ICDDRB: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No.: 2001-022

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: Randomized, Double-blind Controlled Trial of Wheat Flour (*Chapatti*) Fortified with Vitamin A and Iron in Improving Vitamin A and Iron Status in Healthy, School Aged Children in Rural Bangladesh

Theme: ***(Check all that apply)***

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Fortification, Wheat flour, Vitamin A, Iron, School aged children, Controlled trial

Principal Investigator: Dr. Ahmed Shafiqur Rahman **Division: HSRD** **Phone:** 9881661, 8811751-60/2518

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Co-Principal Investigator(s): 1. Prof. David Sack 2. Dr. M A Quayium

Co-Investigator(s): 1. Parveen A Khanum 2. M A Wahed 3. Dr. S K Roy 4. Dr. M S. Alam 5. Dr. Tahmeed Ahmed

Student Investigator/Intern:

Collaborating Institute(s):

Population: Inclusion of special groups (*Check all that apply)*:

Gender

- ☑ Male
- ☑ Females

Age

- ☐ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65
- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- ☑ Others (specify: **6 – 15 years age group**)
- ☐ Animal

Project / study Site (*Check all the apply)*:

- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☐ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☑ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh _____
- ☐ Outside Bangladesh
  name of country: _____
- ☐ Multi centre trial
  (Name other countries involved)
  _____

Type of Study *(Check all that apply)*:

- ☐ Case Control study
- ☑ Community based trial / intervention
- ☐ Program Project (Umbrella)
- ☐ Secondary Data Analysis
- ☐ Clinical Trial (Hospital/Clinic)
- ☐ Family follow-up study
- ☐ Cross sectional survey
- ☐ Longitudinal Study (cohort or follow-up)
- ☐ Record Review
- ☐ Prophylactic trial
- ☐ Surveillance / monitoring
- ☐ Others

Targeted Population *(Check all that apply)*:

- ☑ No ethnic selection (Bangladeshi)
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
  - ☐ Refugee

Consent Process *(Check all that apply)*:

- ☑ Written
- ☐ Oral
- ☐ None
- ☑ Bengali language
- ☑ English language

Proposed Sample size:

Total sample size: ____350_____ ☐

Sub-group _____ ☐

_____ ☐

_____ ☐

_____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☑ Existing data available via public archives/source
- ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

☑ ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ☑ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ☑ a. place the subject at risk of criminal or civil liability?

☐ ☑ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ no risk
- ☑ no more than minimal risk
- ☐ only part of the diagnostic test

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☐ ☑ Is the proposal funded?

If yes, sponsor Name: _____

Yes/No

☑ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) _____MOST, USAID

(2) _____

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support		**Cost Required for the Budget Period ($)**				
(Day, Month, Year - DD/MM/YY)		*a.*	*Ist Year*	*2nd Year*	*3rd Year*	*Other years*
Beginning date	01/11/2001		$129,866	_____	_____	_____
End date	31/10/2002	*b.*	*Direct Cost :* $106,293	*Total Cost :* $129,866		

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Robert Breiman — Name of the Division Director

Signature

3 October 2001 — Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI _____

Date: 03/10/2001

Name of Contact Person (if applicable)

_____

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☐ Check here if appendix is included

ROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. **(TYPE TEXT WITHIN THE SPACE PROVIDED).**

Principal Investigator: Ahmed Shafiqur Rahman

Project Name: **Randomized, Double-blind Controlled Trial of Wheat Flour (*Chapatti*) Fortified with Vitamin A and Iron in Improving Vitamin A and Iron Status in Healthy, School Aged Children in Rural Bangladesh**

Total Budget $129,866 Beginning Date 01/11/2001 Ending Date 31/10/2002

Fortification of an appropriate food is considered to be a cost-effective and sustainable strategy to address micronutrient malnutrition in deficient populations. In many parts of the world, wheat flour is fortified with minerals and vitamins with an aim to improve micronutrient status of a deficient population or maintain an already improved status. In Bangladesh, most of the population groups, and especially those who belong to the lower strata of the society are suffering from micronutrient deficiencies of varying degrees. Vitamin A and iron deficiencies have been identified as two major micronutrient deficiencies that have highly significant public health implications. In the long run, consumption of wheat flour fortified with vitamin A and iron by members of vulnerable families improves the growth and health of the children as well as micronutrient status of others who consume it. Instead of whole wheat or unfortified flour, using fortified wheat flour for feeding programs aiming at improving nutritional status of vulnerable groups seems logical. The purpose of this pilot study is to determine if any effect can be demonstrated, primarily in terms of improved vitamin A status; and also iron status among children consuming *Chapattis* made from flour fortified with vitamin A and iron. A positive impact will justify use of fortified flour in this part of the world.

We propose to conduct a randomized, double-blind controlled trial in a rural area of Chittagong district in Bangladesh. A total of 350 apparently healthy school aged children (6-15 years) will be enrolled from a randomly selected clusters of *baris* (each bari is composed of 5 –6 households having a total of 30 inmates) under the study area. The selected baris will be randomized to receive in a blinded manner either fortified or unfortified wheat flour according to a six-block scheme. Three of these blocks will contain fortified flour and the rest will be unfortified. A bari mother will be selected to prepare and serve the *chapattis* along with a condiment (i.e. Dal/Suji/Sugar) to the study subjects, and should also ensure their daily consumption. The children will consume *chapattis* made from 100g of wheat flour on a daily basis for a period of six months. An assigned house member will keep a written record of *chapatti* consumption. This will be followed by spot checks at least twice a month by field staff. The impact on vitamin A and iron

status will be assessed by determining changes in serum concentrations of retinol, ferritin, transferrin receptor (TfR) and hemoglobin before and after intervention in the treatment and control groups. These markers will be the outcome measures of any change in vitamin A and iron status in the children. A mid-point blood analysis at 3 months will also be done to measure of the onset of a detectable change.

Concerned agencies may use the results of this study for planning food fortification program in Bangladesh as well as in other developing countries. It may also stimulate local food companies to come forward with new strategies in areas of fortification and take an effective role in micronutrient intervention programs. Similarly, the Government of Bangladesh may use this information to formulate policies regarding food fortification.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline/ Specialty	Role in the Project
1. 1. Ahmed S Rahman	Senior Operations Researcher / Nutrition	PI
2. David Sack	Director, ICDDR,B	Co-PI
3. M A Quayium	Senior Operations Researcher / Public Health Research	Co-PI
4. Parveen A Khanum	Operations researcher / Public Health research	Co-Investigator
5. M A Wahed	Associate scientist / Biochemistry Lab	Co-Investigator
6. S K Roy	Scientist / Clinical nutrition	Co-Investigator
7. M S Alam	Field Operations Manager	Co-Investigator
8. Tahmeed Ahmed	Associate Scientist / Paediatric Nutrition	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Consumption of *chapattis* made of wheat flour fortified with vitamin A and iron, in addition to the usual diet, will improve vitamin A and iron status of school aged children.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods **(TYPE WITHIN LIMITS).**

The aim of the study is to evaluate whether an impact of consuming *chapatti* made from vitamin A and iron fortified wheat flour by the school aged children can be demonstrated

1. Primarily, on their vitamin A status reflected in serum retinol concentration at 6 months

2. Secondarily, on iron status reflected in serum ferritin, TfR and hemoglobin concentration at 6 months

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Background:

Other than iodine deficiency which poses risk to more than a billion people worldwide, lack of vitamin A and iron and consequences of their deficiencies are the two most recognized global public health problems (1). In addition to its effects on xeropthalmia, adequate vitamin A nutriture reduce upto a quarter to one-third of infection related childhood mortality (2,3,4,5). When given along with iron, vitamin A improves iron metabolism in anemic persons (6,7). The consequences of iron deficiency anemia include reduced physical capacity, impaired cognitive function and brain metabolism and reduced immunocompetence (1).

Global trend :

Vitamin A deficiency: The latest global estimate suggest that about 3 million children are still suffering from xeropthalmia and another 150 – 200 million children are sub-clinically deficient; most are in South Asia [32 – 60 million](8). VAD is not limited to pre-school children and extends beyond that age into adolescence and the reproductive years for women. Nightblindness is 5 – 25 times more prevalent now in pregnant women than in preschool children and the condition is associated with lower dietary intake of vitamin A, low serum retinol, higher risks of severe anemia, reproductive and infectious morbidity and increased mortality. Each year 13% of pregnant women suffer from night blindness in South Asia alone (8).

Anemia/Iron deficiency: Globally, more than 3 billion people are suffering from anemia and iron deficiency and it is affecting the development of half of those growing up in Africa and Asia (9). Like vitamin A, it is not restricted to children; about 40% of women from the developing world suffer from anemia and the prevalence increases to over 50% during pregnancy, which leads to increased risk of low birth weight and maternal death.

Bangladesh trend:

Vitamin A deficiency: Xeropthalmia in pre-school children has been reduced from 3.5% in 1982-83 to 0.6% in 1997-98 (10). Much of this success has resulted from a national program that distributes high potency vitamin A capsule twice a year to these children. However, 22% of pre-school children were still found to be sub-clinically deficient [serum retinol <0.7 μ mol/L](10). Among school aged children 22% had serum retinol level <0.7 μ mol/L and 74% had less than 1.05 μ mol/L (10). Among adolescent girls working in garment factories, 56% had serum retinol level < 1.05 μ mol/L and 14% below 0.7 μ mol/L (11). A survey conducted in 1997 among women in their different reproductive stages, revealed a prevalence of nightblindness of about ≥ 2% (10). A recent study in ICDDR,B showed that 20% of the children (2-6 years) had low levels of serum retinol [< 0.7 μ mol/L] (12).

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Anemia and Iron deficiency: Nutrition surveys conducted in Bangladesh revealed that all age groups and sexes are affected with anemia and iron deficiency disorders. Rates of anemia [Hb < 11.0 g/dL] among 0 – 5 years of age were 66.5% for boys and 71.3% for girls (10). In the 15–49 years age group, 50% of the

pregnant women had anemia [Hb < 11.0 g/dL] and 47% of the non-pregnant-non-lactating-women had hemoglobin levels < 12.0 g/dL (10). In the 6-14 years group more than 90% had anemia [Hb < 12.0 g/dL] (10). When cut-off level of Hb for anemia was set at 11.0 g/dL, 51.4% of rural children (6-14 years) were found to be anemic and in the urban areas it was 42% (10) . In the study conducted at ICDDR,B, 60% of the women in the age group between 17 – 53 years were anemic [Hb < 11.0 g/dL] and 23% had iron deficiency anemia[IDA](12). In children, 2-6 years of age, 50% were anemic and 19% had IDA (12).

Solution to the problem:

There is no single universally applicable strategy available that can solve the problem of micronutrient malnutrition, especially of vitamin A and iron. Main strategies that can be implemented to solve the problem of micronutrient deficiencies are supplementation, dietary diversification, public health and disease control measures and food fortification. Each of the strategies has its own merits and supplement or complement each other. Food-based strategies, including dietary diversification and food fortification are the most sustainable approaches to increasing micronutrient status of populations. However, supplementation should be in place for high-risk population groups along with sustainable food-based approaches. Moreover, public health measures such as deworming should be combined with supplementation or fortification where parasitic infestation is high.

Food fortification is a type of food processing in which an appropriate food (vehicle) is supplemented with micronutrients at a level (usually 25 – 50% RDA) that do not cause any change in its physical, chemical or biological properties. This approach is justified when widespread coverage is desired or when targeting a specific population group. Fortification has been successful in developed countries and is the most direct approach to eliminating micronutrient deficiencies. In the USA, two-third of the RDA for iron comes from fortified foods. Fortification is primarily a preventive programme, not intended to treat severe disorders in large sections of the population. In Guatemala, a 50% reduction in vitamin A deficiency has been documented over a five year period in preschool children who consumed vitamin A fortified sugar (13). In Venezuela, consumption of fortified flour (maize, wheat) has been shown to improve iron status compared to pre-intervention period (14). In a recent study in the Philippines, vitamin A status of the low and marginally deficient children has been found improved after consuming *pandesal* (bun) made from vitamin A fortified wheat flour for thirty weeks (15). Fortification of staple foods for example cereal powder, sugar, edible oil, salt etc. with vitamins and minerals is technically feasible and also most cost-effective and many countries are currently undertaken this strategy as a means to improve micronutrient status of their population (16).

Until now, no fortification program has been in place nor was one ever tested in Bangladesh. Rice, the major staple in Bangladesh is extremely difficult to fortify with micronutrients. Wheat flour, therefore, has been selected to be the vehicle for the fortification.

Since many of the children in Bangladesh are micronutrient deficient especially in vitamin A and iron, it would seem logical for wheat flour to be fortified for feeding programmes for those groups who tend to have more malnutrition. Wheat is the second major staple food in Bangladesh. Domestic production which is about 20% of the total consumed and consumption have been increased in recent years (17). The wheat production in Bangladesh was 1.8 million MT in the year 2000 and projected wheat production in 2001 is 2.0 million MT (18). The major portion is imported and distributed by the Govt. of Bangladesh under Food for Work and Food for Education programme as whole cereal. Cereals which are consumed in powder form are technically more feasible to fortify with micronutrients and cost effective than cereals which are consumed whole. Moreover, wheat is relatively centrally processed than rice processing in Bangladesh. At present, the flour mills in Bangladesh do not have the technology to fortify wheat flour. This fortification will be done at a local pharmaceutical company which has the necessary equipment to mix the premix fortificants with the flour effectively. The flour for fortification will be purchased locally and supplied by

the donor agency. However, most of the roller mills in Bangladesh is located in big towns and many of them, depending on capacity, can install a feeder (dosifier) need to fortify flour with a cost between US$ 2000 -20,000 (19). On a large and commercial basis, the total cost of fortifying wheat flour with iron alone is only US$ 0.63 per MT (19). However, the cost of the premix to fortify wheat flour in the present study will be US$ 30/kg and 0.255g will be needed to fortify 1 kg flour to the required concentrations which means an amount of Tk. 0.43 for the premix will be spent to fortify 1 kg flour (20). The present study does not allow us to evaluate other costs incurred in the whole process of fortification (costs of equipment, mixing charges, quality assurance, extra personnel etc.). However, economic analyses will be attempted based on available data.

The present study will determine any impact of consuming *Chapatti* made from fortified wheat flour on vitamin A and iron status of school aged children. Although younger children might also benefit from fortified foods, they are currently receiving vitamin A supplements and this programme would confound evaluation of the fortified atta (flour) being introduced in this population.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Overall study design: This is planned as a cluster-randomized, double-blind, controlled trial in a rural area in Mirsarai thana (sub-district) of Chittagong district in Bangladesh. *Chapattis* prepared from wheat flour fortified with vitamin A (retinyl palmitate, 10,000 IU/kg flour), iron (electrolytic elemental iron, 66 mg/kg flour) and few other vitamins and minerals and consumed by the apparently healthy, rural, school aged children daily for six months will demonstrate an impact in serum concentrations of retinol, ferritin and transferrin receptor (TfR) and may show a change in hemoglobin concentration. The composition of the fortified flour and contribution of the fortificant micronutrients to RDA is shown in **Table** .

The wheat flour (Atta) will be produced by a local flour mill and fortified fresh as a "batch" process at a local pharmaceutical company. The quality assurance will be maintained by the fortifying company and also samples tested by another laboratory (Institute of Nutrition and Food Science, Dhaka University). The supplied flour will be kept in a well ventilated dry room and be used within 2-3 weeks.

The study had a one-month pretest to establish procedures and preparation of the field settings including handling, storage of wheat flour, making of *chapattis* and see chapatti consumption pattern. Our pretest reveal that two chapattis made from 100g flour were consumed by all the 43 participants except one and well accepted when served with a condiment (halwa made of suji and sugar). This will be followed by a six month period when participant children will consume two chapattis (100 g flour) daily in addition to their usual food.

An organoleptic analysis of chapatti made from fortified flour was performed byInstitute of Nutrition and Food Science, Dhaka University (INFS,DU) with a group of penalists experienced in organoleptic tests except one (21). The analyses based on hedonic scales of different parameters, reveal that chapatti made from fortified atta (flour) using hot water has a number of qualities with high acceptibility.

The sampling unit will be the bari (composed of an average of five households and a population of about 30 per bari). The selected baris will be randomized to receive in a blinded manner either fortified or unfortified wheat flour and also receive a condiment (i.e., dal, suji, sugar etc.) to increase consumption compliance. Before feeding starts, measures like z-scores for weight-for-age, weight-for-height, height-for-age, blood hemoglobin, serum retinol, serum ferritin and serum transferrin receptor will be taken to see baseline comparability. The outcome measures that are expected to change in the group receiving fortified *chapatti* are serum retinol, serum ferritin and serum transferrin receptor concentrations. Also a change in hemoglobin level is expected. Each of these measures (weight, height, Hb level, serum retinol, serum ferritin, serum transferrin receptor) will be determined at the start (0 month), at the mid-point (3 months) and at the end of the trial (6 months).

Table: Level of fortificants per kg wheat flour and contribution from *chapatti* consumption (100g flour) to micronutrient requirements of children

Nutrient	**Level in flour**	**Contribution of flour to requirements of children 6-15 years old**		
		Nutrient consumed (per day)	**RDA**	**% RDA**
Vitamin A[1]	**10,000 IU/kg**	**212 μg**[2]	**400 - 600 μg**	**53 – 35%**
Iron[3]	**66.0 mg**	**3.3 mg**[4]	**10 - 15 mg**	**33 – 22%**
Thiamin (Vit. B_1)	**6.4 mg**	**0.64 mg**	**1.2 mg**	**53%**
Riboflavin (Vit. B_2)	**4.0 mg**	**0.40 mg**	**1.0 mg**	**40%**
Folic acid	**1.5 mg**	**0.15 mg**	**250 - 400 μg**	**60 – 37.5%**
Zinc oxide	**33.0 mg**	**3.3 mg**	**10 mg**	**33%**
Niacin as niacinamide	**53.0 mg**	**5.3 mg**	**10.4 - 12.5 mg**	**51 – 42%**

[1]Retinyl palmitate, USP-FCC
[2]Accounting for a loss of 30% during storage and food preparation
[3]As electrolytic iron-FCC
[4]Accounting for 50% bioavailability compared to ferrous sulfate

RANDOMIZATION, WHEAT FLOUR DISTRIBUTION, CHAPATTI PREPARATION AND MONITORING:

The baris will be randomized to receive either fortified or unfortified wheat flour according to a 6 block scheme (A, B, C, D, E, F). Three of these designations will be fortified flour and three unfortified flour in a blinded manner[1]. Containers of flour will be prepared centrally with the levels (A, B, etc.) and distributed by field assistants weekly to each of the baris according to the randomization scheme. A table of random numbers will be used to identify the required number of baris from amongst the total listed baris in the area under Mirsarai surveillance. The identified baris will then be randomized to any one of the six schemes.

The wheat flour provided will be sufficient to provide two *chapattis* to all individuals from 6 to 15 years of age. The chapattis will be prepared by bari mothers[2] using the flour provided and preferably with hot water.

Twenty chapattis will be made from one kg of flour. Two *chapattis* (100g flour) will be given to each child in the study daily. Our pretest reveal that two chapattis made from 100g flour were consumed by all participants except one and well acceptable when provided with a condiment (halwa made from suji and sugar).

A written record of *chapatti* consumption will be maintained at the home by another assigned bari person[3], and spot checks will be made by field assistants at least twice monthly to validate that *chapattis* have been distributed and consumed by the participants. In addition, supervisors and one investigator will monitor the whole process of flour distribution including storage, preparation, consumption and recording at the household level at least once a month. The amount of flour distributed will also be recorded and amount remaining at the weekly visit will be recorded in order to ensure that the flour is being used. Spot check samples of flour from the bari mother will be saved for vitamin A and iron assays to verify that the flour being used is the same as that which is being distributed. When these samples are tested, care will be taken to code the samples in a way to protect the double-blinded nature of the study.

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[1]Although the baris could be divided into two groups (A and B), this plan could more easily unblind the code, and the six codes will protect unwisely attempting to unblind the code.

[2] The bari mother is a women from the bari who agrees to cook and distribute *chapattis*. In return, her family will receive additional amount of flour for other members for personal use.

[3] The bari person is a man or women from the bari who will be assigned to keep a written record of the consumption of *chapattis* by the study participants.

SAMPLE and DATA COLLECTION: The following measures will be taken during the study.

Sample and data collection				
	Pre-test	Baseline	3 months	6 months
Demographic listing	×			
Age		×	×	×
Weight		×	×	×
Height		×	×	×
Serum retinol		×	×	×
Hemoglobin		×	×	×
Serum ferritin		×	×	×
Serum-transferrin-receptor		×	×	×

STUDY PROCEDURE:

Stage I: Recruitment and training of study personnel and forming the bari list for enrolment
Stage II: Pretest of measurements, procedures and handling/storage/preparation of flour
Stage III: Enrolment of participants and baseline data collection and initiation feeding
Stage IV: Mid-point sampling and data collection
Stage V: Final sampling and data collection
Stage VI: Data analysis and reporting

SAMPLE SIZE ESTIMATION:

Sample size was estimated by taken into account the ***mean and standard deviation (SD) of serum retinol concentrations***. Unfortunately, there are no data on serum retinol or serum ferritin distributions in this age group in rural Bangladesh on which to base a sample size. However, in a trial in Guatemala [Arroyave et al. 1981] (22), the mean ± SD of serum retinol concentrations were 29.2±10.2 μg/dL and 34.2±9.5 μg/dL before and after 1 year of supplementation with sugar fortified with vitamin A. With this level of change, we require 83 subjects in each group (5% significance level and a power of 90%).

Sample size was also estimated by taken into account the ***mean and standard deviation (SD) of serum ferritin concentrations***. In a trial in Venezuela [Layrisse et al. 1996] (14), the mean ± SD of serum ferritin concentrations were 18.01± 13.8 μg/L and 25.2± 15.3 μg/L before and after supplementation with maize and wheat flour fortified with iron. With this level of change, we require 87 subjects in each group (power of 90% and 5% level of significance).

Considering the greater of the two estimates (87 subjects), the calculated sample size has been doubled to 175 in each group to account for cluster and dropout effects. With a population of 30 in each bari in the study area and 4 eligible children (6-15 years) in each bari we require 44 baris in the experimental and 44 baris in the control group.

Sample size estimation was done by using the formula:

$$\frac{2(SD)^2 \times \text{factor for } \alpha, \beta}{(WD)^2}$$

Where, SD is the standard deviation and WD is the worthwhile difference between the means.

STUDY PARTICIPANTS:

Boys and girls between 6 – 15 years age living in the selected baris will be considered as the study participants.

The inclusion criteria will include: aparently healthy children, informed parental consent to participate in the study

The exclusion criteria include: children with severe malnutrition, taking medication for a chronic condition, refusal to give consent, diagnosed to have any chronic diseases such as tuberculosis, any obvious congenital or acquired disorder such as cerebral palsy.

STAFF TRAINING AND QUALITY CONTROL PROCEDURE:

To ensure the validity of the data, the following steps will be taken.

1. The field assistants will receive training as a group and will carry out the procedures in the study for one month (pre-test) prior to the start of the intervention.
2. The supervisors will spot check each of the baris at least monthly to validate the distribution of the flour, the preparation and the consumption of the chapattis.
3. The quantity of flour distributed and the quantity recovered after one week will be weighed.
4. Samples of flour recovered from the bari mother will be spot checked and preserved for vitamin A and iron assays.

LABORATORY ASSAYS:

Venous blood (5 mL) will be obtained from the subjects and aliquoted into appropriate tubes with or without appropriate anticoagulant for retinol, ferritin, transferrin receptor (TfR), and hemoglobin measurements. These samples will be protected from light, refrigerated and sent to Dhaka within two days for processing in nutrition biochemistry laboratory of the ICDDR,B.

Hemoglobin:

The hemoglobin concentration in whole blood will be determined by methemoglobin method. This is a quantitative and colorimetric technique in which total hemoglobin at alkaline pH is rapidly converted to a cyano derivative when treated with $K_3[fe(CN)_6]$, which is determined by its absorbance at 530-550 nm. The color intensity at this wavelength is proportional to total hemoglobin concentration (23)

Retinol:

Serum retinol will be determined by high performance liquid chromatography (HPLC). 100 µl serum is deproteinized with methanol containing 50 µg/dL retinyl acetate, and retinol extracted into hexane. The hexane layer is transferred to a clean vial, evaporated under nitrogen, redissolved in mobile phase and injected onto the HPLC column. Retinol is separated by reverse-phase HPLC using a C-18 column, and detected at 325 nm. Two plasma pool samples with assigned value set against a standard serum from National Institute of Standard and Technology (NIST) are run with each lot of samples and the concentration of retinol calculated based on the known concentration of retinol in the pool samples. (24,25,26)

Ferritin:

Ferritin will be measured using ELISA based on anti-ferritin antibodies bound to latex reacting with the antigen in the sample to form an antigen-antibody complex (27)

Transferrin receptor (TfR):

Transferrin receptor is measured using ELISA based upon double antibody sandwich method. Serum samples are diluted in buffer and pipetted into microwells pre-coated with polyclonal antibody to TfR. Horseradish peroxidase (HRP) conjugated murine monoclonal antibody specific for TfR is added to wells and incubated for two hours at room temperature. TfR binds to the polyclonal antibodies absorbed to the wells and the HRP-cojugated second antibodies bind to the captured TfR. Any unbound TfR and excess HRP-conjugate are removed from the wells by washing. Enzyme substrate (chromogen TMB) is added to the wells and through the action of HRP forms a blue product. Upon addition of an acid stop solution, the blue product is converted to yellow colour, the intensity of which is measured in a plate reader set at 450 nm (28).

STAFF AND ORGANIZATION OF THE TRIAL:

Bari mothers will be recruited to participate in the study. They will receive the flour at weekly intervals, prepare the *chapattis* and distribute them to the families who are participating in the study. As a motivational factor, in return for this contribution, the bari mothers as incentives will receive a small amount of money and an additional amount of flour for her family.

Bari person will be assigned to keep a written record of the consumption of *chapattis* by the study participants.

Field research assistants (FRAs) will distribute the flour, monitor the usage of the flour in each bari, make spot checks of each bari to confirm that the *chapattis* are being prepared and consumed by the children and collect spot samples of flour/chapatti for analyses of vitamin A and iron

Supervisors (SFRAs) will train and supervise the FRAs to ensure that they understand their job and are collecting the correct data. They will meet the FRAs at least every two weeks to monitor the progress of the study. Supervisors will maintain liaison with the investigators and laboratory personnel at Dhaka.

Code supervisor: One of the supervisors (SFRA) will be in charge of the dummy code (A, B etc.) for the flour to ensure that the FAs are distributing or the families receiving the correct flour.

Co-Investigator (Field Operations Manager) will supervise the activities of the field assistants (FRA) and the supervisors (SFRA) at the field level and maintain liaison with PI and other investigators at Dhaka.

Co-Investigator (Operations Researcher) will assist quality control checking, field level operations and coordination with field and laboratory.

Co-Investigator (Scientist/clinical nutrition) will give training to field staff on anthropometry and assist monitoring for compliance and quality control of data collection.

Co-Investigator (Associate Scientist/pediatric nutrition) assisting in designing the project, monitor and evaluation and trouble shooting during conduction of the study.

Co-Investigator (Laboratory biochemist): This co-investigator will be responsible for organizing blood collection at the field, transporting them to laboratory at Dhaka, carry out analyses, and maintain all results in a data set in PC.

Co-PI (Senior Operations Researcher/Field coordinator) will coordinate all field level activities including supply and logistics.

Co-PI (Professor David Sack, ICDDR,B) will help with designing, decision making and planning of the study

The PI: This individual will be responsible for the overall supervision of the study, specifically checking quality control operations at the field level, and review the progress of the study. Coordination of the study at the field, laboratory and office level including data analyses and reports writing will be done by this person.

TIME SCHEDULE:

Forming demographic listing for participant recruitment	1 month
Pretest of procedures	1 month
Recruitment and baseline observations	3 months
Mid-point sampling and data collection	1 month
Continue feeding programme	2 months
Final sampling and data collection	1 month
Data analyses / Reporting	3 months
Total	**12 months**

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

The study will be conducted in rural villages of Mirsarai thana (sub-district) of Chittagong district where ICDDR,B maintains a field research station. This area has been under demographic surveillance and is considered a "low performing area" having a lower than average health statistics. A total of 88/required number of baris will be randomly selected from a total of 4875 baris to ensure an enrollment of 350 children in the age group of 6 – 15 years. Once the bari's is being selected, a census will be carried out, listing the names, ages, sex of each child between 6 and 15 years, along with other family members to ensure identification of each participant in the study.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

The groups will be compared to ensure the comparability of the groups at the start of the study. The comparisons will include z-scores of weight-for-age (WA), height-for-age (HA) and weight-for-height (WH), hemoglobin, serum retinol, serum ferritin and serum TfR. These should be statistically similar at the start of the study. However, randomization design of the study is also expected to take care of comparability between the two groups. The same data points will be compared after three and six months of consuming *chapattis*. Within six months, it is expected that serum retinol will be significantly increased in the fortified group compared to the unfortified group. We expect that the TfR will be decreased within six months since there will be fewer binding sites available.

Outcome measures including retinol, ferritin and TfR will be compared both within and between groups from start to the end of the trial. Change in nutritional status reflected by z-scores for WA, WH and HA, although not outcome measures, will also be investigated. Means of continuous variables, if normally distributed, will be compared by Student's t test. If the data of continuous variables do not conform to a Gaussian distribution, non-parametric tests including Mann-Whitney test will be used. Difference in categorical variables will be assessed by chi-square test. The confounding will be controlled by multiple regression analyses. An analysis of covariance will be performed for comparing the final levels of the outcome variables making allowances for any difference between the initial or 3-month levels.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Fortification of food has been found to be an effective way of improving micronutrient status of people. Although micronutrient deficiency is very common in Bangladesh, fortification of food with iron, vitamin A and other micronutrients has not been tested in this country. There is, therefore, a logical need to do research in children in Bangladesh.

Partcipation is purely on a voluntary basis. Consent will be obtained in written from the parents/guardians and participants who are 8 years older or above. Rights of subjects are protected as they can withdraw from the study at any point.

The study does not involve any potential risk to the participating children. In addition to taking weight and height measurements, samples of blood (5 mL) will be collected from study subjects three times during the whole study period with utmost aseptic condition. Apart from known complications of blood drawing i.e., temporary discomfort/pain or rare instances of bruising at the site of puncture etc., participation poses no substantial risk to the study subjects. Well-experienced personnel will perform all the sampling procedures.

A-040654

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

1. Underwood BA and S Smitasiri. Micronutrient Malnutrition- Policies and Programmes for Control and their Implications. Annual Review of Nutrition, Vol. 19 pp. 303- (www.blauen-institut.ch/Tx/tt/ttVAO4MiMa.html).
2. Beaton GH et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. ACC/SCN State-of-the-Art Series: Nutrition Policy Discussion Paper No. 13, Geneva: The United Nations 1993.
3. Tonacia JA. Meta-analysis of community trials: Impact of Vitamin A on mortality. Bellagio Meeting on Vitamin A deficiency and childhood mortality. HKI, February 1992.
4. Glasziou PP, Mackerras DEM. Vitamin A supplementation in infectious diseases: A meta-analysis. BMJ, 1993; 306:366-370.
5. Fawzi WW, Chalmer TC, Herrera MG, Mosteller F. Vitamin A supplementation and child mortality. JAMA 1993;269:898-899.
6. Mejia LA, Chew F. Hematological effect of supplementation of anemic children with Vitamin A alone or incombination with iron. Am J Clin Nutr. 1988;48:595-600.
7. Suharno D, West CE, Muhilal, Karyadi D, Hautvast JGAJ. Supplementation with Vitamin A and Iron for nutritional anemia in pregnant women in west Java, Indonesia. Lancet. 1992; 342:1325-1328.
8. Keith West Jr. The magnitude of Vitamin A deficiency disorders (VADD). 25 years of progress in controlling Vitamin A deficiency: Looking to the Future, XX International Vitamin A Consultative Group (IVACG) Meeting held in Hanoi, Vietnam, 12-15 February 2001, Newsletter 1/2001, SIGHT AND LIFE.
9. Nutrition 'at aglance' Series, World Bank Health-Nutrition-Population website:www.worldbank.org/hnp
10. FAO-Nutrition Country Profiles: Bangladesh, March 25, 1999. FAO, Rome.
11. Faruk Ahmed. Vitamin A Deficiency in Bangladesh: A review and recommendations for improvement. Public Health Nutrition 1999; 2(1): 1-14.

12. ASG Faruque et al. Anemia and Vitamin A deficiency in rural Bangladesh: A population based survey. 2000, ICDDR,B (personal communication).

13. Fortifying sugar with vitamin A: A practical solution. Project OMNI (www.jsi.com/intl/omni/home).

14. Miguel Layrisse et al. Early response to the effect of iron fortification in the Venezuelan population. Am J Clin Nutr. 1996; 64: 903-907.

15. Solon F S, Klemm R D W, Sanches L, Darton-Hill I, Craft N E, Christian P, and West Jr K P. Efficacy of a vitamin A-fortified wheat-flour bun on the vitamin A status of Filipino schoolchildren. Am J Clin Nutr. 2000;72: 738-744.

16. Nutrition, The progress of Nations 1996. UNICEF (www.unicef.org/pon96/unfortif.htm).

17. Report on Market Survey of Flour Mills of Bangladesh, WFP, Dhaka, July 31, 2000.

18. FAO/GIEWS – Food outlook No.3 – June 2001.

19. Manual for Wheat Flour Fortification with Iron, Part 1, MOST, The USAID micronutrient Program, October, 2000.

20. Personal communication with MOST, Dhaka.

21. Malek MA, Bhuyan MAH. Report on Organoleptic Test of Fortified Atta. Institute of Nutrition and Food Science, University of Dhaka, August 28, 2001.

22. Arroyave G, Mejia L A, Aguilar J R. The effect of vitamin A fortification of sugar on the serum vitamin A levels of preschool Guatemalan children: a longitudinal evaluation. Am J Clin Nutr. 1981; 34: 41-49.

23. Rice EW. Rapid determination of total hemoglobin as hemoglobin cyanide in blood containing carboxy-hemoglobin. Clin Chim Acta, 18: 89, 1967.

24. Driskell WJ, Neese JW et al. Measurement of vitamin A and vitamin E in human serum by high-performance liquid chromatography. J Chrom, 231: 439-444, 1982.

25. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of α-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr. 32: 2143-2149, 1979.

26. Furr HC, Tanumihardjo SA, Flores H, Olson JA et al. Assessment of human vitamin A status using relative dose response (RDR) and modified relative dose response (MRDR) tests: manual, Dept of Biochemistry and Biophysics. ISU, Ames,USA,1992.

27. Dubois S, McGovern M, EhrhardtV. Eisenstoffwechsel - Diagnostic mit Boehringer Mannheim/Hitachi-Analy-Sensystemen: Ferritin, Transferrin and Eisen. GIT Labor-Mdizin, 9: 468-471, 1988.

28. The clinical measurement of serum transferrin receptor. J Lab Clin Med, 114: 368, 1989.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

- The findings from this study will be disseminated through seminar, reports, and participation in national and international meetings.
- Concerned agencies may use the results of this study for planning food fortification programme in Bangladesh as well as in other developing countries.
- The findings may stimulate local food companies to come forward with new strategies in areas of fortification and take an effective role in micronutrient intervention programmes.
- Government of Bangladesh may use this information to formulate policies regarding food fortification for health and nutritional benefit of people.
- Scientific papers will be written using the results of this study

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

No

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Ahmed Shafiqur Rahman

2 Present position : Senior Operations Researcher

3 Educational background : MBBS, PhD
(last degree and diploma & training relevant to the present research proposal)

List of ongoing research protocols
(start and end dates; and percentage of time)

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2000/008	1/9/2000	28/2/2002	50%
2000/016	1/5/2000	30/6/2001	50%

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	3
b) Peer reviewed articles and book chapters	
c) Papers in conference proceedings	2
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	
d) Working papers	
b) Monographs	

6 Five recent publications including publications relevant to the present research protocol

1) Iron, zinc and copper levels in different tissues of clinically vitamin A-deficient rats. Ahmed S. Rahman, Mieko Kimura, Katsuhiko Yokoi, Tanvir-E-Naher, and Yoshinori Itokawa. Biological Trace Element Research, Vol.49, pp.75-84 (1995).

2) Neurological Disorder and Excessive Accumulation of Calcium in Brain of Clinically Vitamin A-deficient Rats. Ahmed S. Rahman, Mieko Kimura, Katsuhiko Yokoi, Tanvir-E-Naher, and Yoshinori Itokawa. Biological Trace Element Research, Vol.53, No.1-3, pp.57-64 (1996).

3) Testicular Atrophy, Zinc Concentration and Angiotensin Converting Enzyme Activity in the Testes of Vitamin A-deficient Rats. Ahmed S. Rahman, Mieko Kimura, and Yoshinori Itokawa. Biological Trace Element Research, Vol.67, No.1, pp.29-36 (1999).

4) Calcium and Magnesium Concentration in Different Tissues of Clinically Vitamin A-deficient Rats. Ahmed S. Rahman, Mieko Kimura, Katsuhiko Yokoi, and Yoshinori Itokawa. Proceedings of the 12th Symposium on Trace Nutrient Research, pp.33-38 (1995).

5) Effect of Vitamin A-deficiency on Iron, Zinc, Copper and Manganese Status in Rats. Proceedings of the 1st Biomedical Research on Trace Elements, Vol.6, No.3, pp.67 (189) -68 (190), (1995).

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : M A Quaiyum

2 Present position : Field Coordinator

3 Educational background : MBBS
(last degree and diploma & training relevant to the present research proposal)

List of ongoing research protocols
(start and end dates; and percentage of time)

4.1 As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.2 As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3 As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
2000/008	1/9/2000	28/2/2002	50%
2000/016	1/5/2000	30/6/2001	50%

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	2
b) Peer reviewed articles and book chapters	
c) Papers in conference proceedings	
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	
d) Working papers	9
c) Monographs	

6 Five recent publications including publications relevant to the present research protocol

1)

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Parveen A Khanum

2 Present position : Operations Researcher

3 Educational background : M.S.S. and M.A. in Population and Reproductive Health Research
(last degree and diploma & training relevant to the present research proposal)

List of ongoing research protocols
(start and end dates; and percentage of time)

4.1 As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.2 As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3 As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time
99002	Sept. 1999	March 2002	40%

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	6
b) Peer reviewed articles and book chapters	
c) Papers in conference proceedings	
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	
e) Working papers	11
d) Monographs	

6 Five recent publications including publications relevant to the present research protocol

1)

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Dr Tahmeed Ahmed

2 Present position : Associate Scientist and Cordinator
Child health Programme
Clinical Sciences Division

3 Educational background : Ph.D, 1996, University of Tsukuba, Japan
(last degree and diploma & training Training in Pediatrics, 1990-92, Department of Pediatrics, University
of relevant to the present research proposal) Tsukuba Hospital, Japan
Training in Pediatrics, 1989-90, Dhaka Shishu (Children's) Hospital, Dhaka

List of ongoing research protocols
(start and end dates; and percentage of time)

4.1 As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
99-040	January 2000	Dece. 2001	20
2000-12	January 2001	Dec. 2002	10

As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
99-042	January, 2000	December 2001	20

As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	12
b) Peer reviewed articles and book chapters	2
c) Papers in conference proceedings	15
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	3
f) Working papers	
e) Monographs	

6 Five recent publications including publications relevant to the present research protocol

1) Mortality in severely malnourished children with diarrhoea and use of a standardized management protocol. Ahmed T, Ali M, Ullah M, Choudhury I, Haque E, Salam A, Rabbiani G, Suskind R, Fuchs G. Lancet 1999;1919-22

2) Management of severe malnutrition and diarrhoea. Ahmed T, Begum B, Badiuzzaman, Ali M, Fuchs G, Indian J Pediatr 2001:68 (1):45-51

3) Production of safe therapeutic feeds from contaminated water supplies. Roy SK, Seal AJ, Tomkins AM, Shameem T, Islam MS, Ahmed T, Fuchs GJ, Asma A, Parvin N, Begum R. Lancet 2001;357: 1587-88

4) Gastrointestinal Allergy to food: A Review. Ahmed T and Fuchs G. J. Diarrhoeal Dis. Res. 1997; 15:211-223

5) Humoral Immune and Clinical responses to food antigens following acute diarrhea in children. Ahmed T, Suma Zaki R, Shibasaka M, Nagai Y, Shin K, Fuchs GJ , Takita H. J Paediatr Child Health 1998; 34: 229-232

Detailed Budget for New Proposal

Project Title: Randomized, Double-blind Controlled Trial of Wheat Flour *(Chapatti)* Fortified with Vitamin A and Iron in Improving Vitamin A and Iron Status in Healthy, School Aged Children in Rural Bangladesh

Name of PI: Ahmed Shafiqur Rahman

Protocol Number: Name of Division: **Health System Research Division**

Funding Source: **MOST** Amount Funded (direct): **$106,293** Total: **$129,866** Overhead: **$23,573** (**26 %**)

Starting Date: 01/11/2001 Closing Date: 31/10/2002

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support (per anum)			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary in USD**	**1st Yr**	**2nd Yr**	**3rd Yr**
	A S Rahman	PI	100	7481	7481		
	David A Sack	Co-PI	5				
	M A Quaiyum	Co-PI	20	11218	2244		
	Parveen A khanum	Co-Inves.	25	7793	1786		
	S K Roy	Co-Inves.	5	17421	798		
	M A Wahed	Co-Inves.	10	17421	1597		
	Tahmeed Ahmed	Co-Inves.	5	14379	659		
	M Shah Alam	Co-Inves.	30	7787	2141		
	Secretary/Admin Officer (1)	Support	20	4889	896		
	Lab. Technician (4)	Field Staff	100	2065	1032		
	SFRA (4)	Field Staff	100	3383	7894		
	FRA (8)	Field Staff	100	2843	13268		
	Programmer (1)	Support	100	3887	648		
	DMA (1)	Support	100	2730	683		
	Remuneration to Bari mothers (88)	For preparing Chapatti			4714		
	Remuneration to Bari persons(88)	Assisting Bari mothers			2357		
	Sub Total				**48199**		
	Consultants						
	Local Travel				5090		
	International Travel				3000		
Sub Total					**8090**		
Supplies and Materials (Description of Items)							
	Office supplies				750		
	Syringe and Test Tubes				1100		
	Miscellaneous				500		
	Sub Totals				**2350**		

	Other Contractual Services			
	Repair and Maintenance			
	Rent, Communications, Utilities	10412		
	Training Workshop, Seminars	2000		
	Printing and Publication	750		
	Staff Development			
	Sub Total	**13162**		
	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests			
	Microbiological tests			
	Biochemistry Tests	25110		
	X-Rays			
	Patients Study			
	Research Animals			
	Biochemistry and Nutrition			
	Transport			
	Xerox, Mimeographs etc.	500		
	Sub Totals	**25610**		
	Other Operating Costs	**5657**		
	Capital Expenditure	**3225**		

TOTAL DIRECT COST **106293**

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

PI (A S Rahman): The principal Investigator will spent 100% of his time for the study and will be responsible for overall designing and supervision of the study, specially checking quality control, operations at the field level and review the progress of the study. Coordination of the study at the field, laboratory and office level including data analyses and report writing will be done by this person.

Co-PI (Professor David Sack): The Co-Principal Investigator will help with designing, decision making and actual planning of the study (no funds).

Co-PI (M A Quayium): This Co-Principal Investigator will spent 20% of his time for the study and coordinate all field level activities including supply and logistics.

Co-Investigator (Parveen A Khanum): This Co-Investigator will spent 25% of her time for the study and will assist in checking quality control, field level operations and coordination with field and laboratory.

Co-Investigator (SK Roy-5%): This Co-Investigator will train supervisors and field assistants on anthropometry and assist in monitoring compliance and quality control of data collection.

Co-Investigator (M A Wahed, laboratory biochemist-10%): This Co-Investigator will be responsible for organizing blood collection at the field, transporting them to the laboratory at Dhaka, carry out analyses and maintain all results in a data set in PC.

Co-Investigator (T Ahmed-5%): This Co-Investigator will provide assistance in designing the project, monitoring and evaluation and trouble shooting during conduction of the study.

Co-Investigator (M N Alam, Field Operations Manager): This person will spent 30% of his time for the study and coordinate activities at the field and supervise the field assistants (FRA) and supervisors (SFRA) at the field. Also maintain liaison with PI and other investigators.

Supervisors (SFRAs): Four supervisors will be recruited for 100% time. They will train and supervise the FA's to ensure that they understand their job and are collecting the correct data. They will meet the FA's at least every two weeks to monitor the progress of the study. Supervisors will maintain liaison with the investigators and laboratory personnel at Dhaka.

Field research assistants (FRAs): A total of 8 field assistants will be recruited and they will spend 100% of time for the study. They will distribute flour, monitor the usage of the flour in each bari, make spot checks at each bari to confirm that the *chapattis* are being prepared and consumed by the children and collect spot samples for analyses.

Lab Technician: Four lab technician would be recruited for 2 weeks during each round of data collection for the purpose of drawing blood samples.

Programmer: One programmer will be recruited for a total of two months to design data entry programme. Also prepare working files for different data and assist in data analyses.

Bari persons will be assigned to keep a written record of the consumption of *chapattis* by the study participants. As a motivational factor, in return for this contribution, the bari person as incentives will receive a small amount of money.

Bari mothers will be recruited to participate in the study. They will receive the flour at weekly intervals, prepare the *chapattis* and distribute them to the families who are participating. As a motivational factor, in return for this contribution, the bari mothers as incentives will receive a small amount of money and an additional amount of flour for her family.

Condiments: Funds are requested to buy and distribute condiments (dal, suji, sugar etc.) along with the distribution of flour to each bari to increase compliance of *chapatti* consumption

Travel and Transportation: Field visit from Dhaka would be necessary to supervise monthly evaluation and progress at the field level. Travel and transportation would be necessary within field site to distribute flour and for monitoring and supervision of study activities at the bari level every week. International travel would be necessary to disseminate study findings in an international seminar.

Dissemination/publication: Seminars, reports written and publication in national and international journals will require funds for dissemination.

Equipment: A microcomputer with accessories and a printer will be required for handling and analyses of data. A refrigerator will also be required to store blood/serum specimens at the field site before transportation to Dhaka.

Other expenses: Funds are requested to cover printing, photocopying, telephone and other communication, training and community meeting costs; utilities, rent and facility charges in Mirsarai; biochemical analyses as detailed in the attachment.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Randomized, Double-blind Controlled Trial of Wheat Flour *(Chapatti)* Fortified with Vitamin A and Iron in Improving Vitamin A and Iron Status in Healthy, School Aged Children in Rural Bangladesh

Principal Investigator: Ahmed Shafiqur Rahman

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

The ICDDR,B (International Centre for Diarrhoeal Disease Research, Bangladesh) is undertaking a research project, under the direction of Dr Ahmed Shafiqur Rahman, to see the impact of consuming *chapatti* made from wheat flour fortified with vitamin A, iron and a few other vitamins and minerals. We believe that children who eat *chapattis* made from this wheat flour on a daily basis will have improvement in vitamin A and iron status of school aged children. Vitamin A is a vitamin that helps to keep eyes healthy and iron is a mineral needed to prevent weakness and low blood counts. Thus, both Vitamin A and iron are needed by your child. If you agree to participate in this study, the following will apply:

1. Micronutrient fortified wheat flour will be supplied free of cost. This will be used to prepare chapattis for your children who are participating in this project. This supply of flour will continue for a period of six months. One of the mothers from your bari will prepare the *chapattis* and distribute it to you for your child and other children who are participating in the project. In addition, condiments (dal, suji, sugar etc.) will also be provided. Some children will receive chapattis that have extra amounts of vitamins and minerals and other children will receive chapattis made from standard wheat flour without these added vitamins and minerals.

2. Body weight, height of your participating children will be measured at the time of enrolment, at 3 months and at 6 months of the study period. At the same time, 5 ml of blood (about 1 teaspoonful) will be drawn from the vein of the elbow of your children. This is a safe procedure. The blood will be taken to measure the levels of vitamin A and iron to see if there is any effect on the levels following consumption of fortified flour.

3. From time to time, we will visit your house to see if your child is eating the *chapattis* properly. Your participation in the study will allow us to see if fortified flour intake is beneficial for improving the health/nutritional status of children.

4. There are no known side effects from eating the *chapattis* made from this special atta or from participating in this study other than the discomfort of the blood stick.

5. Your child does not have to participate in this research if you do not want to. If you join and wish to discontinue later, you may do this at any time. You are free to ask any questions you may have about the project.

If you agree to participate in the study, then please give your signature or impression of your left thumb below.

Signature of Investigator/ or agents
Date:

Signature of Subject/ Guardian
Date:

International Centre for Diarrhoeal Disease Research, Bangladesh

Title of the Research Project: Randomized, Double-blind Controlled Trial of Wheat Flour (Chapatti) Fortified with Vitamin A and Iron in Improving Vitamin A and Iron Status in Healthy, School Aged Children in Rural Bangladesh

Principal Investigator: Ahmed Shafiqur Rahman

অনুমতি পত্র

আই সি ডি ডি আর,বি পরিচালিত একটি প্রকল্প যা ডঃ আহ্‌মেদ শফিকুর রহমানের সম্পূর্ণ তত্ত্বাবধানে শুরু হতে যাচ্ছে। এই প্রকল্পে ভিটামিন-এ, আয়রন এবং অন্যান্য ভিটামিন ও খনিজ পদার্থ সমৃদ্ধ গমের আটার তৈরী রুটি খাওয়ার পর এর উপযোগিতা জানার জন্য ভিটামিন ও খনিজের পরিমাপ করা হবে। আমরা বিশ্বাস করি যে, যে সব বাচ্চা (যাদের বয়স ৬ থেকে ১৫ বছরের মধ্যে) ঐ রুটি প্রতিদিন নিয়মিত খাবে, তাদের শরীরে ভিটামিন-এ এবং আয়রনের পরিমান বৃদ্ধি পাবে। ভিটামিন-এ বাচ্চাদের দৃষ্টিশক্তি অক্ষুন্ন রাখতে এবং আয়রন প্রধানত শারীরিক দূর্বলতা ও রক্তশূন্যতা দুরীকরনে সহায়তা করে। সেজন্য এই দুই প্রকার ভিটামিন ও খনিজ শিশুর বৃদ্ধির জন্য একান্ত প্রয়োজন। যদি আপনি এই গবেষনা প্রকল্পে সহায়তা করতে সম্মত হন - তবে এই ব্যাপারে আপনাকে কিছু তথ্য প্রদান করতে চাই।

১। ভিটামিন ও খনিজ সমৃদ্ধ/সাধারণ ময়দা বিনামূল্যে সরবরাহ করা হবে। আপনার বাচ্চাদের যারা এই কার্যক্রমে অংশগ্রহন করবে তাদেরকে খাওয়ানোর জন্য ভিটামিন ও খনিজ সমৃদ্ধ এই ময়দা দিয়ে রুটি তৈরী করা হবে। এই ভিটামিন ও খনিজ সমৃদ্ধ ময়দা পূর্ণ ৬ মাস পর্যন্ত সরবরাহ করা হবে। আপনার বাড়ীর একজন মা/মহিলা প্রতিদিন এই রুটি তৈরী এবং বাচ্চার মাদের মাধ্যমে যারা এই কার্যক্রমে অংশগ্রহন করবে তাদের মধ্যে তা বিতরন করবে। তাছাড়াও রুটির সাথে খাবার জন্য ডাল বা সুজি এবং চিনিও সরবরাহ করা হবে। এই কার্যক্রমে যে সমস্ত বাড়ীর বাচ্চারা অংশগ্রহণ করবে তাদের অর্ধেক ভিটামিন ও খনিজ সমৃদ্ধ রুটি ও অর্ধেক সাধারণ ময়দার রুটি গ্রহণ করবে।

২। আপনার বাচ্চা যারা এই গবেষনা কার্যক্রমে অংশগ্রহন করবে তাদের দৈহিক ওজন ও উচ্চতা পরিমাপ করা হবে প্রধানতঃ এই কার্যক্রম শুরু করার সময়, ৩ মাসের সময় এবং শেষে অর্থাৎ ৬ মাসের সময়। প্রতিবার বাচ্চার দৈহিক ওজন ও উচ্চতা মাপার সময় ৫ ml (এক চা চামচ) রক্ত বাচ্চার শিরা থেকে সংগ্রহ করা হবে। নিরাপদ পদ্ধতির মাধ্যমে রক্ত সংগ্রহ করা হবে। বাচ্চার রক্তে ভিটামিন-এ এবং আয়রনের পরিমান জানার জন্য / পরিমাপ করার জন্যই এই রক্ত পরীক্ষা করা হবে।

৩। আপনার বাচ্চা যারা এই কার্যক্রমে অংশগ্রহন করছে তারা নিয়মিত ভিটামিন সমৃদ্ধ ময়দা দ্বারা তৈরীকৃত রুটি খাচ্ছে কি-না তা জানার জন্য আমরা মাঝে মাঝে আপনার বাড়ীতে আসব। এই গবেষনায় আপনার অংশগ্রহনের মাধ্যমে আমরা জানতে পারব যে - ভিটামিন সমৃদ্ধ রুটি খাওয়া বাচ্চাদের স্বাস্থ্য/পুষ্টি উন্নয়নের জন্য উপকারিতা মূলক উপায় কি-না।

৪। এই ভিটামিন সমৃদ্ধ ময়দা দ্বারা তৈরীকৃত রুটি খাওয়ার ফলে জানামত কোন পার্শ্ব প্রতিক্রিয়া / ক্ষতিকর প্রতিক্রিয়া নেই। তবে রক্ত সংগ্রহের সময় কিছুটা কষ্ট অনুভূত হতে পারে।

৫। যদি আপনি রাজি না থাকেন তবে আপনার বাচ্চারা এই কার্যক্রমে অংশগ্রহন নাও করতে পারে। এমনকি এই গবেষণা কার্যক্রমে অংশগ্রহণের পরও যে কোন সময় আপনার সম্পৃক্ততা/অংশগ্রহণ প্রত্যাহার করে নিতে পারেন। এই প্রকল্প সম্বন্ধে যে কোন প্রশ্ন আপনি করতে পারেন।

সবশেষে যদি আপনি এই গবেষনা কার্যক্রমে অংশগ্রহনে রাজি থাকেন তবে অনুগ্রহ করে আপনার স্বাক্ষর বা বাম হাতের বৃদ্ধ আঙ্গুলীর ছাপ প্রদান করুন।

অভিভাবক/অংশ গ্রহণকারীর স্বাক্ষর স্বাক্ষীর স্বাক্ষর গবেষক/প্রতিনিধির স্বাক্ষর

তারিখ .....

Enumeration Form
Health Systems Research Division
ICDDR,B: Centre for Health and Population Research

Bari ID # _____ Household ID # _____
Upazila: _____ Union: _____ Mouza: _____ Village: _____

Religion: 1=Islam, 2=Hindu, 3=Buddist , 4= Christian , 5=others

Participants ID#	SL #	Relationship H/W #	Relationship F #	Relationship M #	Name	Relat. with HHH	Sex M/F	Date of birth Y/m/d	Age (Comp Years)	Yrs. of schooling	Main occupation	Watch TV d/w/m/N	Listen to radio d/w/m/N	Read newspaper d/w/m/N	Comment

Name of data collector: _____ Supervisor's Name : _____ Date : _____

Form No. 1

Intervention on Wheat Flour Fortification
Assessment of Childrens' Nutritional Status
Health Systems Research Division
ICDDR,B: Centre for Health and Population Research

Bari ID # _____ Upazila: _____ Union: _____ Mouza: _____ Village: _____

Religion: 1=Islam, 2=Hindu, 3=Buddist, 4= Christian, 5=others

House hold ID #	Name of the household head	Participants ID#	Name of participant	Relat. with HHH	Sex M/F	Age (Comp Years)	Weight (kg)	Height (cm)	Blood drown y/n	Date of blood collection d/m/y	Comment

Name of data collector: _____ Date : _____

Supervisor's Name : _____ Date : _____

NB: only the children age 6 - 15 will be included in the list

Form No. 2

Intervention on Wheat Flour Fortification
Socio-economic status of household
Health Systems Research Division
ICDDR,B:Centre for Health and Population Research

Bari ID # _____ Household ID # _____

Upazila: _____ Union: _____

Mouza: _____ Village: _____

1. Source of water use

Purpose	Source					
	Shallow tube well/ handpump	Ditch/ canal/ Pond/ River	Open ring well	Closed ring well	Deep tube well	Others (tap etc)
a. Drinking						
b. Dish-washing						
c. Hand washing						
d. Bath						

2. Use of sanitation facility ☐

Code:
1. Sanitary/sealed latrine 2. Pit latrine
3. Hanging latrine 4. Bush/open field
5. Others

3. Do the household currently have electricity supply?

☐ Yes =1 ☐ No =0

4. Household possessions

Items	**Yes=1**	**No=0**	**# of items**
Cows/Buffaloes			
Almirah			
Table/chair/bench			
Watch/clock			
Cot/bed			
Radio			
Television			

Form No. 3

Items	Yes=1	No=0	# of items
Bicycle			
Rickshow/van			
Sewing machine			
Boat for commercial use			
Crushing machine			
Handloom			
Others			

5. Household own any cultivated land (in decimals)? ☐☐☐☐

6. Household involved in agricultural production? ☐ Yes=1, ☐ No =0

7. Quantity of paddy do you/your household members received last year

_____ (in maunds) N/A=88

8. Do your household currently growing vegetable? ☐
(code: No=0, Traditional=1, Developed garden=2)

9. Do your household currently cultivating fish? ☐
(code: No=0, Wild fish only=1, Cultivated fish=2, Wild and cultivated fish=3, Others=4)

10. Do you/your family involve in poultry rearing/farming? ☐
(code= No=0, Domestic use=1, For sale (large scale=2)

11. Total number of living rooms: ☐☐

12. Average no. of family members stay in one room ☐☐

13. Construction materials of the living houses of the household

a. Type of main living house ☐

b. Type of second living house ☐

c. Type of third living house ☐

(Code: Pucca=1, Tin house=2, Semi pucca (tin roof/ and wall pucca) =3, Mud / straw/bamboo wall/ and tin /tally roof=4, Low cost housing = 5,
Others (specify)_____=6)

Form No. 3

14. Status of living houses of the household

	Length (in feet)	Width (in feet)
a. Main living house		
b. Second living house		
c. Third living house		

15. Do you/your family member belong to any GO/NGO credit programme?

☐ No=0, ☐ Yes =1

16. Duration of credit programme membership _____ (in month)

17. Household's average monthly expenditure in last month

Food	Tk	☐☐☐☐☐
Agricultural inputs	Tk	☐☐☐☐☐
Clothing	Tk	☐☐☐☐☐
Medicine	Tk	☐☐☐☐☐
Fuel	Tk	☐☐☐☐☐
Loan payment	Tk	☐☐☐☐☐
Livestock purchase	Tk	☐☐☐☐☐
Household items	Tk	☐☐☐☐☐
Education cost	Tk	☐☐☐☐☐
Taxes	Tk	☐☐☐☐☐
Electricity	Tk	☐☐☐☐☐

Name of data collector: _____ Date: _____

Edited by: _____ Date: _____

Form No. 3

Health Systems Research Division
ICDDR,B: Centre for Health and Population Research

Bari ID # _____ Upazila: _____ Union: _____ Mouza: _____ Village: _____

HH ID #	Part. ID #	Name of participant	Sex M/F	Age (in yrs)	Diarrhoea				Reason for not seeking care	ARI			Night blind ness	Fever		Comments
					Types	Duration	Treatment	Place		Symptom	Treatment	Place		Duration	Treatment	

Name of the data collector: _____ Supervisor's name: _____ Date: _____

(Only the children age 6-15 will be included in this list) NB: Please use code to fill up this form. Code-list is attached

Form No. 4

Weekly Health Record Form
Code-list

Type of diarrhoea:	None=0 Watery=1 Dysentry=2 Simple diarrhoea=3

(Watery diarrhoea= Water like stools without any fecal solid materials. Locally known as **panir motho paikhana**. Dysentery it is stool contained blood, the types of diarrhoea be recorded as dysentery . locally known as **Rakto Amasha.** Simple diarrhoea Soft or semi stools with or without mucus. Locally termed as **Sadharan peter ashoke**).

Duration of diarrhoea	Exact duration in no. of days, N/A=8
Treatment (last)	None=0, Fluid from ORS packet=1, home made sugar-salt water solution (labon-gur) =2, both (labon-gur+ORS) =3, Plain water=4, Any medicine except ORS=5, ORS + any medicine=6 Green Coconut water = 10, Sugar-cane Juice=11, Any fluid (except ORS) + medicine =12, Other =13 Don't know=77, N/A=88
Place	Home=1, Government=2, NGO clinic=3, Kabiraj/traditional/=4, village practitioner =5, Homeopathic=6, qualified medical practitioner = 10, private clinic/private hospital= 11 Volunteer team=12, Religious treatment=13, Other =14, Don't know= 77, N/A=88
Reason for not seeking care	No transport=1, No money=2, No time=3, Nobody to accompany=4, No access=5, Did not think necessary=6, Other (specify)_____=10, Don't know= 77, N/A=88
ARI symptoms	No=0, Nasal discharge/sore throat=1, Bouts of cough=2, first breathing=3, cough and first breating=4,
Treatment for ARI	Yes=1, No=0
Place/providers visited for ARI	Same as for Diarrhoea
Night blind ness	No=0 Yes=1
Fever (duration)	No=0, Exact duration in number of days
Fever (treatment)	No=0, Any medicine =1, N/A=8

Review of ICDDR,B proposal: "Randomized, double blind, controlled trial of wheat flour (chapatti) fortified with vitamin A and iron in improving serum vitamin A and iron status in healthy rural school-aged children in Bangladesh"

PI: A S Rahman

By: Philip Harvey MPH, PhD. Nutrition Advisor, MOST, The USAID Micronutrient Project
Date: 8/23/01

General comments:
This proposal addresses a priority concern to public health in Bangladesh. There is no doubt that micronutrient malnutrition has many and serious adverse effects on the health, education and economic well being of the population. This proposal is an important step towards identifying practical means to reduce this problem.

A randomized clinical trial is proposed in which chapatti fortified with micronutrients (notably vitamin A and iron) will be provided to children 6-15 years of age for 6 months (the 9-month option is well beyond the available budget). Another group will be provided with non-fortified chapatti. Comparisons of micronutrient status of the two groups will be made to determine the impact of the fortified chapatti.

The procedures to carry out the study have well thoroughly thought through and succinctly described. Detail is lacking but it is assumed that this will be added as the study protocols are developed after piloting that is proposed.

Fortified flour: A profile of the proposed fortified flour should be added. Importantly, the amount of addition vitamin A and iron that it is proposed to provide daily to each needs to be added. This is clearly important to estimating the likely impact on biochemical parameters and will be essential to comparing the observations of this study with those made in previous work.

Dr Rahman, as Principal Investigator, is a young investigator for whom this study should provide valuable experience in leading a field-based study. He should be provided with ample support by the two co-Principal investigators and five co-investigators, some of who have extensive experience in undertaking this type of study. The donor assumes that this rather large number of co-investigators each will actively contribute to the study and provide the support that is likely to be necessary.

Specific comments:
The literature reviewed adequately justifies the work and describes previous some investigations of similar questions. One significant and unfortunate gap is the absence of reference to the study by Solon et al. (AJCN) in the Philippines. Solon's work is highly pertinent to the study proposed and should be carefully reviewed by the PI as a matter of priority. A major difference with Solon's study is that a second micronutrient (iron) is added. It is this reviewer's opinion that no referred journal would accept a manuscript from the study proposed without comparisons of results to this recent relevant work in the Philippines.

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Quiyum & Shafiqie

The design described is sound and while limited in some respects, achieves what is feasible given the limited budget available. The 'clinical trial' approach is efficient in that feeding costs are incurred only for children directly contributing data to key comparisons. The study setting seems to be quite appropriate in that it assures a population similar to the likely target group of a subsequent intervention.

The study procedures are not specified in detail but are in general, seem well thought out and entirely feasible. Detailed study protocols will presumably be developed as piloting takes place. These documents will be critical to the success of the study. The most important issue will be that the study remains truly blind, and that there is no crossover in treatments. It is assumed that the large number of experienced co-investigators will ensure that the integrity of the study design is maintained.

The sample size calculation seems reasonable if not specified in an orthodox manner. The proposed analysis is sketchy and appears to have benefited from a statistician's input. Such input is recommended. In particular, some form of multivariate analysis is likely to allow stronger conclusions to be drawn regarding the main hypotheses being tested. It is well established that an initial level of a biochemical indicator is one the strongest determinants of that indicators response to a supplement. Thus initial levels will need to be controlled for. Another variable to consider as a potential confounder to control for in analysis will be age, particularly in girls after menarche.

Treatment of hookworm: The first version of the proposal I saw that hookworm not be treated in all subjects presumptively. The advantage of this is that the findings of the study will be more easily generalized to the current public health situation in Bangladesh. However, the disadvantage is that presence of hookworm may limit response of indicators, particularly hemoglobin. It may be that hookworm is not highly prevalent in the study area and if so, this reviewer would prefer to see the hookworm treatment deleted. If, however, hookworm is highly prevalent (say prevalence > 20%), I would recommend the treatment remain included. It would be ideal if it were possible to include a stool sample for each subject. The second version includes treatment.

Time line: This reviewer believes that two months is an unrealistically short period for data analyses and report writing. It appears that laboratory analysis may also scheduled in this period. I recommend an additional month be allocated for the PI only to allow more time for this critical phase of the study.

- The 9-month study, while scientifically preferable, is well beyond the funds available.
- Cost of the flour (both fortified and unfortified) should be added.
- Add one month for PI.
- International travel item may not be possible to include in the budget for this study. Other mechanisms to pay for travel may be available
- If the budget must be reduced further, I recommend removing the mid-study survey as the most dispensable of the components. I calculate this will reduce direct costs by at least $7,000 and thus total cost by about $9,000. A second option for budget cutting would be to remove the serum ferritin assay.

Name	Position	Date of Birth
Dr. S. K. ROY	Scientist, Clinical Sciences Division, ICDDR,B	November 12, 1950

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location Study	Degree	Year	Field of
Dhaka Medical College. University of Dhaka, Bangladesh	MBBS	1973	
London School of Hygiene and Human Nutrition Tropical Medicine, UK	M.Sc	1984	Nutrition
University of London	Ph. D	1990	Nutrition

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1. 1992-Present Scientist, Clinical Sciences Division, ICDDR,B
2. 1987-92 Associate Scientist, Clinical Sciences Division, ICDDR,B
3. 1981-87 Senior Medical Officer, ICDDR,B

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Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included ☑
2. Approval of the Division Director on Face Sheet ☑
3. Certification and Signature of PI on Face Sheet, #9 and #10 ☑
4. Table on Contents ☑
5. Project Summary ☑
6. Literature Cited ☑
7. Biography of Investigators ☑
8. Ethical Assurance ☑
9. Consent Forms ☑
10. Detailed Budget ☑

To :

Date: 20-10-2001

From :

Subject :