Journal of Food Science and Engineering 5 (2015) 112-121
doi: 10.17265/2159-5828/2015.03.002

Multi-micronutrient Fortified Beverage Delivered through the School-Based System Improved Iron Status and Test Scores of Children

Imelda Angeles-Agdeppa and Clarita Reyes Magsadia

Department of Science and Technology, Food and Nutrition Research Institute, Bicutan, Taguig City 1631, Philippines

Abstract: Fortifying commonly consumed foods and beverages offers a great opportunity of filling the nutrient gap between actual and the needed nutrient intakes of young children. Ready-to-drink beverage as a vehicle for fortification is easy to administer, more consistent and least obstructive, because it can be consumed without further processing/cooking. This study aimed to evaluate the effectiveness of fortified beverage on the improving of iron status and school performance test of schoolchildren. About 5,000 schoolchildren aged 6-12 years old from 19 schools in Quirino province, Philippines were invited to participate in the study. Samples of 4,875 children with parental consent were screened for hemoglobin and serum ferritin levels. About 4,495 children had complete participation in the school performance test, which were developed and administered by school supervisors. Children were fed 200 mL ready-to-drink juice fortified with iron, zinc, lysine, vitamins A and C for 120 d. The beverage was delivered through the baseline. At end of the intervention, only 1,050 children had complete data set and the anemia rate reduced from 100% to 60%. Iron deficiency has increased insignificantly from 3.6% to 4.5%. Mean percentage of test scores increased significantly between base and end of the study: English (40% to 60%), math (35% to 58%) and science (32% to 58%). In conclusion, consumption of 200 mL fortified juice drink had contributed to the reduction of anemia and increased tests scores of children.

Key words: Anemia, iron, school performance, cognition.

1. Introduction

Malnutrition is estimated to contribute to more than one-third of all child deaths, although it is rarely listed as the direct cause. Lack of access to highly nutritious foods, especially in the present context of rising food prices, is a common cause of malnutrition. Poor feeding practices, such as inadequate breastfeeding and not ensuring that the child gets enough nutritious food, contribute to malnutrition. Infection, particularly frequent or persistent diarrhea, pneumonia, measles and malaria, also undermines a child's nutritional status [1].

In developing countries, micronutrient deficiencies tend to exist where diets lack diversity and intake of animal products is minimal. In poor countries, this deficiency is exacerbated by systemic infectious and parasitic diseases that reduce nutrient absorption and biological utilization. Thus, incorporating vitamins and minerals that are present in low amounts in the diets into widely consumed foods has become one of the main interventions used to prevent the consequences of their deficiencies for both poor and wealthy societies. The type and amounts of micronutrients to add may vary from country to country, because the epidemiological realities are different. Therefore, proper data about food/nutrient intake should be available to make an informed decision for designing efficacious and safe food fortification programs [2].

Micronutrient deficiencies do not occur in isolation. Millions of people worldwide suffer from deficiencies of multiple micronutrients at the same time. Iron deficiency anemia is a major problem in developing countries, especially in infants and young children.

Corresponding author: Imelda Angeles-Agdeppa, Ph.D., research field: public health nutrition.

Deficiencies in vitamin A usually coexist with iron deficiency, while like iron, zinc is important for the transport of vitamin A via biological membranes. Zinc deficiency is found to have effects on growth in infants and young children.

In Philippines, anemia rates across population groups are alarmingly high. The recent 2013 national nutrition survey data revealed that the prevalence of anemia among 6-12 years old Filipino children is 11.1% [3]. Government and non-governmental agencies are increasingly considering food fortification with micronutrients as an alternative approach to resolving micronutrient deficiencies. Fortifying commonly consumed foods and beverages offers a great opportunity of filling the nutrient gap between actual intake and the needed required nutrient intake of young children and thereby improving their nutritional status. Ready-to-drink beverage as a vehicle for fortification is easy to administer, more consistent and least obstructive, because it is consumed without further processing/cooking. Therefore, this study aimed to evaluate the effectiveness of fortified beverage on the nutritional status and school performance of schoolchildren through a school-based delivery system.

2. Materials and Methods

2.1 Study Sites, Subjects and Study Design

The study was conducted in 19 selected elementary schools in three districts in Quirino province, Philippines. Five thousand (5,000) schoolchildren aged 6-12 years old were invited to participate in the study. Only 4,875 had consented to participate in the study. Children without parental consent, aged less than 6 years and more than 12 years old, with physical abnormalities were excluded. Only 4,520 children had consented to participate in the school performance test, which were developed and administered by school supervisors. Children were fed 200 mL ready-to-drink juice fortified with iron, zinc, lysine, vitamin A and C for 120 d. The beverage was delivered through the school system by a feeding coordinator (Table 1).

2.2 The Intervention, Implementation and Monitoring

Public health nurses from the Department of Education (DepEd) directly administered albendazole chewable tablets (400 mg) to all targeted children before the commencement of feeding.

The feeding program was implemented from Monday to Friday for a total of 120 feeding days. The orange-flavored fortified juice in 200 mL foil pack was fortified with iron (1.3 mg), zinc (1.4 mg), lysine (212 mg), vitamin C (45 mg) and vitamin A (133 μg) (Table 1) and distributed together with a 30 g bread during the children's morning recess (10:00-10:30 am).

The bread was delivered everyday by a local bakery contracted for the project, while the fortified juice was hauled to each school twice weekly, since there are no large spaces as storerooms in the schools. The pre-identified feeding coordinator of each school allocated the fortified juice and bread per class. The

Table 1 Amount of nutrients in the fortified drink and percent contribution to Recommended Energy and Nutrient Intake (RENI).

Nutrients	Amount of nutrient in a 200 mL pack	RENI for children				Percent adequacy for specific nutrients			
		4-6 years	7-9 years	10-12 years (boy)	10-12 years (girl)	4-6 years	7-9 years	10-12 years (boy)	10-12 years (girl)
Iron (mg)	1.3	9.0	11.0	13.0	19.0	14.4	11.8	10.0	6.8
Zinc (mg)	1.4	5.4	5.4	6.8	6.0	25.9	25.9	20.6	23.3
Lysine (mg)	212	836	1,056	-	-	25.4	20.1	-	-
Vitamin C (mg)	45	30	35	45	45	150.0	128.6	100.0	100.0
Vitamin A (μg)	133	400	400	400	400	33.3	33.3	33.3	33.3
Energy (kcal)	99	1,410	1,600	2,140	1,920	7.0	6.2	4.6	5.2

teacher or their representative student allocated their fortified juice and bread by presenting a list of children beneficiaries for the day.

In the classroom, children washed their hands and prayed before taking their juice and bread. The classroom teacher monitored the consumption. Zero leftover of the juice was maintained from start to end of the feeding period. The classroom teacher accomplished the consumption report and recorded the reasons of children who were absent. This report was submitted monthly to the school feeding coordinator. Public health nurses from the DepEd division office also monitored the implementation of feeding activities in each school for the duration of the program. Accomplished consumption report, stock and issue forms from the feeding coordinator were collected by the research team monthly. Problems encountered as recorded in the forms and as reported by the feeding coordinator were discussed, and appropriate solutions were instituted during field visits. Monthly feedback meetings were conducted by the research team with the DepEd officials.

2.3 Ethical Considerations

The study protocol was reviewed and approved by the Technical Committee and the Institutional Ethics and Review Committee, Food and Nutrition Research Institute of the Department of Science and Technology (FNRI-DOST), General Santos Avenue, Bicutan, Taguig City, Metro Manila, Philippines.

Orientations among school officials at all levels were conducted to equip them with details and purpose of the study. This is also to solicit their active participation in the study and be oriented on their roles and responsibilities. The same activity was conducted among parents of children, who were selected to participate in the study.

2.4 Measurements

A modified semi-quantitative food frequency questionnaire was used to determine the quality of food intake. The questionnaire consisted of list of foods high in iron, vitamins A and C, zinc and lysine. This was administered to only 50% randomly selected study children.

Blood collection was carried out through vein puncture by trained medical technologists from an international standardization organization (ISO) contracted laboratory from 7:30 am to 11:30 am. Blood samples were transported in a cool box and analyzed in a laboratory set up at the Quirino provincial hospital. A 3.5 mL of blood sample was collected for the analysis of hemoglobin (2 mL) and serum ferritin (1.5 mL). For hemoglobin analysis, sample was placed in BD Vacutainer K2 ethylenediaminetetraacetic acid (EDTA) and measured using sodium lauryl sulphate (SLS) hemoglobin method in Sysmex XT-1800i. Meanwhile, serum ferritin was placed in BD Vacutainer SST with no anticoagulant but with gel separator that stood for 30 min after extraction. It was then subjected to centrifugation using Universal 320 that worked at 3,500 rpm for 10 min. It was analyzed using Elecsys 2010 machine by Roche diagnostics that utilized electrochemiluminescence immunoassay (ECLIA) for the *in vitro* quantitative determination of ferritin in human serum [4].

To evaluate the effects of the juice drink, all identified anemic children from the 4,875 children with consent plus 13% random sub-samples with normal hemoglobin level had undergone post-intervention measurement. For serum ferritin, 50% of random sub-samples of anemic children plus 25% to account for drop out was considered for analysis. Anemia was defined as haemoglobin (Hb) < 120 g/L [5], while concentration for serum ferritin (SF) < 20 μg/L indicates iron deficiency anemia [4]. Quality assurance and measures were maintained throughout the analysis based on Westgard multirule.

To measure the school performance, questionnaires were developed by the education supervisors from the DepEd division office in Quirino, Philippines. All questions were assured that these are within the scope of the topics covered by the different subjects and grade level. Subjects covered were English, mathematics and science for grades III to VI; English and mathematics for grade II; and school readiness assessment (SReA) test with the different domains: gross motor, fine motor, receptive/expressive language, numeracy, reading readiness, construction and visual motor integration, sensory discrimination and seriation/classification, and concept formation for grade I. At start, there were only 4,520 out from 4,875 children who gave consent have participated. The 355 children refused to participate. The test was administered by the classroom teachers and supervised by the education supervisors and research team in an unnoticed manner. This was to avoid unwanted fear from the children. Changes in test scores were collected to determine the possible indirect effects of the intervention.

2.5 Data Organization, Processing and Analysis

Data were encoded using the statistical package for social science (SPSS) version 9.0. The one-sample Kolmogorov-Smirnov test was employed to test the normality of the distribution for the hemoglobin and serum ferritin. If the data did not follow a normal curve, non-parametric statistical test was used or logarithmic transformation was performed; hence geometric means of the values were reported [6, 7]. To determine the homogeneity of the groups, two related sample Wilcoxon signed-rank test was used to test within group comparison in the values for hemoglobin, serum ferritin and school performance at baseline and after the 120 feeding days. Paired *t*-test was used for serum ferritin levels after log transformation for within group comparison. Baseline values of data gathered from children who dropped out from the study were analyzed using unpaired *t*-test.

3. Results and Discussion

3.1 Effects of the Intervention

The mean age of children was 9.88 years old. Results showed that anemia prevalence of the 4,875 targeted children was 23% (1,100) (Table 2). This indicates that anemia is a public health problem among schoolchildren in Quirino province, and therefore warrants immediate actions to prevent its deleterious effects. To evaluate the effects of the juice drink on iron status, all identified anemic children (1,100) plus 13% random sub-samples of children with normal hemoglobin level (3,775 × 13% = 490) which was total 1,590, were considered to undergo post-intervention (endline) measurement. However, only 1,050 of the anemic and 454 out of children with normal hemoglobin levels totaling to 1,504 have complete data set. Mean hemoglobin level increased significantly from 115.7 g/L at baseline to 120.4 g/L at endline (P = 0.000). At endline, 40% (420) of the anemic children at baseline had attained normal hemoglobin levels. Children, who were assessed as having normal hemoglobin level at baseline, had remained at normal levels at endline (Table 3). The

Table 2 Distribution of study children by hemoglobin level at screening (*n* = 4,875).

Hemoglobin level	Frequency	Percent (%)
Anemic	1,100	22.6
Non-anemic	3,775	77.4

Table 3 Mean hemoglobin level of study children at baseline and endline (*n* = 1,504).

Hemoglobin (g/L)	Baseline	Endline	*P*-value
Mean	115.7	120.4	0.000*
Standard deviation (SD)	6.3	8.0	

*Means significant difference at α = 0.01.

Table 4 Distribution of study children by serum ferritin level at baseline and endline (*n* = 674).

Serum ferritin (SF) level	Frequency	Percent (%)
Baseline		
Iron deficient (SF < 20 μg/L)	24	3.6
Normal	650	96.4
Endline		
Iron deficient (SF < 20 μg/L)	29	4.3
Normal	645	95.7

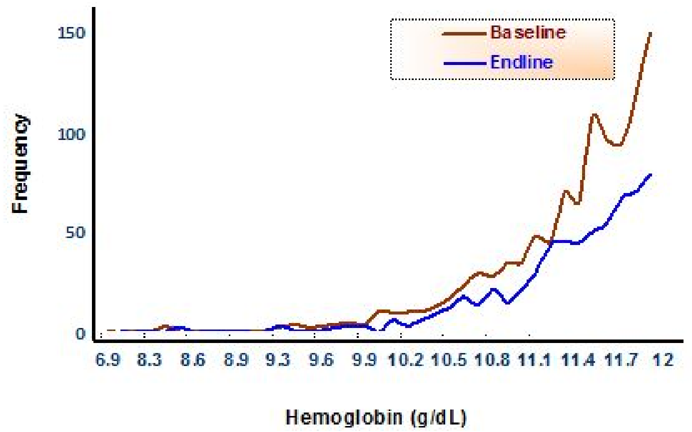

Fig. 1 Hemoglobin level of anemic children at baseline and endline.

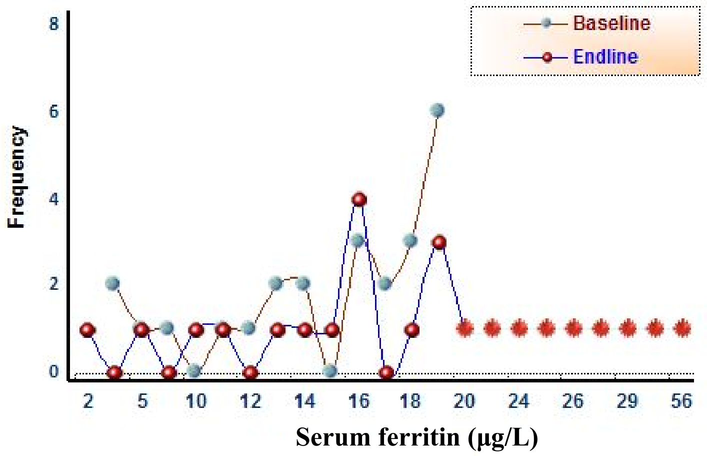

Fig. 2 Distribution of old cases of children with low serum ferritin levels at baseline and endline.

60% (630) who remained still anemic at endline had also manifested an improvement in hemoglobin level, but fall short to be categorized as normal at endline (Hb ≥ 120 g/L) (Fig. 1). Baseline values of dropped-out children were not significantly different from the mean hemoglobin values of the remaining subjects. For serum ferritin analysis, 50% (550) of the randomly sub-sampled anemic children plus 12% (132) giving a total of 682 children to account for drop out was considered for analysis. Only 674 (99%) had a complete data set for baseline and endline. Results revealed that iron deficiency rate had insignificantly increased from 3.6% to 4.3% at base and end of the study, respectively (Table 4). In Fig. 2, nine children had improved from iron deficient to normal serum ferritin levels at endline, but in new cases five were found iron deficient at endline (Fig. 3).

The results indicate that providing fortified juice drink for only 120 d and meeting only 7% to 14% of the daily RENI for specific age groups might not be

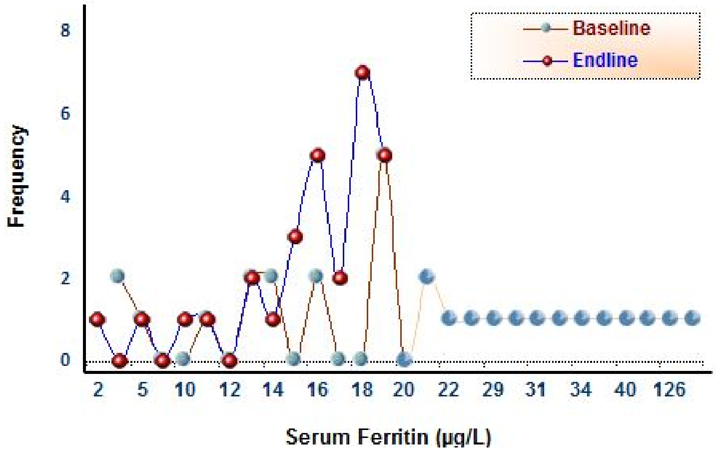

Fig. 3 Distribution of new and old cases of children with low serum ferritin levels at baseline and endline.

adequate to cause iron to be stored among iron depleted study children. It should be noted that the first response to iron intervention is increased in hemoglobin concentrations, which is seen in this study. Serum ferritin levels could increase, if there is a continuous adequate supply of iron from food, while hemoglobin levels are maintained at normal levels. Although there was a significant decrease in ferritin levels between baseline and endline, the distribution curve shows that schoolchildren were achieving higher ferritin levels despite low intake of quality iron-rich foods. Food frequency data showed that, on a daily basis, the commonly consumed foods by children were one pack of crackers (31%), one pack instant noodles (17%), chicken egg (16%), hotdog (9%), pandesal (7%) and pork meat (5%). On a weekly basis, the commonly consumed foods by children were tilapia (84%), chicken meat (77%), chicken eggs (77%), pork meat (76%), hotdog (72%) and noodles (71%). Compliance on juice drinking could had contributed to the results of hemoglobin and serum ferritin. Only 77% completed the 120 feeding days; 16.2% had consumed the juice for < 120-110 d and 9.3% (453) consumed for at least for 100 d.

Increment in hemoglobin values could be explained by increase in iron absorption from the form of nutrients in the juice drink, which is micronized in readily absorbable form; the presence of vitamin C also enhances iron absorption [8] and the vitamin A increases the use of iron in hemoglobin synthesis [9]. The deworming drugs given at baseline had increased the hemoglobin level of schoolchildren and significantly improved iron status [10, 11], and thus absence of intestinal parasites contributed to positive iron balance and facilitated iron absorption [12]. Furthermore, regular consumption of multiple micronutrient fortified beverage improved hemoglobin concentration and effectively decreased the prevalence of anemia and iron deficiency [13]. Similar findings in Refs. [14-17] suggested that multiple micronutrient supplementations were effective, had improved hemoglobin and ferritin levels and reduced the prevalence of anemia which can be attributable to the iron component. In the same manner, studies on multiple micronutrient-fortified beverages [18-20], iron-fortified candies [21], biscuits [22] and drinking water [23] showed positive response to intervention by increased hemoglobin levels, serum ferritin or decreased prevalence of anemia among subjects after the intervention period. The multiple micronutrient intervention was effective in reducing the prevalence of anemia in children [24-29].

On the contrary, studies on iron-fortified soup [30] and micronutrient-fortified beverage [31] showed no significant improvement in hemoglobin values. Differences in the outcome can be attributed to the dose of iron added in the product, duration of the intervention and the study design. Findings in efficacy trials relate to single interventions, whereas many effectiveness large scale programs were implemented as multi-component, integrated programs under normal existing environmental and socio-economic exposures.

3.2 School Performance of Study Children

Study children's mean percentage of test scores in English, mathematics and science taken by grades II to VI had significantly increased from baseline to endline. The highest mean score increased was in science at 25.16% (Table 5).

At baseline, anemic study children had lower mean test scores in all three subjects compared to study children with normal hemoglobin level, although not significant. At endline, study children with normal hemoglobin level had higher mean test scores compared to anemic study children except in math, though not significant (Table 6). Meanwhile, the mean test scores of children in grade I who took the SReA text revealed significant increase in gross and fine motor, receptive and expressive language, sensory discrimination and seriation/classification, concept formation, numeracy, reading readiness, and construction and visual integration from baseline to endline (Table 7).

Table 5 Mean percentage of test scores of grades II to VI children at baseline and endline (*n* = 3,610).

		Baseline	Endline	*P* value
Subject	Percent mean scores	40.01	61.76	
	SD	13.43	17.88	0.000*
	Corr.	0.453**		
Math	N	3,578		
	Percent mean scores	35.12	57.84	0.000*
	SD	11.94	18.36	
	Corr.	0.349**		
Science	N	2,842		
	Percent mean scores	32.46	57.62	0.000*
	SD	11.18	19.99	
	Corr.	0.283**		

*Significance at $\alpha = 0.05$; **significance at $\alpha = 0.01$.

Table 6 Mean percentage of test scores of grade II to VI anemic and study children with normal hemoglobin at baseline and endline.

Subjects	Baseline	Endline
English		
Anemic (711)[a] (443)[b]	37.74 [12.5][1]	59.05 [17.2]
Normal (326)[a] (613)[b]	38.96 [13.4]	59.81 [18.4]
Mean difference	1.22	0.76
P value	0.113	0.538
Mathematics		
Anemic (706)[a] (443)[b]	33.98 [11.0]	55.89 [17.5]
Normal (319)[a] (612)[b]	34.14 [11.9]	55.47 [18.1]
Mean difference	0.16	-0.42
P value	0.950	0.735
Science		
Anemic (493)[a] (317)[b]	31.85 [10.9]	55.96 [19.2]
Normal (265)[a] (462)[b]	32.78 [11.5]	56.58 [21.0]
Mean difference	0.93	0.62
P value	0.412	0.826

[1]Means the value of standard deviation; a—baseline value; b—endline value.

Table 7 Mean percentage of test scores in SReA of grade I study children at baseline and endline (*n* = 885).

Categories	Baseline		Endline		Change	*P* value
	Mean	SD	Mean	SD	Mean	
Gross motor (five items)	4.35	1.28	4.86	0.61	-0.52	0.000
Fine motor (five items)	3.67	1.77	4.76	0.76	-1.09	0.000
Receptive/expressive language (five items)	2.17	1.92	4.15	1.37	-1.97	0.000
Sensory discrimination and seriation/classification (five items)	2.54	1.99	4.28	1.29	-1.74	0.000
Concept formation (five items)	2.58	1.90	4.07	1.45	-1.48	0.000
Numeracy (five items)	2.63	1.98	4.09	1.45	-1.46	0.000
Reading readiness (10 items)	3.51	3.30	6.31	3.31	-2.80	0.000
Construction and visual integration (five items)	2.33	1.92	4.17	1.84	-1.84	0.000

The relationship between micronutrient deficiency and early cognitive development had captured recent attention, because micronutrients were related to specific physiological and neurological processes [32] and had been shown to be related to various domains of childhood development [33]. As micronutrient deficiencies coincided and synergistic effects of micronutrients may indirectly affect cognition, children supplemented with multiple micronutrients could have advantages over single micronutrient [34].

Earlier studies concluded that oral iron and folic acid had improved IQ scores for both anemic and non-anemic children [35, 36]. Children with iron deficiency anemia had poorer learning outcomes particularly in mathematics, while children with normal iron status performed better on standardized math tests than children with iron deficiency [37]. Anemia was associated with disturbances in attention and perception, which resulted in poor academic achievement [38, 39]. Iron deficient children first enrolled in school were disadvantaged in terms of aptitudes, and this disappeared once they become iron-replete through supplementation [40]. Similar findings showed that multiple micronutrient fortified food was effective in improving micronutrient status and cognition [41, 42], and vitamin and mineral deficiencies limit the child's optimal ability in cognition and intelligence [43]. In contrast, micronutrient supplementation during preschool years had no effect on aspects of intellectual, executive and motor function at 7-9 years old, suggesting no long-term developmental benefit during 12-35 months of age [44].

4. Conclusions

Feeding 200 mL ready-to-drink juice fortified with iron, zinc, lysine, vitamin A and C for 120 d through a school-based feeding program has contributed to the improvements of hemoglobin levels and test scores of children. However, a well-controlled trial following rigid inclusion criteria to get valid information on the cause-effect relationship of anemia on school performance should be conducted. Single micronutrient interventions may not be enough to improve the cognition of schoolchildren, nevertheless a multiple micronutrient fortified foods could be considered in feeding programs through an organized school-feeding system. Food based approach, such as multiple micronutrient fortification, could serve as an effective strategy to combat deficiencies and promote better school performance and well-being of children.

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