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Original Article

Double-blind cluster randomised controlled trial of wheat flour *chapatti* fortified with micronutrients on the status of vitamin A and iron in school-aged children in rural Bangladesh

Ahmed S. Rahman*, Tahmeed Ahmed*, Faiz Ahmed*, Mohammad S. Alam*, Mohammad A. Wahed* and David A. Sack†

**International Centre for Diarrhoeal Diseases Research, Bangladesh; Dhaka, Bangladesh, and †Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA*

Abstract

Food fortification is a cost-effective and sustainable strategy to prevent or correct micronutrient deficiencies. A double-blind cluster (*bari*) randomised controlled trial was conducted in a rural community in Bangladesh to evaluate the impact of consumption of *chapatti* made of micronutrient-fortified wheat flour for 6 months by school-aged children on their vitamin A, haemoglobin and iron status. A total of 43 *baris* (group of households) were randomly selected. The *baris* were randomly assigned to either intervention or control group. The intervention group received wheat flour fortified with added micronutrients (including 66 mg hydrogen-reduced elemental iron and 3030 μg retinol equivalent as retinyl palmitate per kilogram of flour), while the control group received wheat flour without added micronutrients. A total of 352 children were enrolled in the trial, 203 in the intervention group and 149 in the control group. Analyses were carried out on children who completed the study (191 in the intervention group and 143 in the control group). Micronutrient-fortified wheat flour *chapatti* significantly increased serum retinol concentration at 6 months by 0.12 μmol L^{-1} [95% confidence interval (CI): 0.06, 0.19; $P < 0.01$]. The odds of vitamin A deficiency was significantly lower for children in the intervention group at 3 months [odds ratio (OR) = 0.26; 95% confidence interval (CI): 0.07, 0.89; $P < 0.05$] and 6 months (OR = 0.21; 95% CI: 0.06, 0.68; $P < 0.01$). No demonstrable effect of fortified *chapatti* consumption on iron status, haemoglobin levels or anaemia was observed. Consumption of fortified *chapattis* demonstrated a significant improvement in the vitamin A status, but not in iron, haemoglobin or anaemia status.

Keywords: controlled trial, Bangladesh, micronutrient fortification, vitamin A, iron, school-aged children.

Correspondence: Dr Ahmed Shafiqur Rahman, Centre for Nutrition and Food Security, International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b), 68 Sheheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka-1212, Bangladesh. E-mail: ashafiq@icddrb.org

Introduction

Vitamin A [World Health Organization (WHO) 2009] and anaemia or iron-deficiency anaemia (IDA; WHO 2008) are two of the most recognised micronutrient-related persisting global public health problems (Underwood & Smitasiri 1999). In addition to its well-known effect on prevention of xerophthalmia, adequate vitamin A nutriture may reduce up to a quarter to one-third of all infection-related childhood mortality (Fawzi *et al*. 1993; Glasziou & Mackerras 1993). Whereas evidence indicates that IDA is associated with impaired mental and physical function in children, including reduced physical coordination and capacity, delayed mental development, reduced cognitive abilities and reduced social and emotional development (UNICEF *et al*. 1999).

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World Health Organization (WHO)'s estimates of 2005 suggested that globally, around 5.2 million pre-school children had been suffering from xerophthalmia and around 190 million from vitamin A deficiency (VAD) in countries with Gross Domestic Product less than USD 15 000 (WHO 2009). In Bangladesh, night blindness, due to lack of vitamin A, among pre-school children has been reduced from 3.6% in 1983 to 0.04% in 2005 (HKI & IPHN, 2006) as a result of Government initiated massive dose vitamin A supplementation programme. However, the latest national survey conducted in Bangladesh during 2011–2012 still implies VAD as a severe public health problem because as of 2012, prevalence of VAD was still about 21% among pre-school and school-aged children (National Micronutrient Survey 2011–2012, unpublished data).

Another form of nutritional deficiency currently ravaging throughout the developing nations is anaemia. It has recently been estimated that globally 1.62 billion people suffered from anaemia, with the highest number of 315.4 million in Southeast Asia region (WHO 2008). In Bangladesh, over one-third of the school-aged children (5–11 years) or adolescents (12–19 years) were considered anaemic in the last decade (HKI & IPHN 2002); however, recent survey shows that the prevalence has been reduced to 19.1% (6–11 years) and 17.1% (12–14 years) among the school-aged children (National Micronutrient Survey 2011–2012, unpublished data).

Nonetheless, globally, it has been assumed that every year, around 27 million DALYs (Disability Adjusted Life Years) are lost due to VAD-related disorders (Rice *et al*. 2004) and 35 million DALYs lost due to iron-deficiency disorders (Stoltzfus *et al*. 2004). The huge quantity of DALYs lost due to the two nutritional deficiency is subsequently hindering the progress towards attaining MDG 4 (Millennium Development Goal 4) for the developing nations.

Other than large-scale supplementation programme, fortification of food with micronutrients can be an effective strategy to combat vitamin A and iron deficiency and its related disorders in children. Fortification of foods, such as margarine, milk and bread, has long been practiced in Western countries to combat against deficiency of iodine, iron, vitamin A, D and several B vitamins. In 1920s, Switzerland has introduced the concept of salt iodisation, and sooner many Western countries followed their direction (Burgi *et al*. 1990). In Guatemala, fortification of sugar with vitamin A substantially improved the vitamin A status of pre-school children (Arroyave *et al*. 1981). In Venezuela, consumption of fortified flour (maize, wheat) showed to improve the iron status of its population (Layrisse *et al*. 1996). Nevertheless, at present, 75 countries globally are using iron/folic acid for fortification of wheat flour (The Flour Fortification Initiative 2012). In Bangladesh, however, the efficacy of food-based vitamin A or iron fortification with any cereal grain has not been assessed. Rice, the major staple food of Bangladesh, would be a suitable vehicle for fortification. However, rice is often processed from paddy fields in the community at the household level or at small-scale rice mills, limiting the opportunity for fortification control and safety. On the other hand, wheat, with an increasing trend in consumption among Bangladeshi population, is more often centrally processed, and hence is considered to be a more feasible candidate for fortification.

The primary objective of this study was to evaluate the impact of daily consumption of *chapattis* made from wheat flour fortified with micronutrients including vitamin A and iron for a duration of 6 months by

Key messages

- Consumption of wheat flour *chapatti* fortified with multiple micronutrients, including vitamin A (retinyl palmitate) and iron (hydrogen-reduced elemental iron), improves vitamin A status of school-aged children but may not improve anaemia or iron status.
- To be efficacious, iron fortificant depends largely on careful choice of the iron compound, dose and other environmental factors.
- Much needs to be learnt in defining best fortification strategy for reducing anaemia and iron deficiency.

school-aged (6–15 years), rural Bangladeshi children on their vitamin A status as reflected in serum retinol (SR) concentration. Furthermore, the secondary objective was to evaluate the impact of fortified *chapatti* on VAD, haemoglobin (Hb) concentration, anaemia and iron status.

Materials and methods

Study design

This was a double-blind cluster (*bari*) randomised controlled trial. The cluster design was chosen to avoid cross-contamination of the two types of wheat flour among the participants.

The study sites included 7 out of the total 16 unions (approximately 65 villages) of Mirsarai sub-district in the south-eastern part of Bangladesh that houses one of the icddr,b.'s demographic surveillance field sites with a population of around 172 300 at that time. All the *baris* (usually composed of 5–6 adjoining households with a population of about 30–35 relatives) in the study area were listed. There were a total of 4875 *baris* in the selected unions.

The 80% extracted wheat flour used in the trial was produced by a flourmill with half the amount fortified as a 'batch process' at a pharmaceutical company; both infrastructures were located in Dhaka, the capital of Bangladesh. The flour was fortified with multiple micronutrients including 66 mg hydrogen-reduced elemental iron and 3030 μg retinol equivalent as retinyl palmitate per kilogram of flour. The pharmaceutical company maintained the quality assurance of the fortified flour. Both the fortified and the unfortified flour were packed in identical polyethylene bags, each containing 700 g of flour, and the bags were labelled with blinded code to indicate flour type. Equal numbers of bags containing fortified and unfortified flour were produced. The wheat flour fortifying company sent the flour bags to the study site every 2 weeks.

A pre-testing of field procedure for collecting information, lasting for 1 month, was conducted in the non-intervention unions of Mirsarai. Information including handling and storage of wheat flour, making of *chapattis* by mothers and observing *chapatti* consumption patterns of the subjects were recorded. The pre-test revealed high compliance [97.6% ($n = 43$)] of *chapatti* consumption by the participating children.

Throughout the intervention period, children belonging to the *baris*, who received fortified flour, were designated as the intervention group, while *baris*, who were allocated unfortified flour to be consumed, were considered as the control group.

The primary outcome measure was vitamin A status at 6 months, determined by SR concentration. The secondary outcome measures were SR concentration at 3 months, haemoglobin, serum ferritin (SF) and serum transferrin receptor (STfR) concentrations and proportion of children with VAD, anaemia and iron deficiency at 3 and 6 months. Anthropometric and all other variables mentioned earlier were recorded for baseline comparison.

Sample size and randomisation of *baris*

In order to calculate sample size, following previously published studies, a difference of 0.175 μmol L^{-1} in SR concentration between groups, with a standard deviation (SD) of $\pm$0.34 μmol L^{-1} within groups (Arroyave *et al*. 1981) and a difference of 7.19 μg L^{-1} in SF concentration between groups with a SD of $\pm$14.5 µg L^{-1} within groups (Layrisse *et al*. 1996) at the end of 6 months of intervention, along with 95% level of significance and 90% statistical power, was considered. Accordingly, a sample of 83 and 87 children per group for SR and SF, respectively, was computed. To adjust for clustering, a design effect of 2 was applied, and finally a sample size of 175 children per group, for a total of 350 children, was calculated.

Children aged below 6 years were excluded as they receive vitamin A supplementation every 6 months on national immunisation or vitamin A days. Severely ill children were also excluded from the study. Furthermore, assuming that 7–9 eligible children (6–15 years) would be available from each *bari* and using a statistics book generated random number table, a total of 44 *baris* were randomly selected from the total listed *baris* for distribution of the flour. Among the 44 selected *baris*, 22 *baris* were randomly assigned to the intervention group and 22 *baris* to the control group (control).

A person not involved with the study assigned the *baris* to six different codes of flour (A, B, C, D, E and F) for distribution of the flour bags to the *baris*. During analysis of data, the principal investigator was informed that codes A, C and F were lumped into 'group A'; and B, D and E into 'group B'. It was only after completion of the analysis, the groups were unblinded.

Ethical approval

The study was approved by the Institutional Review Board of International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b). Written informed consents were obtained from the head of the *baris* and/or parents and assents were obtained from children >8 years before their enrolment. If a *bari* head refused to participate at the time of enrolment, an additional *bari* was randomly selected.

Conduct of the study

Throughout the trial period, the project staff distributed the flour once every week. In order to prevent participants sharing of *chapattis* with other members of a *bari*, the same amount of flour was also allocated to other members of that *bari* during this period. Thus, all residents of the *bari* were eating the *chapattis*, although data on consumption were collected only from children enrolled in the study. To improve *chapatti* consumption compliance, participants were also supplied with condiments [*suji* (semolina) and sugar to prepare *halwa*] each week along with the flour.

A total of 65 *bari* mothers were selected to prepare *chapatti* and *halwa* daily for distribution among the participating children. A measuring cup with a capacity of 100 g flour was supplied to the *bari* mother to ensure that the proper amount of flour was used. Children received *chapattis* made from 100 g of fortified or unfortified wheat flour daily for 6 months. It was assumed that taking *chapattis* would not significantly alter their routine dietary intake, even if it did, equal effect was expected to be produced on both groups. A different *bari* adult (not the *bari* mother) was assigned to monitor *chapatti* consumption by the participants during morning feeding sessions and to document the number of *chapatti* consumed by each participant. This was recorded on a form supplied by the study staff. Study staff visited the *baris* at least once per week to monitor *chapatti* consumption during feeding sessions and to collect the forms after verifying them by interviews with *bari* adult, participants and mothers. In addition, samples of flour and *chapatti* were collected from the participating *baris* and were sent to the Institute of Nutrition and Food Science, Dhaka University for analyses. On average, the moisture content of the flour and *chapattis* were 5.09% and 32%, respectively. Vitamin A content in the fortified flour and chapatti were 100% and 89%, and iron content were 90% and 90% of the added amount on the dry weight basis, respectively. The *chapatti*-feeding programme concluded by collection of blood at the end of the 6-month intervention period.

Measurement

Baseline blood samples were drawn from 352 children to measure SR concentration (vitamin A status) along with SF concentration and transferring receptor concentration (iron status) and haemoglobin concentration. Mid-point (3 month) and endpoint (6 month) blood samples were collected from 343 (97%) and 334 (95%) subjects, respectively.

About 4.5 mL of blood was collected from the participating children at these time points by venipuncture, and an aliquot (4 mL) was immediately put into a vial covered with aluminum foil to prevent exposure to light and was kept in a rack at room temperature until clotted. Another aliquot (0.5 mL) was placed in a tube coated with ethylenediaminetetraacetic acid for the estimation of haemoglobin. All the samples were transported immediately to a nearby temporary laboratory set-up at the International Centre for Diarrhoeal Diseases Research, Bangladesh (icddr,b) surveillance centre in Mirsarai for centrifugation, serum preparation and temporary storage. Blood and serum samples were then transported to the Nutritional Biochemistry Laboratory of icddr,b in Dhaka twice a week. Haemoglobin concentration was determined in whole blood immediately thereafter by methemoglobin method (Rice

1967), and serum was stored at –20°C until analysis. The precision of assay, based on coefficient of variation (CV), for haemoglobin was <1%. SR was determined by high-performance liquid chromatography (Driskell *et al*. 1982). The CV for SR was <2%. Both SF and transferrin receptor concentration were measured by immunoturbidimetric methods using commercial kits (Tina-quant Ferritin; Tina-quant Transferrin Receptor; Roche Diagnostics, Mannheim, Germany). The CV for both tests was <5%.

Study definitions

Anaemia was defined as a haemoglobin concentration <115 g L^{-1} for children <12 years and <120 g L^{-1} for those ≥12 years; iron deficiency as SF concentration <20 μg L^{-1} considering the possibility of subclinical infection in this population and/or transferrin receptor concentration >5 mg L^{-1}. While VAD was defined as SR concentration <0.70 μmol L^{-1}.

Data analysis

All the collected data were checked for inconsistencies. Any discrepancies in recording the data were immediately addressed before finalizing the data set. Distributions of the continuous variables were verified using histograms. SR and Hb were normally distributed, whereas SF and STfR followed approximately normal distribution. Body weight and height measurements were converted to body mass index-for-age *z*-score (BAZ) using WHO AnthroPlus 2007, v 1.0 software (WHO, Geneva, Switzerland). As the assumption of independence among the subjects was violated due to clustering effect of the individuals nested within *baris*, multi-level analyses were performed by incorporating the cluster (*bari*) as random effects in the mixed-model analyses. All models were adjusted for child's sex, age and baseline values. Mixed-model linear regression analyses were performed to assess the intervention effect on the continuous outcomes, while mixed-model logistic regression analyses were carried out to understand the intervention effect on the status of VAD along with anaemia and iron deficiency using Stata Statistical Software, version 11 (Stata Corp., 2003, College Station, TX, USA). All the analyses were for 3 and 6-month interval; no other interim interval data were available.

Results

The participants were enrolled for the study during February–March 2002; flour distribution commenced during the last week of March and the consumption of *chapatti* started during the first week of April 2002. However, the primary starting point of the trial was the day when the respective participants of a *bari* started consuming *chapattis* made from the supplied wheat flour, while the primary endpoint was considered to be the day when the participants finished consuming the last bag of the wheat flour provided (total duration 6 months). The last episode of drawing blood from the participants happened within 1–2 days after they finished consuming the last bag of flour provided.

The trial profile is illustrated in Fig. 1. During the baseline data collection, one of the selected *baris* withdrew their consent, and thus a total of 43 *baris* participated in the study. Finally, 352 children aged 6–15 years, living in the 43 *baris*, were included in the study. In total, 203 children were enrolled from 22 *baris* in the intervention group and 149 children from 21 *baris* in the control group. The number of children in each *bari* was not equal, which was accounted for the uneven distribution of children in the two groups. Analysis was carried out on 334 children who completed the study (191 in the intervention and 143 in the control group). Table 1 shows baseline characteristics of the selected children by treatment groups. There was no significant difference between groups with respect to age, sex, weight, height and the outcome variables (SR, SF, STfR, Hb, VAD, anaemia and iron deficiency based on SF), except for iron deficiency based on STfR and nutritional status (BAZ).

The amount of micronutrients added to fortified flour and its contribution to the recommended dietary allowances among the intervention group is shown in Table 2.

The numbers of *chapattis* (mean ± SD) consumed by the intervention and control groups were 351 ± 21

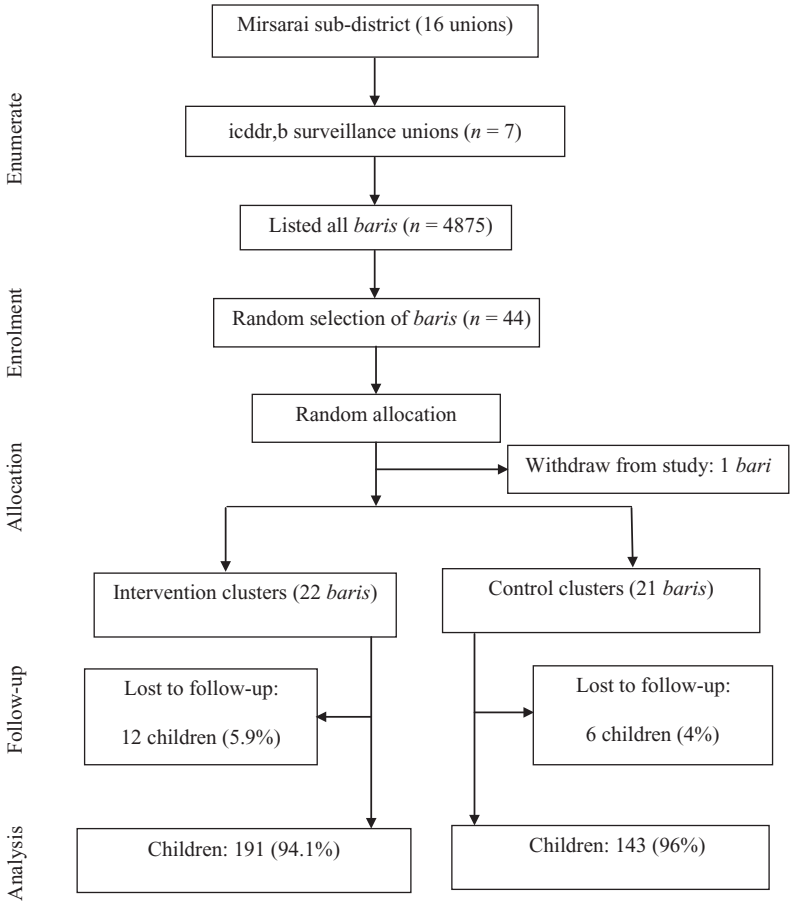

Fig. 1. Trial profile.

and 355 ± 16, respectively. Considering the highest possible intake of 366 *chapattis*, equal or greater than 90% (≥329 *chapattis*) compliance were achieved by 89% and 93% of the children in fortified and control groups, respectively, and there were no statistical differences in the mean *chapatti* intake or compliance between the groups.

Vitamin A statuss

There was no significant difference in the mean SR concentration between intervention and the control groups at baseline. However, compared with the control group, SR concentration was significantly higher in the intervention group at 6 months by 0.12 μmol L^{-1} [95% confidence interval (CI): 0.06, 0.19; $P < 0.01$] (Table 3). Moreover, the odds of VAD was significantly lower for children in the intervention group at 3 months [odds ratio (OR) = 0.26; 95% CI: 0.07, 0.89; $P < 0.05$] and at 6 months (OR = 0.21; 95% CI: 0.06, 0.68; $P < 0.01$) (Table 4). The prevalence of low SR concentration (<1.05 μmol L^{-1}) has decreased from 67% (at baseline) to 48% (at 6 months) in the intervention group and from 63.6% (at baseline) to 60.3% (at 6 months) in the control group, whereas the odds of low SR concentration (<1.05 μmol L^{-1}) was significantly lower for children in the intervention group at 6 months (OR = 0.38; 95% CI: 0.17, 0.87; $P < 0.05$).

Iron, haemoglobin and anaemia status

The mixed-model linear regression analyses showed no statistically significant effect of fortified *chapatti* on haemoglobin, SF and transferrin receptor concentrations at 3- or 6-month interval (Table 3). The

Table 1. Baseline characteristics of the study children by treatment group

Characteristic	Intervention	Control
Number of clusters (*baris*)	22	21
Number of children, *n* (%)	191 (57.2)	143 (42.8)
Male, *n* (%)	95 (49.7)	74 (51.7)
Average number of children in the *baris**	8.7 ± 6.8	6.8 ± 4.9
Age (years)*	10.4 ± 2.68	10.3 ± 2.86
Weight (kg)*	25.3 ± 8.2	27.0 ± 9.5
Height (cm)*	129.6 ± 15.3	131.4 ± 16.7
Body mass index-for-age *z*-score (BAZ)†	−1.63 ± 0.07‡	−1.28 ± 0.07
Serum retinol (μmol L^{-1})†	0.96 ± 0.02	0.98 ± 0.02
Haemoglobin (g dL^{-1})†	12.2 ± 0.07	12.1 ± 0.08
Serum ferritin (μg L^{-1})†	39.9 ± 1.79	36.4 ± 1.75
Serum transferrin receptor (mg L^{-1})†	3.69 ± 0.06	3.74 ± 0.09
VAD, % (95% CI)	13.6 (8.7, 18.5)	15.4 (9.5, 21.3)
Anaemia, % (95% CI)	24.3 (18.2, 30.5)	30.3 (22.7, 37.8)
Iron deficiency:		
Serum ferritin <20 μg L^{-1}, % (95% CI)	19.4 (13.8, 25)	24.5 (17.4, 31.5)
Serum transferrin receptor >5 mg L^{-1}, % (95% CI)	4.2 (1.3, 7)‡	10.5 (5.5, 15.5)

CI, confidence interval; VAD, vitamin A deficiency. *Mean ± SD. †Mean ± SE. ‡Significantly different from control group at $P < 0.05$.

Table 2. The amount of micronutrient fortificants in the *chapattis* (100 g flour) and their contribution (%) to daily requirements

Nutrient	Contribution of 100 g of flour in meeting dietary requirements of 6–15-year-old children		
	Amount	RDA	% RDA
Vitamin A*	212 μg†	400–600 μg	35–53%
Iron‡	6.6 mg	12.6–29.2 mg§	23–52%
Thiamin (vitamin B1)	0.64 mg	1.2 mg	53%
Riboflavin (vitamin B2)	0.40 mg	1.0 mg	40%
Folic acid	0.15 mg	0.25–0.4 mg	37–60.5%
Zinc oxide	3.3 mg	10 mg	33%
Niacin as niacinamide	5.3 mg	10.4–12.5 mg	42–51%

RDA, recommended dietary allowance. *Retinyl palmitate, S/N, United States Pharmacopoeia-Food Chemical Codex (USP-FCC). †Assuming 30% loss during storage and chapatti preparation. ‡As hydrogen-reduced elemental iron, USP-FCC. §Assuming 5% bioavailability.

mixed-model logistic regression analyses also showed no significant effect of fortified *chapatti* on the occurrence of anaemia or iron deficiency among the children (Table 4).

Furthermore, although more than a quarter of children were anaemic at baseline (Table 1), no marked change was observed at 6 months (26.1% in intervention and 24.8% in control group were anaemic). At baseline, iron deficiency based on SF level was present in about 19% and 24.5% children in the intervention and control groups, respectively. At 6 months, iron deficiency was still prevalent among 19% of children in both groups. However, iron deficiency was less prevalent in these children if based on transferrin receptor level rather than SF level; nonetheless, 8.4% and 14% children in the intervention and control groups, respectively, had iron deficiency at 6 months based on transferrin receptor (Table 4).

Discussion

The double-blind randomised controlled efficacy trial of *chapattis* made from micronutrient-fortified wheat flour on 6–15-year-olds resulted in a significant improvement in vitamin A status, as reflected in increased SR concentration at 6 months and decreased odds of VAD at both 3 and 6 months. The odds of low SR concentration (<1.05 μmol L^{-1}) was also significantly lower in children in the intervention group at 6 months, emphasizing the 19% reduction in the prevalence of low SR concentration in the intervention group compared to a reduction of only 3.3% in the control group from the baseline prevalence. Nonetheless, 48% children in the intervention group still had SR concentration <1.05 μmol L^{-1} at 6 months. It could be assumed that a longer duration of supplementation of fortified *chapatti* could have further improved the vitamin A status of the children. However, fortified *chapatti* had shown no statistically significant effect on haemoglobin, anaemia or iron status.

It should be acknowledged that although the observed effect of fortified *chapatti* on SR at 6 months was lower than we presumed by citing a previous published study (Arroyave *et al*. 1981), a significant physiological effect of fortified *chapatti* on SR concentration was observed at 6 months. In addition, it should also be mentioned that the study was not powered for VAD, a larger sample might have been needed to test the hypothesis associated with this

Table 3. Effect of fortified wheat flour *chapatti* on serum retinol (SR), serum ferritin (SF), serum transferrin receptor (STfR) and haemoglobin concentrations at 3- and 6-month interval

Interval	SR concentration*				
	Control ($n = 143$) Mean ± SE (μmol L^{-1})	Intervention ($n = 191$) Mean ± SE (μmol L^{-1})	Intervention effect (95% CI), (μmol L^{-1})†	Estimated intracluster correlation coefficient†	*P*-value
3 months	1.04 ± 0.03	1.07 ± 0.02	0.04 (–0.02, 0.12)	0.07	0.23
6 months*	0.94 ± 0.02	1.06 ± 0.02	0.12 (0.06, 0.19)	0.13	0.00
Interval	SF concentration				
	Control ($n = 143$) Mean ± SE (g L^{-1})	Intervention ($n = 191$) Mean ± SE (μg L^{-1})	Intervention effect (95% CI) (μg L^{-1})†	Estimated intracluster correlation coefficient†	*P*-value
3 months	44.0 ± 2.14	44.8 ± 2.4	–1.42 (–7.0, 4.1)	0	0.62
6 month	45.6 ± 2.5	47.9 ± 2.3	0.12 (–5.8, 6.0)	0.02	0.97
Interval	STfR concentration				
	Control ($n = 143$) Mean ± SE (mg L^{-1})	Intervention ($n = 191$) Mean ± SE (mg L^{-1})	Intervention effect (95% CI), (mg L^{-1})†	Estimated intracluster correlation coefficient†	*P*-value
3 months	3.86 ± 0.09	3.84 ± 0.09	0.07 (–0.22, 0.35)	0.18	0.64
6 months	3.84 ± 0.1	3.79 ± 0.07	–0.01 (–0.15, 0.13)	0	0.89
Interval	Haemoglobin concentration				
	Control ($n = 143$) Mean ± SE (g dL^{-1})	Intervention ($n = 191$) Mean ± SE (g dL^{-1})	Intervention effect (95% CI) (g dL^{-1})†	Estimated intracluster correlation coefficient†	*P*-value
3 months	12.1 ± 0.07	12.1 ± 0.07	–0.04 (–0.18, 0.11)	0	0.62
6 months	12.3 ± 0.08	12.3 ± 0.07	–0.16 (–0.4, 0.08)	0.2	0.2

CI, confidence interval. *Primary outcome. †Mixed-model linear regression analyses with cluster (*bari*) as random effects, adjusted for age, sex and baseline value of the outcome.

outcome. Due to the lack of any statistically significant effect of fortified *chapattis* on iron status, haemoglobin concentration and anaemia, it would be noteworthy to mention that several factors may have contributed to the lack of impact, such as the amount of iron consumed by the intervening children from fortified flour was 6.6 mg day^{-1} which might not be sufficient to reduce iron deficiency and anaemia as their iron consumption from regular diet might also be low. We did not collect dietary intake data as we assumed that because of randomisation of the sample, both groups would have approximately equal amount of micronutrient consumption from their usual diet. In addition, the bioavailability of the iron used to fortify wheat flour might have been lower than originally presumed. The iron compound used in this efficacy trial was hydrogen-reduced elementary iron. It is evident, being water insoluble and poorly soluble in dilute acid, the bioavailability of this form of iron is less than other forms, e.g. ferrous sulphate (Hurrel 2002). A review of earlier studies observed a wide variability in the bioavailability of hydrogen-reduced iron ranging from 13% to 148% relative to ferrous sulphate (Hurrel 2002). In addition, another efficacy trial reported that the RBV (relative bioavailability) of hydrogen-reduced iron is 49% in human subjects (Zimmermann *et al.* 2005). Moreover, infection caused by *Helicobacter pylori*, common in Bangladesh (Sarkar *et al.* 1997), was found to be associated with iron deficiency (Seo *et al.* 2002) and anaemia (Annibale *et al.*, 1999, Ashorn *et al.* 2001; Choe *et al.* 2001) or hypochlorhydria in children (Sarker *et al.* 2004). Existence of *H. pylori* infections was not taken under consideration during the design of this trial and

Table 4. Effect of fortified wheat flour *chapatti* on VAD, iron deficiency and anaemia at 3- and 6-month interval

Interval	VAD				
	Control ($n = 143$)%	Intervention ($n = 191$)%	OR (95% CI)*	Estimated intracluster correlation coefficient*	*P*-value
3 months	16.2	7.9	0.26 (0.07, 0.89)	0.25	0.03
6 months	22.5	7.4	0.21 (0.06, 0.68)	0.29	0.009
Interval	Iron deficiency (SF $\leq$ 20 μg L^{-1})				
	Control ($n = 143$)%	Intervention ($n = 191$)%	OR (95% CI)*	Estimated intracluster correlation coefficient*	*P*-value
3 months	15.4	15.3	1.37 (0.53, 3.54)	0.19	0.52
6 months	18.9	18.8	1.03 (0.41, 2.59)	0.19	0.94
Interval	Iron deficiency (STfR > 5 mg L^{-1})				
	Control ($n = 143$)%	Intervention ($n = 191$)%	OR (95% CI)*	Estimated intracluster correlation coefficient*	*P*-value
3 months	14.0	9.52	0.9 (0.41, 1.97)	0.01	0.79
6 months	14.0	8.38	0.76 (0.34, 1.7)	0.003	0.51
Interval	Anaemia				
	Control ($n = 143$)%	Intervention ($n = 191$)%	OR (95% CI)*	Estimated intracluster correlation coefficient*	*P*-value
3 months	30.8	26.1	0.89 (0.52, 1.53)	0.005	0.68
6 months	24.8	26.1	1.54 (0.72, 3.35)	0.1	0.27

CI, confidence interval; VAD, vitamin A deficiency. *Mixed model logistic regression analysis with cluster (*bari*) as random effects, adjusted for age, sex and baseline value of the outcome.

hence remains untreated and therefore could be responsible for lack of any improvement in iron status among the subjects. The variations in iron bioavailability may also depend on the iron particle size, presence of hypochlorhydria or other existing causes of iron malabsorption (Hurrel 2002).

Our findings are in concordance to a study in Sri Lanka that failed to demonstrate any improvement in reducing anaemia among different age groups with reduced and electrolytic iron-fortified flour (Nestel *et al*. 2004). Moreover, a recent review reported that efficacy trials conducted in four different countries that used hydrogen-reduced elementary iron for fortification of wheat or maize products did not show any discernible impact on iron and/or haemoglobin status of the participants. Therefore, due to the lack of evidence of significant beneficial effect of the currently available hydrogen-reduced iron powders on iron status, the reviewers recommended not to use reduced iron powders for the fortification of wheat or maize flours (Hurrell *et al*. 2010).

It is conceivable that the success of iron fortification programmes depends largely on the careful choice of the iron compound as well as dose and duration of supplementation, along with other environmental factors such as infection or parasitic infestation. It should also be noted that the children of this study did not receive anthelmintic drug since deworming has not been a routine practice in Bangladesh. Therefore, it could be speculated that deworming prior to administration of fortified *chapatti* might has improved iron status.

Finally, it should be acknowledged that the average per capita consumption of wheat was only

20 g in rural Bangladesh in 1998 (HKI & IPHN 1999), which was far less than the amount of flour (100 g) used in our study. However, wheat production, import and consumption have increased over the years, and in 2011, per capita wheat consumption was estimated to be above 70 g day^{-1} (Hussain 2012). Moreover, an organoleptic test of *chapattis* based on hedonic scales of different parameters such as colour, flavour, taste, mouth-feel and overall satisfaction of consuming *chapattis* made from fortified wheat flour was found to be highly acceptable (Malek & Bhuyan 2001). Furthermore, vitamin A content retained in the fortified flour, even after baking *chapatti* was still high (89% of the added amount of vitamin A). Therefore, wheat flour could be a suitable vehicle to be fortified with vitamin A including other micronutrients for targeted as well as general population of this country.

The findings of this study are consistent with those of previous community trials in developing countries where staple food products have been fortified with vitamin A such as substantial improvement in vitamin A status from the use of fortified condiments, like sugar (Darnton-Hill 1998), monosodium glutamate (Muhilal *et al*. 1988), margarine (Solon *et al*. 1996) and wheat flour bun [pandesal] (Solon *et al*. 2000). Unlike our study, community trials of iron-fortified staple foods or condiments in developing countries resulted in very small, but statistically significant improvements in the haemoglobin and or iron status (Stuijvenberg *et al*. 1999; Sari *et al*. 2001; Zimmermann *et al*. 2003; WHO 2009).

There is great interest on provision of important micronutrients through food fortification, including initiatives such as the GAIN programme (Global Alliance for Improved Nutrition 2003). However, the results of our study suggest that much needs to be learnt in defining best fortification strategy for reducing anaemia and iron deficiency.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

ASR, TA, MSA, MAW and DAS contributed to the study design. ASR was responsible for field implementation. Data collections were performed by ASR, MSA, FA and TA. The laboratory analysis was carried out by MAW. ASR did the statistical analysis with contribution from TA, FA and DAS. The manuscript was written by ASR and edited by TA, FA, MSA, MAW and DAS.

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