### **Original Article**

# Fortified juice drink improved iron and zinc status of schoolchildren

Imelda Angeles-Agdeppa PhD, Clarita R Magsadia MPS-FNP, Mario V Capanzana PhD

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

Energy and micronutrient deficiency remain prevalent among Filipino children. Juice drinks are commonly consumed and could be a viable vehicle for fortification to supplement the nutrient gap. This study determined the effects of a newly developed non-carbonated fortified juice drink on the iron, zinc and nutritional status of schoolchildren. One hundred randomly selected anemic children were randomly allocated into two groups in a doubly-masked placebo controlled manner: Group 1 received the fortified juice, Group 2 received the nonfortified juice for 100 days, five days a week under strict supervision. The juice drink was fortified with vitamin A, zinc, iron, vitamin C and lysine. The non-fortified juice was fortified only with vitamin C. All children were dewormed prior to the intervention. Hemoglobin, plasma ferritin and plasma zinc, weight and height were assessed using standard methods before and after intervention. A two-day 24-hour food recall was also collected. The basal prevalence of anemia was significantly reduced in both the fortified group (100% to 13%) and the non-fortified group (100% to 40%) at endline. The mean plasma ferritin levels were similar in both groups at baseline and endline. At endline, mean plasma zinc in the fortified group has significantly increased by 20  $\mu g/dL$ from a baseline value of 83.9  $\mu g/dL$  to 103.9  $\mu g/dL$ , while the non-fortified group remained at similar levels with baseline. Basal weight and height significantly increased among all children at endline. The fortified juice drink was effective in reducing the prevalence of anemia and improved the zinc status of children.

Key Words: anemia, hemoglobin, plasma ferritin, zinc status, fortified juice drink

#### INTRODUCTION

Iron deficiency anemia (IDA) in the Philippines among six to 12 year-old children is 20.8%. On the other hand, among six to 10 year olds, the prevalence of underweight and stunting are 25.6% and 33.1%, respectively.<sup>1</sup>

The Food and Nutrition Research Institute – Department of Science and Technology (FNRI–DOST) is the research arm of the government in terms of nutrition, food and other science and technology activities and is mandated to continuously pour its efforts into finding solutions and appropriate technologies to combat nutrient deficiencies and other nutritional problems. In a publicprivate partnership mechanism, a private industry has collaborated with FNRI and requested to develop a nutritious product, specifically a beverage. In the Philippines, results of the National Food Consumption Survey showed that beverages such as fruit juice drinks (orange) was one of the 30 food items most commonly consumed by children.<sup>2</sup>

Fortifying commonly consumed foods and beverages offer a great opportunity of increasing the nutrient intake of young children, thereby, improving their nutritional status. Beverage as a vehicle for fortification is easy to administer, more consistent and least obstructive because it is consumed without further processing/cooking. Evidences of previous studies showed significant improvements in hemoglobin and serum retinol concentration, as well as increased weight and height in children consuming beverages fortified with ten micronutrients.<sup>3,4</sup>

The present study made use of a juice drink fortified with iron, vitamins A, C, zinc and lysine because of the complementary role of these nutrients. Vitamin A has been implicated in the synthesis of transferrin that makes iron available for hematopoesis,<sup>5</sup> Vitamin C and zinc improve immune response, and zinc also improves growth like lysine.<sup>6</sup>

This study was carried out to investigate the effects of the multi-nutrient fortified juice drink on the iron, zinc and anthropometric indices of children.

#### MATERIALS AND METHODS

#### Study design and sampling size

This study was conducted at Pinaglabanan Elementary School in San Juan, Metro Manila. This is a government school in the National Capital Region with high prevalence of anemia.<sup>7</sup>

All children aged 6 to 9 years old were invited to participate in the study however, only children with written parental consent were selected for screening. Children not suffering from any infections (diarrhea, fever, acute respiratory infections) during the time of examination and for

Email: imelda\_agdeppa@yahoo.com

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**Corresponding Author:** Dr Imelda Angeles-Agdeppa, Food and Nutrition Research Institute-Department of Science and Technology, Gen. Santos Ave. DOST Compound, Bicutan, Taguig City, Philippines.

Tel: (632) 837-2071 ext 2280; Fax: (632) 837-3164

the last two weeks prior to the assessment period were further assessed for hemoglobin (Hb). Children found to be anemic with Hb >70 g/L to <120 g/L and with normal weight-for-age z-score (WAZ <-2 to +2 SD) were included as subjects of the intervention study.<sup>8</sup> Children with illness like cough and colds, fever, diarrhea, who were severely underweight (WAZ – 3 SD) and/or severely anemic (Hb <70 g/L) were excluded from the study and referred by the medical team to the nearest health facility for management.

Thirty five children per group were needed for this study. The sample size was calculated to detect a minimum change of 7 g/L in Hb concentrations with an estimated SD of 9 g/L, a confidence interval of 95%, and a power of 90%. To allow for 30% attrition rate, the sample size was increased to 50 per group.<sup>9,10</sup>

The 100 randomly selected anemic children were ran-

domly allocated into two groups in a double-masked placebo controlled manner: group 1 received the fortified juice drink; and group 2 received the non-fortified juice drink. The juice drink was administered for 100 days, five days a week under a supervised regimen. Figure 1 shows the operational flow of the study.

#### The intervention

The juice drink was fortified with 133.3 µg vitamin A, (Vitamin A Palmitate 250 cold water soluble, US Pharmacopeia (CWS, USP); 1.4 mg zinc (zinc sulfate monohydrate); 1.3 mg iron (micronized dispersible ferric pyrophosphate or Sun-Active FeP80, Food Chemical Codex (FCC); 45 mg vitamin C (Ascorbic acid USP); and 200 mg lysine (L-lysine monohydrochloride, USP) (Table 1). The non-fortified juice was fortified only with vitamin C (Ascorbic acid, USP). Both juice drinks contain 106 ki-



Figure 1. Operational flow diagram of the study

Nutrient	Amt. of nutrient in a 200 ml /pack —	RENI for children		% adequacy for specific nutrients	
		4 – 6 y	7 – 9 y	4 – 6 y	7 – 9 y
Iron (mg)	1.3 (2.6)	9	11	28.9	23.6
Zinc (mg)	1.4 (2.8)	5.4	5.4	51.9	51.9
Lysine	200 (400.0)	836§	1056§	47.8	37.8
Vitamin C <sup>*</sup> (mg)	45.0 (90.0)	30	35	300	257
Vitamin A (µg)	133.3 (166.6)	400	100	41.6	41.6
Energy (kcal)	106 (212)	1410	1600	15.0	13.2

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

† () amount of nutrients in 2 packs of juice

‡ the same amount was also added in the non-fortified drink

§ http://www.umm.edu/altmed/ConsSupplements/Lysinecs.html

localories. Dosages of the different nutrients were determined based on the food fortification policy of the Philippines on processed foods which states that nutrients to be added should at least meet 30% of the Recommended Nutrient Intake (RNI) of specific target groups.<sup>11</sup>

Before the start of the study, 400 mg of Albendazole was administered directly to all study children by the school physician and nurses. Administration of the intervention started a week after the deworming. The fortified juice drink is a non-carbonated, orange flavored juice, packed in 200 ml foil packs. The beverage foil packs were color coded to correspond to the color codes of the IDs of the two treatment groups in study. The coding was done by a non-member of the research team. These codes were revealed to the principal investigator after the data analysis.

Children had to consume the two packs of juice drinks from 8:00 to 11:00 A.M. with no left-over in a designated feeding room. Children who cannot finish the two packs come back to the feeding room during recess to finish their left-over.

#### Monitoring of intake

Feeding was administered by two research staff under a strictly supervised regimen. All empty foil packs were retrieved from the children after feeding. Daily consumption was recorded in a structured form. Attendance of children was checked and recorded every day. Reasons for absenteeism, e.g. due to illness, domestic problems, or unavoidable circumstances were also recorded including the type and duration of illness experienced by the children.

Children who were absent received their juice drink on the day they reported to school in addition to the juice drink they have to consume for the day, hence children were given two packs of drinks in the morning and two in the afternoon. For children who were absent for longer days (e.g. two or three days), their juice allocation were given on a staggered days with a maximum of four packs per day.

#### Measurements

Weight of children was measured using a Detecto weighing scale (Webb City, Mo. U.S.A) and recorded to the nearest 0.1 kg. Study children were weighed in lightweight clothing without shoes. Height was measured barefoot and was recorded to the nearest 0.1 cm using a microtoise (Depose, France) posted flat against a wall. All equipment used were calibrated before every use.

Anthropometric z-scores were computed for all children relative to the NCHS/WHO international reference standard using the Epi-info Nutrition Package (CDC 2004). In this study, the cut-off of WAZ <-2 SD; HAZ <-2 SD; WHZ <-2 SD was used to define underweight, stunting, and wasting respectively.<sup>12</sup>

A 24-hour food recall was collected at baseline and towards the end of the study from parents who were interviewed face-to-face by a well-trained nutritionist. Food intakes were transformed to nutrients using the individual dietary evaluation system (IDES). The IDES is a software developed by the FNRI–DOST. Adequacy of intakes was computed as the percent of actual nutrient intake as against the values in the RNI of the Philippine Recommended Energy Nutrient Intake (RENI).<sup>13</sup>

Trained medical technologists from FNRI–DOST conducted blood collection and processing for Hb, plasma ferritin, and plasma zinc analyses. Blood samples of children were collected from 7:30 am to 11:30 am by fingerprick using sterile, disposable lancets. About 1 to 1.5 mL of free flowing blood (after discarding the first drop) was drawn into two EDTA polypropylene Becton and Dickinson microtainer tubes.

Hb was measured using the cyanmethemoglobin method. Twenty  $\mu$ L of blood was pipetted using a disposable pipette into 5 mL cyanmethemoglobin reagent. Absorbance was read in a portable spectrophotometer (Odyssey DR-2400 by Hach) at 540 nm and converted to equivalent hemoglobin concentration using the regression curve generated from Hemoglobin Reference preparation.<sup>14</sup> Anemia in this study was defined as Hb <120 g/L.<sup>8</sup>

The remaining samples in two microtainer tubes were centrifuged within 1-2 hours after the draw at 2500 to 3000 rpm for 15-20 minutes. Plasma was transferred into two separate labeled polypropylene microcentrifuge tubes, one tube for plasma ferritin, and the other tube for plasma zinc analysis. The samples were properly packed and stored in -20°C freezer at the biochemical laboratory of the Institute. Single assay day analysis protocol of baseline and endline sample of each subject was observed for plasma ferritin and plasma zinc determinations.

Plasma ferritin concentration was measured by immunoradiometric assay of Ramco Laboratories using Gamma Counter (Packard). A tri-level, human serumbased immunoassay control and pooled serum control sample were analyzed in every assay day to monitor interassay precision and accuracy. Concentration below 20  $\mu$ g/L indicates iron deficiency anemia.<sup>15</sup>

Plasma zinc levels were determined by flame atomic absorption spectrophotometry (AAS, Buck). The suggested lower cut-off for the assessment of zinc status in children <10 years is 65 µg/dL.<sup>16</sup>

#### Statistical analysis

All data were encoded and analyzed using SPSS 9.0.<sup>17</sup> Data were rigorously scrutinized for errors and lack of consistency between raw and encoded data, and all errors were corrected by comparison of all records referring to the same child.

The one-sample Kolmogorov-Smirnov test was employed to test the normality of distribution for the Hb, plasma ferritin and plasma zinc values, weight and height measurements. If data did not follow a normal curve, then, logarithmic transformation was performed; hence, geometric means of these values were reported.<sup>9,10</sup>

T-test was used to test between group comparisons in the values for biochemical and anthropometric indicators at baseline to determine the homogeneity of the groups. The same analysis was used at baseline and at the end of the feeding period for between group comparisons for biochemical, anthropometric measurements. Within group differences of these variables were tested using paired t-test.<sup>9,10</sup>

A chi-square test was used to compare data expressed in proportions and categories (e.g. prevalence of anemia, low ferritin, low Zn, etc).<sup>9,10</sup>

#### Ethical considerations

School officials, teachers and parents were oriented about the purpose and details of the study. Individual signed consent was sought from the parents of each of the selected children. The study protocol was guided by the Council for International Organizations of Medical Sciences Ethical Guidelines for Biomedical Research Involving Human Subjects and the National Guidelines for Biomedical/Behavioral Research.<sup>18,19</sup> This was reviewed and approved by the Technical Committee of FNRI–DOST and the Institutional Ethics and Review Committee.<sup>20</sup>

#### RESULTS

The total number of children screened in this study was 639. The prevalence of anemia was 19.9% (n=127). However only 100 children consented for blood testing at baseline. At endline only 4 from the fortified group and 7 from the non-fortified group dropped – out from the study

**Table 2.** Percent adequacy of energy and nutrient intake of study children at baseline and after the 100-day feeding period, by treatment group

	Fortified group	Non-fortified froup	<i>p</i> -value
Energy (%)			
Base	100.0	90.0†	0.267
6-mo	65.5	75.4	0.126
Difference	-34.5	-14.6	0.066
<i>p</i> -value	< 0.001	0.050	
Protein (%)			
Base	109.7	91.8	0.111
6-mo	76.4	86.4	0.201
Difference	-33.2	-5.4	0.042
<i>p</i> -value	0.002	0.548	
Vitamin A (%)¶			
Base	46.4	50.5‡	0.758
6-mo	32.2	43.4	0.264
Difference	-14.2	-7.1	0.954
<i>p</i> -value	0.436	0.468	
Vitamin C (%)			
Base	34.5	32.0‡	0.447
6-mo	13.4	21.6	0.178
Difference	-21.1	-10.4	0.686
<i>p</i> -value	0.057	0.336	
Iron (%)			
Base	82.9	72.7	0.258
6-mo	58.6	57.3	0.878
Difference	-24.4	-15.4	0.450
<i>p</i> -value	0.010	0.047	
Calcium (%)¶			
Base	37.7	31.0§	0.191
6-mo	24.6	26.6	0.593
Difference	-13.1	-4.4	0.144
<i>p</i> -value	0.005	0.113	

† Mean

‡ Median

§ Geometric mean

¶ Comparison of vitamins A and C between groups were done by Mann-Whitney U tests while calcium was analyzed on log transformed value.

during the implementation phase because they either transferred to other schools (n=4), moved to another province (n=2) or were absent during the data collection (n=5).

The recorded illness during the course of the study revealed that cough and colds were similarly experienced by study children in the fortified group (58.1%) and non-fortified group (58.7%). Episodes of fever and diarrhea were also similar in both groups.

The mean percent adequacy of intake of energy, protein, iron, vitamin A, vitamin C and calcium were similar at baseline in both the fortified and non-fortified groups. At endline, mean energy intake decreased in both groups by 14.6% in the non-fortified and 34.5% in the fortified group. Protein, iron, vitamin C and calcium significantly decreased in the fortified group while only iron intake decreased in the non-fortified group. Vitamin A intake had insignificantly decreased in both the non-fortified and fortified groups (Table 2).

The consumption of fortified foods was also similar in both the fortified and non-fortified groups. The two commonly consumed fortified foods were noodles and powdered juice drinks. The main reason for consuming these fortified foods was likeness or acceptability of the taste. Other reasons given were: makes the child healthy and accessible.

Basal hemoglobin levels in both the fortified and nonfortified groups were similar at baseline. At endline, the mean hemoglobin levels in both the fortified (126 g/L) and non-fortified groups (121 g/L) had significantly increased (p = 0.001). However, mean change in the fortified group (12.5 g/L) was significantly higher than the non-fortified group (7.2 g/L) (p = 0.002) (Table 3). The rate of anemia in the fortified group significantly reduced from 100% to 13.0%; while in non-fortified group, from 100% at baseline to 39.5% at endline (p = 0.007) (Table 4).

Basal plasma ferritin levels of the fortified (27.6  $\mu$ g/L) and non-fortified (28.8  $\mu$ g/L) groups were similar at baseline. At endline, significant increases in plasma ferritin levels were achieved by both groups and mean increments were not significantly different between groups with 23.0  $\mu$ g/L for the fortified group and 21.6  $\mu$ g/L for the non-fortified group (Table 3).

At baseline plasma zinc level in the non-fortified (96.2  $\pm$  24.1 µg/dL) group was significantly higher than in the fortified (83.9  $\pm$  23.3 µg/ dL) group (p = 0.020). At endline, the mean change in zinc level of the fortified group significantly increased by 20.0 µg/dL while the nonfortified group remained at similar level as the baseline

Table 3. Biochemical measures of study children by treatment group

	Fortified group n=46	Non-fortified group n=43	<i>p</i> -value*
	Mean (SD)	Mean (SD)	
Hemoglobin (g/L)			
Baseline	114 (5.5)	114 (5.8)	0.878
Endline	126 (6.4)	121 (8.4)	0.001
Diff	12.5 (8.7)	7.2 (6.4)	0.002
p-value	< 0.001	< 0.001	
Serum Ferritin (µg/L)			
Baseline	27.6 (22.1, 34.4)	28.8 (22.9, 36.1)†	0.819
Endline	50.6 (44.1, 58.0)	50.4 (41.0, 61.8)	0.913
Diff	23.0	21.6	0.730
p-value*	< 0.001	< 0.001	
Plasma Zinc (µg/dL)			
Baseline	83.9 (23.3)	96.2 (24.1)	0.020
Endline	104 (26.1)	96.6 (22.7)	0.185
Diff	20.0 (25.9)	0.4 (26.3)	0.001
p-value*	<0.001	0.922	

† Geometric mean (95% confidence interval of the mean), analysis was done on log transformed values

\* *p*-value of t-test

Table 4. Prevalence of anemia, iron deficiency and zinc deficiency of study children by treatment group

	Fortified Group n=46	Non-Fortified Group n=43	<i>p</i> -value*
	n (%)	n (%)	
Anemia (Hb <120 g/L)			
Baseline	46 (100)	43 (100)	-
Endline	6 (13.0)	17 (39.5)	0.007
Iron deficiency (SF $< 20 \mu g/L$ )			
Baseline	13 (28.3)	9 (21.4)	0.460
Endline	2 (4.3)	3 (7.1)	0.572
Zinc deficiency (PZ $< 65 \mu g/dL$ )			
Baseline	9 (19.6)	2 (4.7)	0.033
Endline	1 (2.2)	3 (7.0)	0.350

\*p-value of chi-square test

	Fortified group	Non-fortified froup		
	(n=46)	(n=43)	<i>p</i> -value*	
	Mean (SD)	Mean (SD)		
Weight (kg)				
Baseline	21.7 (3.3)	21.7 (3.2)	0.915	
Endline	23.4 (3.6)	23.3 (3.5)	0.900	
Diff	1.7 (0.9)	1.7 (0.8)	0.904	
p-value**	< 0.001	<0.001		
Height (cm)				
Baseline	120 (7.4)	120 (5.8)	0.988	
Endline	123 (7.4)	123 (5.9)	0.979	
Diff	3.3 (0.6)	3.3 (0.7)	0.902	
p-value**	< 0.001	< 0.001		
WAZ				
Baseline	-1.01 (0.66)	-1.09 (0.73)	0.613	
Endline	-0.88 (0.69)	-0.96 (0.73)	0.607	
Diff	0.13 (0.20)	0.13 (0.19)	0.950	
p-value**	< 0.001	< 0.001		
HAZ				
Baseline	-1.15 (0.86)	-1.21 (0.60)	0.727	
Endline	-1.02 (0.85)	-1.08 (0.61)	0.704	
Diff	0.13 (0.09)	0.13 (0.11)	0.825	
p-value**	<0.001	<0.001		

Table 5. Anthropometric measures of study children by treatment group and nutritional status

\* *p*-value of t-test

\*\* p-value of paired t-test

#### (0.4 µg/dL) (Table 3).

No between group differences was found in weight and height measurements from baseline to endline, however significant increments within groups were observed overtime (Table 5). The mean increment in weight (1.7 kg) and height (3.3 cm) were similar in the fortified and the non-fortified groups.

#### DISCUSSION

Numerous published studies on the negative consequences of IDA in children have shown delayed motor and mental development,<sup>21</sup> cognitive function impairment and slowed growth.<sup>22,23</sup> The signing of the Food Fortification Law of the Philippines (RA 8976) which mandates the fortification of rice and other staples with iron as well as voluntary fortification of commonly consumed foods is one of the strategies to respond to this public health problem.<sup>11</sup>

Food fortification is a viable approach to reach the atrisk segments of the population at minimal cost.<sup>24,25</sup> Fortifying commonly consumed foods and beverages offer a great opportunity to increase the nutrient intake of children, thereby, improving their nutritional status. Beverages as vehicle for fortification are easy to administer, more consistent and least obstructive because they are consumed without further processing/cooking.<sup>3</sup>

Since, diet-related micronutrient deficiencies rarely occur in isolation and that no single fortified food is likely to provide an adequate intake of supplemental vitamins and minerals,<sup>4</sup> this study has considered multiple micronutrients to be added to the juice drink. Studies have shown that multiple micronutrient intervention have a greater impact on nutritional status than administration of the supposed key deficient single micronutrient.<sup>24,26</sup> The juice drink was fortified with zinc, vitamins A, C, iron, and lysine. Zinc was added because of its role in improving physical growth and improved immune response,<sup>16</sup> vitamin A on the effective utilization of iron,<sup>27,28</sup> vitamin C for its role in improving immunity and in enhancing iron absorption,<sup>29,30</sup> iron to improve iron status and reduce anemia and lysine to promote growth.<sup>6</sup>

The significantly higher increase of Hb levels in the fortified than in the non-fortified group could be attributed to the combined effects of nutrients added in the juice drink and the positive effect of deworming.<sup>8,31,32</sup> Parasitic infection is a common cause of iron loss, hence, the effect of iron fortification is greater when combined with deworming.<sup>33</sup> The increase in Hb levels of the nonfortified group between baseline and at endline measurements could be due to the effect of deworming given to all study children. Studies have shown that deworming alone had significantly improved iron status of schoolchildren in Thailand and Africa.<sup>33,34</sup> Several studies have shown a strong association between intensity of hookworm infection and anemia.35-37 Similarly, intestinal parasitoses contribute to negative iron balance through occult gastrointestinal blood loss<sup>38</sup> and may interfere with iron absorption.<sup>35</sup> Moreover, the vitamin C that is present in the juice drink of the non-fortified group may have acted on improving the immune response of children as evidenced by similar frequency in the occurrence of illness between the two groups.

The evident effect of fortification was shown in the significant decline of anemia prevalence (100% to 13%) in the fortified group compared to the non-fortified (100% to 40%) from baseline to endline.

The significant increase in Hb levels among anemic children consuming the fortified drink could be explained by the mechanism of iron absorption wherein, iron absorption is more effective when iron is depleted. The insignificant difference in plasma ferritin concentrations between the fortified and non-fortified groups of anemic children showed that providing fortified beverage for only 100 days and meeting only 20% (2.6 mg) of the RENI might not be adequate to cause iron to be stored among iron depleted children because the first response to iron intervention is the increase in Hb concentrations as observed in this study. Should the duration of intervention be extended, we can extrapolate to achieve greater benefits.

The presence of anemia (13%) despite normal concentrations of plasma ferritin implies that anemic children had low grade anemia with Hb level ranging from 118 g/L to 119 g/L. This level has not caused the utilization of ferritin for Hb formation.<sup>39</sup> The use of the cut-off for anemia as 115 g/L for children aged 5-11 years might be more appropriate to be used in efficacy studies to get evident results.<sup>40</sup>

Plasma zinc levels had significantly increased in the fortified group while a slight increase was observed in the non-fortified group. Plasma zinc levels in the nonfortified group remained at similar values as baseline after 100 days. The prevalence of zinc deficiency has been reduced with an absolute difference of 17.4 percentage points among fortified group. The inhibitory effect of zinc in iron absorption and vice - versa was considered and prevented in this study by having the ratio of zinc to iron as 1:1.41 In this study, it showed that zinc fortification is more beneficial among children with depleted iron stores. Previous studies on zinc supplementation on plasma zinc vielded different results. Daily supplementation of Bangladeshi and Guatemalan children resulted in increased plasma zinc concentrations but supplementation among Iranian children showed no increase in plasma zinc.42-44 The differences in the results are attributed to the limitations of zinc concentrations used as indicator for the diagnosis of actual zinc status,45 different nutritional status of subjects, duration of the intervention and different study designs.

The micronutrients contained in the fortified juice drink were reasonably well absorbed, as indicated by the increase in hemoglobin, plasma ferritin and plasma zinc concentrations in the fortified group. Therefore, in multimicronutrient supplementation, the amount of nutrients should then be carefully considered because of the existence of nutrient – nutrient interactions.

The increase in weight and height in the non-fortified group could be attributed to their significantly higher protein food intake than the fortified group. Moreover, the non-fortified group had generally slightly higher vitamin A, and vitamin C intakes as compared with the fortified group. Deworming drugs, which were also given at baseline, might also have played a role in improving nutritional status. Improvement in anthropometric indices due to deworming also manifested in previous studies.<sup>46,47</sup> Furthermore, efficacy trials have shown that deworming improves growth and appetite of Kenyan schoolchildren.<sup>48,49</sup> Deworming may have reduced the parasites and thereby decreased competition in the utilization of nutrient from the diets of the children.

The average increase in weight and height among the fortified and non-fortified groups, however, were similar despite the fact that lower nutrient adequacy was observed in the fortified group. It can be deduced that the added nutrients (fortificants) in the beverage might have played a role in improving growth velocity in the fortified group. Furthermore, the maximum positive effect of the micronutrients on growth was not attained probably because of the short duration of the feeding period and the juice being a low – calorie drink which provided only 13-15% adequacy as compared with the total requirement of children for energy in this age group.

#### Conclusion

The micronutrients contained in the fortified juice drink were reasonably effective, as indicated by the significant reduction in anemia rates and significant increments in plasma zinc level from baseline to post intervention, among the fortified group compared to the non-fortified group. Therefore, fortification efforts should carefully consider the interaction and or complementary role of nutrients. The intervention had also significantly increased weight and height of both groups. However, the roles of lysine and zinc in growth velocity were not explicitly shown in this study maybe because of the relatively low dose, short duration of intervention and because the beverage was a low-calorie food. The administration of deworming drugs prior to intervention was beneficial in improving the hemoglobin concentration of children consuming the non-fortified juice drink, but greater effects were achieved on the iron and zinc status of children given the fortified juice drink.

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#### AUTHOR DISCLOSURES

Imelda Angeles-Agdeppa, Clarita R. Magsadia and Mario V. Capanzana have no conflicts of interest.

#### REFERENCES

- Food and Nutrition Research Institute Department of Science and Technology (FNRI-DOST). The Seventh National Nutrition Survey Initial Results; 2009.
- Food and Nutrition Research Institute Department of Science and Technology (FNRI-DOST). Philippine Nutrition Facts & Figures 2003. 2006;5-39
- Ash DM, Tatala SR, Frongillo EA Jr, Ndossi GD, Latham MC. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. Am J Clin Nutr. 2003;77:891-8.
- Abrams SA, Mushi A, Hilmers DC, Griffin IJ, Davila P, Allen L. A multinutrient-fortified beverage enhances the nutritional status of children in Botswana. J Nutr. 2003;133: 1834-40.
- Mejia LA, Arroyave G. The effect of vitamin A fortification of sugar on iron metabolism in preschool children in Guatemala. Am J Clin Nutr. 1982;36:87-93.
- Overbeck S, Rink L, Haase H. Modulating the immune response by oral zinc supplementation: a single approach for multiple diseases. Arch Immunol Ther Exp (Warz). 2008;56,15-30.
- Food and Nutrition Research Institute Department of Science and Technology (FNRI-DOST). Philippine Nutrition Facts & Figures 1998. 2001;70-80.
- International Nutritional Anemia Consultative Group (IN-ACG). Why iron is important and what to do about it: A new perspective. Hanoi, Vietnam International Nutritional Anemia Consultative Group; 2001.

- Dawson Saunders B, Trapp RG. Basic and clinical biostatistics. 2nd ed. A Lange Medical Book: Prentice Hall International Inc.USA; 1994.
- Norman GR, Streiner DL. Biostatistics: The bare essentials. Farell R, editor. Mosby; 1994.
- Republic Act No. 8976 An Act Establishing the Philippine Food Fortification Program as For Other Purposes. Available from: http://www.bfad.gov.ph/oldsite/republicacts/ RA 8976-Food Fortification Law.pdf
- Gibson RS. Principles of nutritional assessment. New York: Oxford University Press; 1990.
- 13. Food and Nutrition Research Institute-Department of Science and Technology (FNRI-DOST). Recommended energy and nutrient intakes (RENI). 2nd ed. Philippines; 2002.
- International Committee for Standardization on Hematology (ICSH). Recommendation for method for hemoglobinometry in human blood standard 6/2 and specifications for international hemoglobincyanide reference preparation EP6/3: 1977. J Clin Pathol. 1978;31:139-43.
- Halberg L. Screening for iron deficiency: an analysis based on bone-marrow examinations and serum ferritin determinations in a population sample of women. Br J Haematol. 1993;85:787-98.
- Hotz C, Brown KH. International Zinc Nutrition Consultative Group (IZINCG), Technical Document #1. Assessement of the risk of zinc deficiency in populations and options of its control. In: FNB. 2004;25:1(S2):S146.
- Norusis MJ. SPSS for Windows Base System User's Guide. Advanced Statistics Release 6.0.USA; 1993.
- 18. Council for International Organizations of Medical Sciences (CIOMS).International ethical guidelines for biomedical research involving human subjects. Council for international organizations of medical sciences in collaboration with World health Organization, Geneva; 2002.
- Philippine Council for Health Research and Development. National Guidelines for Biomedical/Behavioral Research. Philippine Council for Health Research and Development, Department of Science and Technology, Bicutan, Taguig, Metro Manila; 1996.
- 20. Food and Nutrition Research Institute-Department of Science and Technology (FNRI-DOST). Institutional ethics and review committee (IERC). National Guidelines for Biomedical/Behavioral Research. Philippine Council for Health Research and Development, Department of Science and Technology, Bicutan, Taguig, Metro Manila; 2004.
- Aukett MA, Parks YA, Scott PH, Wharton BA.Treatment with iron increases weight gain and psychomotor development. Arch Dis Child. 1986;61:849-57.
- Sungthong R, Mo-suwan L, Chongsuvivatwong V. Effects of haemoglobin and serum ferritin on cognitive function in schoolchildren. Asia Pacific J Clin Nutr. 2002;11:117-22.
- Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. J Nutr. 2001;131:568S-80S.
- Hurrel, RF, Cook JD. Micronutrient fortification of food: Technology and quality control. In: Strategies for iron fortification of foods. FAO/WHO. 1992;1(9):56-61.
- Lynch S. Food iron absorption and its importance for the design of food fortification strategies. Nutr Rev. 2002;60(S1):S3-6.
- Barclay D. Multi-fortification of beverages. FNB. 1998;19 (2):168-71.
- 27. Mejia LA, Chew F. Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. Am J Clin Nutr. 1988;48:595-600.
- Suharno D, West CE, Muhilal, Karyadi D, Hautvast JG. Supplementation with vitamin A and iron for nutritional

anaemia in pregnant women in West Java, Indonesia. Lancet. 1993;342:1325-8.

- Hallberg L, Rossander L. Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulfate on iron absorption from a simple Latin American-type of meal. Am J Clin Nutr. 1984;39:577-83.
- Monsen E. Iron nutrition and absorption: dietary factors which impact iron bioavailability. J Am Diet Assoc. 1988; 88:786-90.
- Pollitt E, Hathirat P, Kotchabhakdi NJ, Missel L. Balyasevi A. Iron deficiency and educational achievement in Thailand. Am J Clin Nutr. 1989;50:687-97.
- Cook JD, Reusser ME. Iron fortification: an update. Am J Clin Nutr. 1983;38:648-59.
- Hathirat P, Valyasevi A, Kotchabhakdi NJ, Rojroongwasinkul N, Pollitt E. Effects of an iron supplementation trial on the Fe of Thai schoolchildren. Br J Nutr. 1992;68:245-52.
- Hercberg S, Galan P, Dupin H. Iron deficiency in Africa. World Rev Nutr Diet. 1987;54:201-36.
- Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. Nutr Rev. 1997;55:223-32.
- 36. Dreyfuss ML, Stoltzfuss RJ, Shrestha JB, Shrestha JB, Pradhan EK, LeClerq SC, Khatry SK, Shrestha SR, Katz J, Albonico M, West KP, Jr. Hookworm, malaria and vitamin a deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. J Nutr. 2000;130: 2527-36.
- 37. Moretti D, Zimmerman MB, Muthaya S, Thankachan P, Lee T, Kurpad AV, Hurrell RF. Extruded rice fortification with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial. Am J Clin Nutr. 2006;84:822-9.
- Brooker S, Bethony J, Hotez PJ. Human hookworm infection in the 21st century. Adv Parasitol. 2004;58:197-288.
- International Nutritional Anemia Consultative Group. Guidelines for the eradication of iron deficiency anemia. INACG, Washington, DC; 1997.
- Chwang LC, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr. 1988;47:496-501.
- Solomons NW. Competitive Interaction of Iron and Zinc in the diet: Consequences of human nutrition. J Nutr. 1986; 116;927-35.
- 42. Khanum S, Alam AN, Anwar I, Akbar Ali M, Mujibur Rahaman M. Effect of zinc supplementation on the dietary intake and weight gain of Bangladeshi children recovering from protein-energy malnutrition. Am L Clin Nutr. 1988; 42:709-14.
- 43. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a longitudinal zinc intervention trial. Am J Clin Nutr. 1993;57:344-52.
- 44. Mahloudji M, Reinhold JG, Haghsenass M, Ronaghy HA, Fox MRS, Halsted JA. Combined zinc and iron compared with iron supplementation of diets of 6 to 12 year old village schoolchildren in Southern Iran. Am J Clin Nutr. 1975; 28:721-5.
- Gibson RS. Zinc nutrition in developing countries. Nutr Res Rev.1994;7:151-73.
- 46. Sarkar NR, Anwar KS, Biswas KB and Mannan MA. Effect of deworming on nutritional status of Ascaris infested slum children of Dhaka, Bangladesh. Indian Pediatr. 2002;39: 1021-6.
- 47. Gupta M, Jood S, Yadav SK, Khetarpaul N. Effect of supplementation on haemoglobin and serum retinol level and

nutritional status of school children of northern India. Nutr Health. 2001;15(2):97-111.

48. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, Trichuris trichiura and Ascaris lumbricoides infections are improved four months after a single dose of albendazole. J Nutr. 1993:123:1036-46.

### Original Article

49. Stephenson LS, Latham MC, Adams EJ, Kinoti SN, Pertet A. Weight gain of Kenyan school children infected with hookworm, Trichuris trichiura and Ascaris lumbricoides is improved following once-or-twice-yearly treatment with albendazole. 1993b. J of Nutr. 1993:123:656-65.

## Fortified juice drink improved iron and zinc status of schoolchildren

Imelda Angeles-Agdeppa PhD, Clarita R Magsadia MPS-FNP, Mario V Capanzana PhD

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

## 強化果汁改善學齡兒童體內鐵和鋅的狀態

菲律賓的兒童至今仍然有能量和微量營養素缺乏的問題。果汁是常吃的食物, 因此可以當作營養強化的載體,來填補營養素的缺口。本篇研究的目的是測定 一種新開發非碳酸強化果汁對學齡兒童體內鐵、鋅和營養狀況的影響。從貧血 兒童中隨機抽出 100 位,再隨機分配至 2 組並採雙盲、安慰劑控制的試驗。第 1 組接受強化果汁,第 2 組接受非強化果汁。在嚴格監督下,每週 5 天,共進 行 10 天。強化果汁添加的物質有維生素 A、鋅、鐵、維生素 C 及離胺酸;非 強化果汁只添加維生素 C。所有兒童在介入之前已驅完蟲。介入前後利用標準 化測量方試來評估血紅素、血漿鐵蛋白和鋅、體重及身高,另外也蒐集了 2 天 的 24 小時飲食回憶記錄。試驗結束時,強化組(100%降至 13%)與非強化組 (100%降至 40%)的貧血盛行率均顯著下降。強化組的血漿鐵蛋白平均值顯著增 加了 20 μg/dL,從試驗前的 83.9 μg/dL 增加至 103.9 μg/dL,但非強化組的數值 則和試驗前相同;所有兒童的身高、體重均有顯著增加。強化果汁可以有效地 降低貧血的盛行並且改善兒童體內的鋅狀態。

關鍵字:貧血、血紅素、血漿鐵蛋白、鋅狀態、強化果汁