.24 (SD 2.41)	2.06 (SD 2.47)	1.34 (SD 0.93)	2.09 (SD 1.99)	1.74 (SD 1.19)
Iron storage (/kgBW) *	1.63 (SD 1.19)	1.84 (SD 1.24)	1.74 (SD 1.18)	1.84 (SD 1.13)	2.44 (SD 2.43)	2.59 (SD 1.82)
Dietary Intake						
Calorie **	1022.01 (SD 272.53)	972.77 (SD 287.62)	1103.64 (SD 268.50)	1200.28 (SD 28.15)	1115.12 (SD 705.79)	1182.13 (SD 250.35)
Iron *	7.57 (SD 6.39)	5.28 (SD 4.70)	9.55 (SD 3.73)	6.33 (SD 1.90)	10.53 (SD 2.74)	7.16 (SD 2.76)
Zinc **	4.01 (SD 1.40)	3.53 (SD 1.30)	5.27 (SD 1.24)	4.48 (SD 0.90)	5.52 (SD 1.10)	6.26 (SD 2.72)
Calcium **	241.69 (SD 150.66)	194.34 (SD 132.28)	462.12 (SD 148.59)	509.17 (SD 113.23)	444.42 (SD 127.98)	439.06 (SD 163.71)
Vitamin C **	23.30 (SD 20.72)	16.86 (SD 17.51)	35.21 (SD 20.87)	39.22 (SD 20.87)	30.23 (SD 15.20)	31.60 (SD 22.59)

MANOVA (repeated measurement):
* Significantly different from three times measurements in baseline, midline, end line
** Significantly different from three times measurements in baseline, midline, end line and correlated by milk intervention
Mann Whitney test:
Significantly different between two groups

Table 5. Effects of milk supplementation on morbidity (mean (SD))

	Milk A	Milk B	P
First three months (month 0-3)			
Number of URTI episodes	0.98 (SD 1.12)	0.98 (SD 1.14)	0.995
Number of fever episodes	0.29 (SD 0.52)	0.22 (SD 0.46)	0.114
Number of episodes of gastrointestinal infection	0.05 (SD 0.22)	0.04 (SD 0.23)	0.724
Total number of episodes of illness	1.32 (SD 1.34)	1.24 (SD 1.29)	0.514
Second three months (month 3-6)			
Number of URTI episodes	0.44 (SD 0.65)	0.35 (SD 0.56)	0.134
Number of fever episodes	0.25 (SD 0.50)	0.24 (SD 0.46)	0.759
Number of episodes of gastrointestinal infection	0.05 (SD 0.25)	0.02 (SD 0.15)	0.269
Total number of episodes of illness	0.74 (SD 0.94)	0.62 (SD 0.90)	0.162
Six months (month 0-6)			
Number of URTI episodes	1.42 (SD 1.40)	1.34 (SD 1.42)	0.515
Number of fever episodes	0.54 (SD 0.80)	0.46 (SD 0.71)	0.223
Number of episodes of gastrointestinal infection	0.10 (SD 0.34)	0.07 (SD 0.30)	0.341
Total number of episodes of illness	2.07 (SD 1.79)	1.86 (SD 1.77)	0.229

Table 6. Correlation between change of ferritin plasma level with morbidity (total of episodes of illness) during milk intervention

Morbidity	Ferritin serum	
	r	P
Total of episodes of illness during first three months	0.01	0.89
Total of episodes of illness during second three months	0.13	0.01*
Total of episodes of illness during six months	0.04	0.46

Discussion

In this study, milk supplementation decreased the prevalence of underweight, stunted and wasted. Prevalence of iron deficiency anemia among the study population also decreased at three months and six months of milk intervention. However, there are no significant difference between fortified group and standard groups. This study indicated that milk provided nutrient benefits for underweight school children. The fact is that additional 200 kcal provided by the milk has led to the lower prevalence of underweight, stunted, wasted, anemia and iron deficiency.

The anemia (15%) and iron deficiency prevalences (14%) in this population was surprisingly lower than those reported earlier. Since most of the subjects had normal hemoglobin and serum ferritin, no major changes that can be obtained after supplementation. We found that the subjects who received fortified milk had significantly better speed processing, even though they had similar result in other cognitive measurements such as working memory, verbal attention and visual attention. Iron is required by enzymes involved in specific brain functions for myelination and synthesis of the neurotransmitters serotonin (tryptophan hydroxylase) and dopamine (tyrosine hydroxylase).[23] Zinc also contributes to the cells growth including 300 of human enzymes. Zinc also required for the synthesis of neurotransmitters.[5] The neurotransmitters are important to mantain adequate cognitive function.[5,23]

In this study, milk supplementation provided positive effect on physical fitness. Since the positive effect on the physical fitness also occurred in both groups, the result is considered to be not related to the micronutrients supplementation as mentioned by other studies.[24] Consequently, subjects which consumed fortified milk had higher iron intake than before. However, consuming fortified milk is not the only cause to have better iron status and iron storage; other factors can affect the ferritin level, such as infection and inflammation. Therefore, the study also showed the influence of the infectious diseases on the ferritin level. Morbidity and inflammation may increase the iron status. This study showed that there was an increase of iron status after six months of supplementation in both groups. By chance, the total illness episodes in both groups had increased after six months of milk supplementation; however the difference of each increment is not significant between the two. One possible factor that effecting the morbidity was that there was unexpectedly flooding disaster in both sites of the study (Jakarta and Solo) during the last three months of milk supplementation. We found that there was a weak correlation between episode of morbidity and serum ferritin level in the last three months of supplementation. Likewise, other study showed a positive effect after a year of fortified milk supplementation with 9.6 mg iron and 7.8 mg zinc in reducing the morbidity;[25] the duration of supplementation and level of micronutrients might also plays a role.

Many factors seem to influence the outcome of micronutrient supplementation. In this study, we found that subjects who received standard milk tended to have higher calcium and vitamin C intake. However, based on the study result, both nutrients do not influence the iron and zinc utilization and its absorption. Based on the theory, higher calcium intake could inhibit iron utilization.[26] Moreover, vitamin C intake might enhance the iron absorption.[26] However, no impact of those nutrients was found in this study.

Interactions between zinc and iron were mostly found when the micronutrients were given by using the water solution medium. Several studies have shown that fortified milk has positive impact on the prevalence of anemia and physical growth[11,12] thus, iron and zinc that supplemented in powder milk using adequate ratio might be an efficient way to provide micronutrient fortification. However, in order to get more evidences, further studies on the effect of iron and zinc supplementation in milk to the iron status are needed.[20,21]

The iron status of the children in the study population was within normal range. Most of the children did not suffer from iron deficiency. As stated earlier, supplementation of iron-zinc fortified milk has positive effects on functional outcome such as growth and cognitive performance. Furthermore, both milk supplementations i.e. fortified milk and standard milk also have positive effects on iron status and physical fitness. It seems that the human body could utilize the milk ingredients well and get positive functional outcomes. The evidence showed that iron-zinc fortified milk and standard milk supplementations are able to increase the iron status. However, vitamin C intake also seems to affect the iron status of children by standard milk. It may also indicates that vitamin C has an important role as an enhancer to iron utilization that will affect the iron status of children who consume standard milk.[18]

Moreover, the fortified milk supplementation is effective to increase height, sitting height and speed processing. All cells require iron (Fe) and zinc to grow, replication, respiration, and DNA synthesis. Iron and zinc are required for critical proteins within the mitochondrion that involved in electron transport and energy production.[27] These findings support several other studies that found no independent negative effect either of iron-zinc supplementation or even a beneficial effect on growth and cognitive performance.[13,27] In conclusion, milk supplementation provides significant benefits for the underweight school children. It reduces the prevalence of anemia and iron deficiency. The addition of 6.3 mg of iron and 1.5 mg of zinc intake per day is effective in improving the speed processing score, height and sitting height. Thus, milk fortified with iron and zinc, in the proper ratio, can be recommended for improving school children's health.

Acknowledgments

Financial support for this research is gratefully acknowledged from the PT. NESTLE Indonesia. Appreciation also goes to Dra. Diffah Hanim for her insights and constructive suggestions in this research. In particular, we would like to extend our very deep appreciation to Dwi Hidayah MD, Pediatrician; Annang Giri Moelyo MD, Sri Wahyu Herlinawati MD, who had volunteered their time and expertise to come to the assistance of this research. We thank the children and parents who participated in this research, and the head masters and teachers who were so kind and supportive. Special thanks and appreciation are due to PRODIA Clinical Laboratory for taking blood and chemical analyses.

References

1. Angeles IT, Schultink WJ, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. Am J Clin Nutr. 1993;58:339–42.
2. Harahap H, Jahari AB, Husaini MA, Saco-Pollit C, Pollitt. Effects of an energy and micronutrient supplement on iron deficiency anemia, physical activity and motor and mental

development in undernourished children in Indonesia. European Journal of Clinical Nutrition. 2000;54:S114-S119.
3. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931-945.
4. Brown KH, Peerson J M, Rivera J, Allen L. Effect of supplemental zinc on the growth and serum concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002;75:1062–1075.
5. Black MM. The evidence linking zinc deficiency with children's cognitive and motor functioning. J Nutr. 2003;133:1473S–1476S.
6. Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA, et al. Zinc Supplementation affects the activity patterns of rural Guatemalan infants. Am J Clin Nutr. 1997;127:1333-8.
7. Sazawal S, Black RE, Menon V P, Dingra P, Caulfield LE, Dingra U, Bagati A. Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized controlled trial. Pediatrics. 2001;108:1280–6.
8. Penny ME, Marin RM, Duran A. Randomized controlled trial of the effect of daily supplementation with zinc or multiple micronutrients on the morbidity, growth, and micronutrient status of young Peruvian children. Am J Clin Nutr. 2004;79:457–65.
9. Lind T, Lönnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nutr. 2004;80 :729–6.
10. Lind T, LönnerdalLind T, Lönnerdal B, Stenlund H, Ismail D, Seswandhana R, et al. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nutr. 2003;77:883–90.
11. Wieringa FT, Berger J, Dijkhuizen MA, Hidayat A, Ninh NX, Utomo B, et al. Combined iron and zinc supplementation in infants improved iron and zinc status, but Interactions reduced efficacy in a Multicountry Trial in Southeast. Asia J Nutr. 2007; 137: 466-71.
12. Sandstrom B. Micronutrient interactions: effects on absorption and bioavailability. Br J Nutr. 2001;85:S181–5.
13. Dijkhuizen MA, Wieringa FT, West CE, Martuti S, Muhilal. Effects of iron and zinc supplementation in Indonesian infants on micronutrient status and growth. J Nutr. 2001;131:2860-5.
14. Friel JK, Serfass RE, Fennessey PV, Miller LV, Andrews WL, Simmons BS, et al. Elevated intakes of zinc in infant formulas do not interfere with iron absorption in premature infants. Journal of Pediatric Gastroenterology & Nutrition. 1998;27:312-6.
15. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clinical Nutrition. 2006;84:1261-76.
16. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr. 2001;131:616S–635S.
17. Lestari ED, Hartini TNS, Hakimi M, Surjono A. Nutritional status and nutrient intake from complementary foods among breastfed children Purworejo District, Central Java, Indonesia. Paediatr Indones. 2005;45:31-39.
18. Lestari ED, Moelya AG, Rohana E, Wiboworini B. Relation of complementary foods and anemia in urban underprivileged children in Surakarta. Paediatr Indones. 2007;47:196-201.
19. Walker CF, Kordas K, Stoltzfus RJ, Black RE. Interactive effects of iron and zinc on biochemical and functional. Am J Clin Nutr. 2005;82:5–12.
20. Villalpando S, Shamah T, Rivera JA, Lara Y, Monterrubio E. Fortifying milk with ferrous gluconate and zinc oxide in a public nutrition program reduced the prevalence of anemia in toddlers. J Nutr. 2006;136:2633-7.
21. Peres-exposito AB, Vilalpando S, Rieviera JA, Griffin IJ, Abrams SA. Ferrous sulfate is more bioavailable among pre-schoolers than other forms of iron in a milk-based weaning food distributed by PROGRESA, a national program in Mexico. J Nutr. 2004;135:64-9.
22. Stephenson LS, Latham MC, et al. Physical fitness, growth, and appetite of Kenyan school boys with Hookworm, Trichuris trichiura, Ascaris lumbricoides infections are improved four months afer a single dose of albendazole. J Nutr. 1993;123:1036-46.
23. Hazhihume M, Kunii O, Sasaki S, Shimdo T, Wakai S, Mazhitova Z, et al. Anemia and iron deficiency among school children in Aral Sea Region Kazakhtan. J Trop Pediatr. 2003;49:172-7.
24. Le HT, Brouwer ID, Verhof FI, Nguyen KC, Kok FJ. Anemia and intestinal parasite infection in school children in rural Vietnam. Asia Pac J Clin Nutr. 2007;16:716-23.
25. Sawazal S, Dhingra U, Hiremath G, Kumar J, Dhingra P, Sarkar A, et al. Effects of fortified milk on morbidity in young children in north India: community based, randomized, double masked placebo controlled trial. BMJ. 2007;334:140-5.
26. Perales S, Barberaa R, Lagarda MJ, Farrea R. Fortification

of milk with calcium: Effect on calcium bioavailability and interactions with iron and zinc. J Agric Food Chem. 2006;54:4901-6.

27. Ponka P, Beaumont C, Richardson DR. Function and regulation of transferrin and ferritin. Semin Hematol. 1998;35:35-54.