Extruded Rice Grains Fortified with Zinc, Iron, and Vitamin A Increase Zinc Status of Thai School Children When Incorporated into a School Lunch Program\(^1\)–\(^3\)

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Abstract

Iron (Fe), zinc (Zn), and vitamin A (VA) deficiencies are common among children in developing countries and often occur in the same individual. Rice is widely consumed in the developing countries of Asia and the low phytate in polished rice makes it ideal for Zn and Fe fortification. Triple-fortified rice grains with Zn, Fe, and VA were produced using hot extrusion technology. The main objective of the present study was to determine the impact of triple-fortified extruded rice on Zn status in school children in Southern Thailand. Although serum zinc was the main outcome indicator, Fe and VA status were also assessed. School children with low serum zinc (\(n = 203\)) were randomized to receive either triple-fortified rice (\(n = 101\)) or natural control rice (\(n = 102\)) as a component of school lunch meals for 5 mo. Serum Zn, hemoglobin, serum ferritin, serum retinol, and C-reactive protein were measured at baseline and at the end of the study. After the intervention, serum Zn increased (\(P < 0.05\)) in both the fortification (11.3 ± 1.3 μmol/L) and control (10.6 ± 1.4 μmol/L) groups, most likely due to the proper implementation of the school lunch and school milk programs, with the increase greater in the group receiving the triple-fortified rice (\(P < 0.05\)). Because the children were not Fe or VA deficient at baseline, there was no change in Fe or VA status. We conclude that Zn fortification of extruded rice grains is efficacious and can be used to improve Zn status in school children. J. Nutr. 143: 362–368, 2013.

Introduction

Deficiencies in iron (Fe), zinc (Zn), and vitamin A (VA)\(^6\) still remain major public health problems in developing countries (1). These deficiencies have adverse health consequences on growth and cognitive development, pregnancy outcome, immune and reproductive function (2,3) and additionally increase mortality and morbidity from infections, including measles, diarrhea and malaria (4). Moreover, the coexistence of micronutrient deficiencies is common (5) and is often found in Thailand. In northeast (NE) Thailand, 60% of school children are at risk of 2 or more coexisting micronutrient deficiencies (6).

Although the national prevalence of Zn deficiency in Thailand is currently unknown, the most recent study in NE Thailand, based on serum zinc concentrations reported that 57% of school children were Zn deficient (6), while in the same region, 20% of the same children had marginal VA deficiency (VAD) (7). Anemia, however, still remains the major public health problem and the latest Thai national nutrition survey in 2003 (8) reported 56 and 26% of 6- to 11-mo-old and 1- to 5-y-old children, respectively, were anemic, a higher prevalence than in the previous survey. Several studies conducted in NE Thailand indicated that thalassemia and hemoglobinopathies rather than iron deficiency (ID) are the major causes of anemia (7,9,10). The latest Thai national nutrition survey also found the highest prevalence of stunting, a potential sign of Zn deficiency, in children in the southern region of Thailand (8).

Due to its wide consumption by poor population groups, rice is a promising vehicle for food fortification. Rice provides ~41% of total energy in Thailand (11). In addition, polished rice is an advantageous vehicle for Zn and Fe fortification because of its relatively low phytate content. In previous studies, we successfully fortified artificial rice grains with micronized ground ferric pyrophosphate using hot extrusion technology (12). These artificial, Fe-fortified rice grains, when mixed with natural rice and fed as part of a school lunch program, improved the Fe status of Indian children (13).

Adding Zn and VA, together with Fe, to rice grains is a potential strategy to control all 3 major micronutrient deficiencies.
Additionally, their simultaneous addition to the same grain may have an additional benefit because of interactions between these micronutrients at the metabolic level. Zn, VA, and Fe (14) all substantially contribute to improved immune competence and there are several potential interactions of VA in Fe metabolism (15). These include the need for VA within the erythropoietin pathway (16) and for the mobilization of Fe from the ferritin stores (17). Fe is likewise needed to recover VA from its liver stores (18). Although Fe can decrease Zn absorption in the absence of food, there is no effect of Fe on Zn absorption in the presence of food (19). We recently produced artificial rice grains triple fortified with Fe, Zn, and VA by hot extrusion technology (20). Storage tests in tropical conditions demonstrated relatively low and acceptable VA losses.

Previous studies of Zn supplementation in pharmacological Zn doses reported increased serum zinc (21), increased growth (22,23), and a decrease in the incidence of diarrhea (23). Previous reports on the efficacy of Zn-fortified foods, however, are inconsistent (24) and several studies have failed to show an improvement in serum Zn with regular consumption of Zn-fortified foods (21,25,26). One study in NE Thailand, however, did report a significant increase in serum zinc in children with low serum zinc at baseline after regular consumption of a Zn-containing, micronutrient-fortified spice mix (27).

The aim of the present study was to test whether adding artificial rice grains, fortified with Zn, Fe, and VA, to daily meals within a school lunch program would improve serum zinc in school children in southern Thailand. Fe and VA status were also monitored.

Subjects and Methods

Study site. The study was conducted in Satun province, which is located on the west coast of southern Thailand, where the majority of the population is Muslim. The study was performed in 8 primary schools in the Muang district, which included mainly children from low-income families. The schools had 4- to 12-y-old children (kindergarten to grade 6) who were provided with a school lunch program (5 d/wk), which was partly subsidized by the government. Lunch menus were prepared in rotating order and usually consisted of rice together with chicken or fish and occasionally with vegetables. The schools also provided free milk (200 mL) daily to all children. Weekly Fe supplementation, which had been given to the children by health officers/village health volunteers before the intervention, was not provided during the intervention. This was to improve the chance of showing an improvement in Fe status even though it was not the primary outcome measure. The study protocol was approved by the ethics committees of the ETH Zurich, Switzerland and Mahidol University, Thailand.

Pilot study to measure Zn, Fe, and VA intake and status. One year prior to the efficacy study (March 2008), a small, 3-d, food intake survey and a micronutrient status study were conducted in 2 schools (of the 8 schools used later in the intervention study) during the school vacation so as to measure current Zn, Fe, and VA intake and status, the main dietary components, and the suitability of such schools for a Zn intervention study.

Dietary assessment. Three-day weighed food records were conducted in all members of 20 families. The households were randomly selected from families with children aged between 7 and 12 y. The assessment was done on 3 consecutive days (including 2 weekdays and 1 weekend day) and during that time, the participants were asked to maintain their usual food habits. Edible portions of all foods and beverages were weighed during preparation and consumption using food scales with a precision of ±1 g. After the meal, the food that was not eaten was weighed. Food consumed outside the home was weighed by asking the participant to buy the same amount of food they have eaten (money given by the staff) or by recalling the food consumed.

Nutrient intake was calculated using the software program INMUCAL (Mahidol University, Thailand, version WD 2.1). Zn bioavailability was estimated based on the phytic acid:Zn molar ratio (28).

Micronutrient status. Children (n = 92) aged between 7 and 12 y with no visible signs of infectious disease and not taking micronutrient supplements were randomly selected from both schools. The number of subjects was based on 25% of total children in the 2 schools. Informed consent was obtained from all participants as well as their parents/guardians. Before obtaining informed consent, the details as well as the risks and benefits of the study were explained to the participants during the school meeting. Anthropometric measurements were collected before ~5 mL blood was drawn from all participants by venipuncture. The blood was equally divided into 2 tubes: one trace element-free tube and one EDTA-containing tube. The whole blood/serum samples were used to measure the following biochemical indicators: hemoglobin (Hb), serum Zn, serum ferritin (SF), serum retinol (SR), and C-reactive protein (CRP).

Efficacy study: preliminary screening and intervention. The intervention study was conducted ~1 y after the pilot study to estimate micronutrient intake and status. A total of 744 children participated in the baseline screening (July to August 2009). As in the earlier pilot study of micronutrient status, informed consent was obtained from participating children and parents/guardians. Height and weight were measured and ~5 mL of whole blood (divided in 2 tubes: one trace element-free and one EDTA-containing tube) was collected by venipuncture for determination of Hb, serum Zn, and CRP. Sample size calculations indicated that 76 children were needed in each group, based on 90% power to detect a difference of 50 μg/L (or 0.76 μmol/L) in the mean serum Zn concentration with a significance level of 0.05 (2-tailed) and SD of the serum Zn concentration from the prescreening survey. Anticipating a drop-out rate of 10–15%, at least 88 subjects were required in each group.

The efficacy study was a double-blind, randomized, controlled trial with Zn status as the main outcome indicator. All children with Zn deficiency (low serum zinc concentration) were invited to join the intervention study. Zn deficiency was defined as serum Zn <9.9 μmol/L for children <10 y, <10.1 μmol/L for female subjects ≥10 y, and <10.7 μmol/L for male subjects ≥10 y, respectively, from a morning nonfasting blood sample (2). Children showing severe anemia (Hb <80 g/L) or VAD (Bitor's spot or ocular signs of xerophthalmia as determined by a local doctor) or serum zinc concentration <8.3 μmol/L were excluded and treated according to local policy. From the original 744 children who participated in the baseline screening, 203 children met the inclusion criteria and agreed to participate. They were randomized into 2 groups. One group was given the triple-fortified rice containing Zn, Fe, and VA (intervention group) and the other group was given nonfortified rice (control group). SF and SR were additionally measured in the Zn-deficient children at baseline. All variables (serum zinc, CRP, Hb, SF, and SR) were measured again at the end of the intervention. The study was conducted from July 2009 to March 2010. Figure 1 shows an outline of the Zn efficacy study.

Rice fortification. Triple-fortified extruded rice grains were produced at ETH Zurich using the hot extrusion technology as described in Pinkaew et al. (20). The extrusion process was done at 50°C and 80°C at barrel numbers 1 and 5 and numbers 2, 3, and 4, respectively. After extrusion, the rice grains were air dried for 2 nights in a dark room before packing into aluminum foil bags under vacuum.

The fortification level of the extruded rice grains was 10 mg Fe, 9 mg Zn, and 1050 μg VA/g extruded rice. These levels were based on a consumption of ~140 g cooked rice per school lunch meal per child, which corresponds to ~50 g of dry uncooked rice, diluting the fortified rice 1:30 with natural rice, and the current intakes of Zn, Fe, and VA as measured in the pilot study. They were estimated to increase the intake of these micronutrients in 97.5% of the school children to above their Estimated Average Requirement (EAR) values when the EARs for Fe and Zn are based on a 10% bioavailability for Fe and a moderate bioavailability for Zn (28,29). For VA, a 40% loss was assumed to.

Rice fortified with zinc, iron, and vitamin A
have taken place during extrusion, drying, cooking, and storage under tropical conditions (20), although during the intervention, the fortified rice was refrigerated and stored in light-protected vacuum packaging. We estimated that the children received an extra 10 mg Fe, 9 mg Zn, and ~890 µg VA per school feeding day from the triple-fortified rice during the intervention. These amounts remain under the tolerable upper intake level (29).

Preparation and feeding of the lunch meal. Natural rice, as consumed in the study region, was mixed with the triple-fortified extruded rice grains at a ratio of 50:1 as described by Moretto et al. (12). For monitoring, at the beginning of each month, Zn was measured in 140 g of cooked rice. The fortified rice was mixed with the natural rice and cooked by local cooks at a central kitchen in Satun town, which had been specifically set up for the study. The cooked rice was weighed into individual portions of 140 g into a color-coded container that was labeled with the child’s name. The weight was regularly controlled by research assistants. The rice was transported to the 8 schools by the research assistants and the 140 g of cooked rice (triple-fortified rice or unfortified rice) was given to each child. The rice was consumed with foods such as soup or curry, which was provided by the school lunch program. The lunch meals contained mainly chicken (2–4 times/wk), whereas egg and fish were served less frequently (1–2 times/wk). Vegetables such as cabbage, cucumber, and yard long beans were used in some menus. Nonfortified-school milk was given to all children daily separately from the lunch meal at the morning or afternoon break. The feeding was constantly monitored by research assistants and teachers. The leftover rice from every child was estimated and recorded every second day. The study duration was 6 mo with a break of 1 mo due to school holidays (feeding time of 5 mo). The rice meal was fed 5 d/wk. After completion of the study, all children who remained deficient in any of the micronutrients in either group received supervised treatment of the respective micronutrient(s) according to local policies.

Laboratory analysis. Blood samples were collected in trace element-free tubes and stored in a cold box immediately after the blood was taken. The separation of serum from whole blood for determination of serum Zn and CRP was done in the field within 30–40 min according to the instructions recommended by International Zinc Nutrition Consultative Group (IZINCG) (2). Separation of plasma from EDTA-containing tubes for the analysis of SF and SR was done at the hospital laboratory after analysis of Hb on the same day. Serum samples were stored at ~20 °C prior to analysis of SR at the Institute of Nutrition, Mahidol University. SF, serum Zn, and CRP were measured at ETH Zurich.

Hb was measured using an electronic complete blood count (Cell Dyn 3700, Abbott Diagnostic) and 3-level controls provided by the manufacturer (Cell Dyn calibrator and control, Abbott Diagnostic). Anemia was defined as Hb <120 g/L in children ≥12 y old and Hb <115 g/L in children 5–11 y old (29). SF and CRP were measured using a chemiluminescent immunometric assay (IMMULITE, Diagnostic Products) together with a 3-level serum control (Diagnostic Products) with every set of measurement. ID was defined as SF <15 µg/L (29) and a cutoff of >5 mg/L for CRP was used to indicate the presence of inflammation or infection (30). Serum zinc was measured by flame atomic absorption spectrophotometry (SpectraAA-400, Varian). Controls (Seronorm Trace Elements Serum level 2, I.G Instrument-Gesellschaft) were used to check the precision and accuracy of the serum zinc analysis. Zn deficiency was defined as described above. SR was measured using HPLC according to the method of The International Vitamin A Consultative Group (31) with reference material from the National Institute of Standard and Technology. VAD was defined as SR <0.7 µmoll/L (4).

Statistical analysis. Statistical analyses were performed using SPSS software (version 18, SPSS Institute). Height-for-age Z-scores, weight-for-age Z-scores, and BMI-for-age Z-scores were calculated by using WHO Anthro Plus software (WHO, 2009) with WHO references 2007. Baseline characteristics were compared by group using unpaired t tests for continuous variables and Pearson chi-square test for proportions. Subjects with elevated CRP (≥5 mg/L) were excluded from the analysis of serum zinc, SF, and SR. The normality of data was checked before analysis with the Kolmogorov-Smirnoff test and graphically by evaluating box plots and Q-Q plots while not normally distributed data were log-transformed. Descriptive statistics are reported as mean ± SD for normally distributed data and as median (quartile 1, quartile 3) for data not normally distributed. Two-factor, repeated-measures ANOVA was done to compare the effects of time × group for Hb, SF, serum Zn, and SR. Post hoc comparisons were done using unpaired t tests between groups and paired t tests within groups and adjusted for multiple comparisons (Bonferroni correction). For variables with persisting skewed distribution after log transformation, comparisons were done using the Mann-Whitney test between groups and Wilcoxon’s test within groups. The group effect for the binary variables of anemia, ID, and Zn deficiency, and VAD was tested using Pearson’s chi-square test and the time effect was tested by using McNemar’s test. Differences were considered significant at P < 0.05.

Results

Pilot study of Zn, Fe, and VA intake and status. Table 1 shows Zn, Fe, and VA intake and status of school children from the study area. The intakes are also given as percent EAR. The phytic acid:Zn molar ratio was 1.7, indicating a high-bioavailability diet (28,29) and not the moderate-bioavailability diet on which we had based our Zn fortification level. The mean intake of Zn at 2.2 mg/d corresponded to 71% of the EAR for a high-bioavailability Zn diet or 42% of the EAR for a moderate-bioavailability Zn diet (28,29). Assuming 10% Fe bioavailability, Fe intake corresponded to 81% of the Fe EAR and VA intake corresponded to 52% of the VA EAR.

Rice and rice products, such as rice noodles and fermented rice noodles, were the main staple foods (data not shown). The diet contained 63% of total energy as carbohydrate, 26% as fat,
and 11% as protein. Because the majority of people were Muslim, chicken was the most consumed animal protein source, followed by mackerel, beef, and egg, while rice and rice products provided the highest contribution of plant proteins. Traditional curry, soup, and stir fry were commonly prepared with vegetables such as cabbage, cucumber, and Chinese cabbage. Protein foods such as chicken, beef, egg, fish, and seafood were major sources of Zn, contributing 53% of total zinc intake. The main individual Zn sources, however, were rice and chicken, which contributed 27 and 17%, respectively, to the total Zn intake. Rice and egg were the main sources of Fe and VA, respectively. Milk and milk products were little consumed at the household level.

Biochemical indices of status from the pilot study were variable in their agreement with expected deficiencies based on the measured dietary intakes (Table 1). ID and VAD were less prevalent than predicted by intake measurements, which were 20 and 50% of their EAR, respectively. Only 5% of the children presented with ID and 1% with VAD. Depending on whether the diet was of high- or moderate-Zn bioavailability, the measured Zn intake was ~30–60% of the EAR and 44.3% of the children presented with Zn deficiency based on their serum zinc concentrations.

**Efficacy study: subject characteristics.** Of the 744 children who were recruited in the prescreening phase, 203 eligible children were invited and agreed to participate in the efficacy trial (Fig. 1). A total of 101 and 102 subjects were randomly assigned to the triple-fortified rice (intervention) or the unfortified rice group (control), respectively. At baseline, subject characteristics including gender ratio, age, and anthropometric measurements (Table 2) as well as the biochemical indicators of status (Table 3) did not significantly differ between the intervention and the control group. Of all participating children, 14.5% were stunted (HAZ < −2.0) and 7% underweight (WAZ < −2.0) (32).

The dropout rate was very low (total ~3%, triple-fortified rice ~2%, control ~4%) and was mainly due to loss of interest. Fourteen children (5 in the triple-fortified group, 9 in the control group) were excluded from data analysis because of low school attendance (<80% during the feeding period) as was planned in the protocol. One child in the control group was sick on the day of the final blood taking. In total, 21 subjects (~10%) were lost for final analysis and 182 completed the study according to the protocol. There was a low infection rate in this area, with only 4% of children showing elevated CRP concentrations.

**Compliance with feeding.** On average, the subjects received the test meal for 130 ± 15 d and this was comparable between the intervention (129 ± 15 d) and control (131 ± 15 d) groups. The mean serving size of cooked rice (both triple-fortified and unfortified rice) was 142 g/d. Taking into account the leftovers of rice that were monitored and estimated by research assistants, the estimated average amount of rice consumed by subjects was 123 ± 18 g/d and there was no significant difference between intervention (124 ± 18 g/d) and control group (122 ± 18 g/d). This corresponds to the provision of an additional amount of 8 mg/d Zn, 8.8 mg/d Fe, and ~780 μg/d VA for children in the intervention group.

**Biochemical indicators of nutritional status.** The results of biochemical indicators before and after the intervention are shown in Table 3. We found a significant time \( \times \) treatment interaction for serum zinc concentration only. Serum zinc increased in both groups \( (P < 0.05) \) at the end of the 5-mo feeding period. In addition, at the end of the study, the serum zinc concentration was higher in the intervention group than in the control group \( (P = 0.018) \). The results for SR, Hb, and SF indicated that VAD, anemia, and ID were not widespread in this population group. At baseline, the incidence of VAD was 3%, anemia 10%, and ID 9%. At the end of the study, the intervention and control groups did not differ in these indicators. However, in the triple-fortified group, the SR concentration improved over time \( (P = 0.006) \), whereas there was no change in the control group. The Hb concentration decreased in both groups at the end of the study, but the decrease was only in the intervention (129 ± 15 d) and control (131 ± 15 d) groups.

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**TABLE 1** Pilot study to estimate approximate intake of Fe, Zn, VA, and phytic acid and the micronutrient status of school children in Satun province

<table>
<thead>
<tr>
<th>Nutrient intake</th>
<th>n</th>
<th>Male:female, n</th>
<th>Fe, mg/d</th>
<th>Fe, % EAR</th>
<th>Zn, mg/d</th>
<th>Zn, % EAR</th>
<th>VA, mg/d</th>
<th>VA, % EAR</th>
<th>Phytic acid, mg/d</th>
<th>Nutritional status measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>92</td>
<td>43:49</td>
<td>7.3 ± 2.5</td>
<td>81</td>
<td>2.2 ± 1.1</td>
<td>42</td>
<td>203 ± 144</td>
<td>52</td>
<td>37.6 ± 18.3</td>
<td></td>
</tr>
</tbody>
</table>

1 Data are mean ± SD or percentage. To convert serum Zn to μg/dL, divide by 0.153. EAR, Estimated Average Requirement; ID, iron deficiency; Hb, hemoglobin; SF, serum ferritin; SR, serum retinol; VA, vitamin A; VAD, vitamin A deficiency.

2 EAR (28,29) of children 7–12 y, based on 10% bioavailability for Fe and moderate bioavailability for Zn. EAR of Fe is 8.9, 8.8, and 10.4 mg/d for children 7–9 y and 10–18 y, females 11–14 y (premenarche), and males 11–14 y, respectively. EAR of Zn (moderate bioavailability) is 4.7, 6, and 7.2 mg/d for children 7–9 y, females 10–18 y, and males 10–18 y, respectively. EAR of VA is 357 and 429 μg/d for children 7–9 y and 10–18 y, respectively.

3 Molar ratio phytic acid:iron, 0.4; molar ratio phytic acid:Zn, 1.7.

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**TABLE 2** Age, gender, and anthropometric characteristics of the children that were supplemented with unfortified rice or triple-fortified rice at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>101</td>
<td>102</td>
</tr>
<tr>
<td>Male:female, n</td>
<td>49:52</td>
<td>49:53</td>
</tr>
<tr>
<td>Age, y</td>
<td>9.5 ± 1.8</td>
<td>9.5 ± 1.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>28.4 ± 8.2</td>
<td>27.7 ± 8.7</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.3 ± 0.12</td>
<td>1.3 ± 0.11</td>
</tr>
<tr>
<td>Weight-for-age Z-score</td>
<td>−0.6 ± 1.1</td>
<td>−0.7 ± 1.3</td>
</tr>
<tr>
<td>Height-for-age Z-score</td>
<td>−0.8 ± 1</td>
<td>−0.8 ± 1.1</td>
</tr>
<tr>
<td>BMI-for-age Z-score</td>
<td>−0.2 ± 1.2</td>
<td>−0.4 ± 1.3</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD. Groups did not differ significantly.

Rice fortified with zinc, iron, and vitamin A
after the intervention remained higher than the highest cutoff for anemia (>120 g/L). The SF values were relatively high at the beginning of the study and did not change over time in either the intervention or control group.

At baseline, the prevalence of Zn, Fe, and VA deficiencies did not significantly differ between the control and intervention groups. Anemia prevalence was slightly higher in the intervention group (2.2%; P = 0.039). These findings were thought to be related to better dietary quality of food given at school and to an increased awareness of teachers and parents of children's nutrition.

We think the improvement in the Zn status of the control children in our study can be explained by the extra Zn provided by the school milk. In our pilot study, the estimated Zn intake of children not receiving the school meals was 2.2 mg/d (0.73 mg/mal), which is lower than the EAR. The EAR of Zn for a high-Zn bioavailability diet is 2.7, 3.6, or 4.3 mg/d for children 7–9 y, females 10–18 y, or males 10–18 y, respectively (28). We estimate that the school lunch and free milk (200 mL/d) provided an extra 1.7 mg/d (1 mg from school lunch and 0.7 mg from milk). The calculation was based on portion sizes and food items described for the standard Thai school lunch (34) together with the menus recorded during the study period. The Zn content was calculated using the nutrient database of Thai foods (INMUCAL, Institute of Nutrition, Mahidol University, version WD 2.1). The additional Zn contained in the school meals and the free milk was consumed by both the children receiving the fortified rice and those receiving the nonfortified rice. This extra 1.7 mg Zn should increase the Zn intake of the control group to ~3.2 mg/d, which

### Discussion

The serum zinc concentrations of the Zn-deficient Thai school children consuming the triple-fortified rice increased markedly after the 5-mo intervention, with only 29% of the children remaining Zn deficient at the end of the study (Fig. 2). Surprisingly, the control group fed the unfortified rice also had a marked increase in their serum zinc concentrations and 39% remained Zn deficient at the end of the study. The improvement of the children's Zn status with the Zn-fortified rice was significantly better than that with the nonfortified rice, demonstrating the efficacy of the Zn-fortified product.

The improvement in Zn status in the control group could be due to the high Zn intakes provided by the ongoing school milk and school lunch programs compared with low Zn intake from the home-based diet during the 2-mo summer vacation, which was immediately prior to the intervention. Food is often scarce over the summer period. The Thai government subsidizes both school lunches and free milk, but these programs are not always consistently provided. During our intervention study, however, the children regularly received white meat and fish with their rice for school lunch and they consumed free milk each school day. Other studies in Thailand in which Zn was added to school lunch programs have also reported a significant improvement in the serum zinc concentrations in the control groups (27,33). These findings were thought to be related to better dietary quality of food given at school and to an increased awareness of teachers and parents of children's nutrition.

The prevalence of Zn deficiency, VAD, anemia, and ID in children who received triple-fortified rice (I) and nonfortified rice (C) for 5 mo in an efficacy trial. *Different from baseline, P < 0.05 (McNemar's test); **different from control group at the same time point, P < 0.05 (Pearson chi-square test). Anemia was defined as Hb <120 g/L, <115 g/L for children ≥12 y old, and children 5–11 y old, respectively (29). ID was defined as SF <15 μg/L (29). VAD was defined as SR <0.7 μmol/L (4). Zn deficiency was defined as serum Zn <9.9 μmol/L for children <10 y, <10.1 μmol/L for female subjects ≥10 y, and <10.7 μmol/L for male subjects ≥10 y, respectively, from a morning nonfasting blood sample (2). To convert serum Zn to μg/dL, divide by 0.153. Hb, hemoglobin; ID, iron deficiency; SF, serum ferritin; SR, serum retinol.

#### TABLE 3

<table>
<thead>
<tr>
<th>Biochemical indicator</th>
<th>Intervention</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Zn, μmol/L</td>
<td>9.4 ± 0.6</td>
<td>9.4 ± 0.6</td>
</tr>
<tr>
<td>n</td>
<td>101</td>
<td>102</td>
</tr>
<tr>
<td>Endpoint 11.1 ± 1.3 *</td>
<td>10.6 ± 1.4 *</td>
<td>82</td>
</tr>
<tr>
<td>n</td>
<td>90</td>
<td>82</td>
</tr>
<tr>
<td>SR, μmol/L</td>
<td>1.01 (0.61, 2.52)</td>
<td>1.07 (0.57, 2.32)</td>
</tr>
<tr>
<td>n</td>
<td>101</td>
<td>102</td>
</tr>
<tr>
<td>Endpoint 1.09 (0.67, 1.83)</td>
<td>1.07 (0.55, 2.53)</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>91</td>
<td>84</td>
</tr>
<tr>
<td>Hb, g/L</td>
<td>127 (80, 141)</td>
<td>126 (99, 152)</td>
</tr>
<tr>
<td>n</td>
<td>101</td>
<td>102</td>
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<tr>
<td>Endpoint 125 (102, 147)</td>
<td>124 (103, 149)</td>
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</tr>
<tr>
<td>n</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td>SF, μg/L</td>
<td>36.6 ± 25.7</td>
<td>34.9 ± 25.5</td>
</tr>
<tr>
<td>n</td>
<td>91</td>
<td>98</td>
</tr>
<tr>
<td>Endpoint 37.1 ± 17.9</td>
<td>34.8 ± 28.4</td>
<td>84</td>
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</table>

1 Data are mean ± SD for serum zinc, geometric mean ± SD for serum ferritin, or median (quartile 1, quartile 3). To convert serum Zn to μg/dL, divide by 0.153.

<table>
<thead>
<tr>
<th>Endpoint</th>
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<td>SD</td>
<td>6</td>
</tr>
<tr>
<td>91</td>
<td>82</td>
</tr>
<tr>
<td>25.7</td>
<td>34.9 ± 25.5</td>
</tr>
<tr>
<td>91</td>
<td>84</td>
</tr>
</tbody>
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#### FIGURE 2

The prevalence of Zn deficiency, VAD, anemia, and ID in children who received triple-fortified rice (I) and nonfortified rice (C) for 5 mo in an efficacy trial. *Different from baseline, P < 0.05 (McNemar's test); **different from control group at the same time point, P < 0.05 (Pearson chi-square test). Anemia was defined as Hb <120 g/L, <115 g/L for children ≥12 y old, and children 5–11 y old, respectively (29). ID was defined as SF <15 μg/L (29). VAD was defined as SR <0.7 μmol/L (4). Zn deficiency was defined as serum Zn <9.9 μmol/L for children <10 y, <10.1 μmol/L for female subjects ≥10 y, and <10.7 μmol/L for male subjects ≥10 y, respectively, from a morning nonfasting blood sample (2). To convert serum Zn to μg/dL, divide by 0.153. Hb, hemoglobin; ID, iron deficiency; SF, serum ferritin; SR, serum retinol; VAD, vitamin A deficiency.
is within the range of their recommended Zn intakes of 2.7–4.3 mg/d based on the EAR and could thus explain the increase in serum zinc. The fortified group received an additional 8 mg Zn/d, which exceeds their requirement and could explain the further improvement in serum zinc and Zn status. In support of our suggestion, Hess et al. (35) reported that serum zinc markedly decreased at Zn intakes of 2–3 mg/d but rose sharply with additional Zn, reaching a plateau at the intake around 25–30 mg/d. In our study, it is likely that in the absence of the school lunch and free milk, the fortified rice alone would have resulted in a much stronger increase in serum zinc.

Most previous Zn supplementation studies in young children reported an increase in serum zinc concentration (21), whereas there has been the lack of response of serum zinc to Zn-fortified foods, even though they contained the same or slightly higher amounts of total Zn than the supplements. This suggests that Zn may be less well absorbed in the presence of foods (21,26,36). Serum zinc, however, is not a robust measure of Zn status. Recent studies in men and young children (37,38) reported that serum zinc responded within 2 wk in subjects receiving supplements but not in subjects consuming Zn-fortified foods, indicating that serum zinc may not be as useful for monitoring short-term fortification programs (37). Other reasons for the failure to show the efficacy of Zn-fortified foods include increased growth and infections in the study subjects, relatively high serum zinc concentrations at baseline, and a food vehicle inhibitory to Zn absorption (24). Our success in demonstrating an increase in serum zinc by incorporating the triple-fortified rice into the school lunch program could be due to the low phytate content of the fortification vehicle and the meals, the low Zn status of the study children at baseline, a relatively long 5-mo intervention, and a low level of infections. A previous study in NE Thailand, in which a Zn-containing micronutrient-enriched seasoning powder was added to low-phytate rice/noodle lunch meals, also reported a significant increase in serum zinc (27). On the other hand, Zn added to high-phytate wheat and maize products did not improve Zn status (26,39,40). Hess et al. (24) suggested that Zn-fortified foods only increase serum zinc when not co-fortified with Fe; however, our results do not support this suggestion, because we found a positive impact on serum zinc concentration with a rice that was co-fortified with Zn, Fe, and VA. Because our subjects were not growth restricted at baseline (mean HAZ, −0.8 and mean WAZ, −0.6) (Table 2), we did not measure the impact of the triple-fortified rice on growth. Previous Zn supplementation studies have shown a growth response only when children have an initial mean HAZ of < approximately −1.5 at baseline (22).

We previously reported that similar extruded rice grains fortified with micronized ground ferric pyrophosphate increased Fe stores and decreased the prevalence of ID in Indian school children with low Fe stores (13). The current study was not designed to show an improvement in Fe status, because most of the Zn-deficient children in the study had adequate iron stores at baseline. Nevertheless, at the end of the study, there was a small but significantly lower prevalence of ID in the intervention children than in the control group (Fig. 2), and the blood Hb concentration had fallen slightly but significantly in the control children but not in the intervention children (Table 3). Similarly, the study was not designed to demonstrate the efficacy of the retinol fortification, as the prevalence of VAD was <5% in all children at baseline. However, there was a small but significant increase in SR in the intervention group but not the control group, which confirms a good stability of VA in the triple-fortified rice grains.

In addition to the hot extrusion technology used in this study, cold extrusion and coating technologies have been used to produce artificial rice grains that are mixed with natural rice. The advantages and disadvantages of these technologies as well as the technical and economic feasibility of rice fortification in developing countries needs careful reflection and was recently reviewed by the United States Academy for Educational Development in 2008 (41). It was concluded that hot extrusion was the most expensive technology but gave the highest quality product with the best consumer acceptance. Although business models need to be defined, a 2–4% price increase due to fortification would not be a problem with branded products but could be a constraint for implementing mass fortification programs.

In conclusion, this study demonstrated the efficacy of zinc sulfate in hot extruded rice grains triple fortified with Zn, Fe, and VA. It suggests that serum zinc can be used to monitor Zn efficacy studies with fortified foods, provided the subjects have low serum zinc and low infection rates at baseline; the meals are low in phytate and the study is of a sufficiently long duration (in this case, 5 mo). It also suggests that effective implementation of the current Thai school lunch programs containing meat and fish and free school milk distribution will substantially improve the Zn status of school children in southern Thailand.

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