International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

LSD
2001

Memorandum

21 November 2005

To : Mr. M. A. Wahed
Principal Investigator of research protocol # 2001-025
Laboratory Sciences Division (LSD)

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

Sub : Approval for an addendum to research protocol # 2001-025

Thank you for your memo dated November 15, 2005 and the proposal for an addendum to your research protocol # 2001-025 titled "The efficacy of vitamin A-rich small fish in improving vitamin A status in children in Bangladesh" for consideration of the ERC. I have the pleasure to inform you that the proposal for the addendum has been approved.

Thank you once again.

Copy: Director, LSD

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Memorandum

To : Professor A K M Nurul Anwar
Chairman
Ethical Review Committee (ERC)

From : Dr. Md. Yunus
Sr. Scientist & Head, MHRC
& Member, ERC

Date : 20th November, 2005

Subject: Expedited Review

Thank you for allowing me to review an addendum proposal entitled " Availability of A2 from Mola Fish in human serum after Mola Fish meal" to the Protocol # 2001-025 titled "The efficacy of Vitamin A- rich small fish in improving vitamin A status in children in Bangladesh" by Mr. M.A. Wahed.

Objective: To demonstrate the presence of A2 in the serum after eating Mola Fish.

I have reviewed the proposal. The PI has given a strong-justification to measure Vitamin –A2 in serum after eating Molla Fish in this addendum proposal. However, I have the following comments:

1. In "Methods and materials" the PI has stated that the volunteers will be discouraged to eat any other fish particularly small fish like Mola during the 6-weeks study period. They will also be requested to refrain from taking any vitamin Pills which may contain Vitamin A. However, the PI has not mentioned anything about intake of foods of animal origin which contain preformed Vitamin A and is more efficiently absorbed. Not relevant.

2. The PI has enclosed the English version of consent form and mentioned in his memo to ERC Chair that since the 5 study participants (including the PI) are the employers of ICDDR,B the consent form is not required to be in Bangla but will be in English only. There is no objection in principle to have consent form in English provided the study participants can comprehend English well.

   The PI has not specified who are the potential 5 volunteers (except the PI) and their educational level. Without knowing the educational level it is difficult to have an idea

only those volunteers have proficiency in English would be selected. m.a. wahed

about their comprehension of consent form in English. The PI is required to provide information in this regard.

There is no other ethical concern with regard to this addendum proposal. Subject to clarifying the above two points, the proposal is recommended for approval.

Thank you again

(1) One reviewer might have confused with Pro-formed vitamin (which is A1) with that of A2 which is only available (to our best knowledge) from fish & small fish and nola is one of them)

Sanil

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Memorandum

15 November 2005

To : Dr. Md. Yunus
Member
Ethical Review Committee (ERC)

From: Professor AKM Nurul Anwar
Chairman, ERC

Sub : **Request for undertaking an expedited review**

Attached please find a proposal from Mr. M. A. Wahed, Principal Investigator (PI) of research protocol # 2001-025 titled "The efficacy of vitamin A-rich small fish in improving vitamin A status in children in Bangladesh" for an addendum to his research protocol. Since the PI has time constraint and that there is minimal risk to the study participants, the proposal has been accepted for expedited review. The proposal and the original research protocol are attached.

It would be much appreciated if you could undertake review of the proposal and provide your comments as early as possible to make decision on it

Thank you.

Copy: Members, ERC

November 15, 2005

The Chairman,
ERC, ICDDR,B

Through: Director and Head, Laboratory Sciences Division.

Subject: ERC approval for an addendum protocol "Availability of A2 from Mola Fish in human serum after mola fish meal"

Enclosed please find herewith the above protocol which has been approved by RRC. We would like the **expedite approval** from ERC as well. We plan to start our activities with effect from December 1, 2005. Since the study participants are from the Centres' employees, we do hope that you will agree with us that the consent form is not required to be in Bangla. As such we attached the English version consent form only.

Thanking you.

Yours sincerely,

M A Wahed
P.I Protocol #2001-025

Encl: As stated

Availability of A2 From *Mola* Fish in Human Serum After *Mola* Fish Meal.

Principal Investigator: M A Wahed

Background

Mola fish has a high content of vitamin A (>2500 RE/100gm edible portion). Out of whole vitamin A in mola fish, about 20% is A1 and 70-80% A2 (didehydroretinol). A2 is a viscous alcohol that has less bioactivity (maximum 40% i.e. it competes with RBP) as compared to A1. Function wise, both A1 and A2 are almost same with the exception of the role of A1 on the retina. In our study (Protocol no. 2001-25), we fed sufficient mola fish to children who are marginally vitamin a deficient and expected an improvement of their vitamin A status (increase in serum retinol). The study is completed .It looks like that improvement in vitamin A status measured as serum retinol (A1) was not as expected.

	Serum Retinol umol/L		
	Mola Fish Gr	**Rui with added Vit A(+Control)**	**Rui (-ve Control)**
Pre	0.59±0.11	0.59±0.10	0.60±0.11
Post	0.60±0.18	0.84±0.20	0.65±0.25
	NS	***<0.001***	***NS***

Note: Preliminary data are being corrected for Acute Phase Protein.

However, none of the children in the mola fish group appeared to be clinically vitamin A deficient neither during study period nor at the end. Then the question comes to our mind that whether there was any absorption of A2 which is the major proportion of vitamin A in mola fish and that could have been demonstrated in the human blood. To our knowledge, there is no report documenting the presence of A2 from fish/food in the human blood although synthetic A2 could be measured after an oral dose. Measurement of A2 in the serum was not part of our original protocol. Normally A2 is also not found in the human blood.

Objective: To demonstrate the presence of A2 in the serum after eating mola fish.

Methods and materials:

Five adult volunteers (ICDDR,B employees) including the investigator will eat lunch with mola fish for 6 days per week for 6 weeks. Volunteers will be discouraged to eat any other fish, particularly small fish like mola during the 6 weeks' period. It should be noted that most of the big fishes do not have A2. Also they will be requested to refrain from taking any vitamin pills which may contain vitamin A. We shall also collect information about any illnesses and medication during the 6 weeks period.

Consent Form

Availability of Vitamin A2 from mola fish in the human blood after mola fish meal.

Mola fish is being promoted to help improving vitamin A status considering its high content of vitamin A, but out of total vitamin A, 80% is A2 (didehydroretinol) and rest A1. Other than action of A1 on retina, functionally both from of vitamin A is similar. There is neither any report documenting the availability of A2 in the human blood nor showing any increase of A1 after mola fish meal. In our recently conducted study in children in Mirpur slum, serum retinol (Vitamin A1) was not increased as expected after mola fish meal. But we do not know whether there was any A2 from mola fish being absorbed as could be evidenced from A2 in serum, because we did not analyze A2 in blood.

Now, we propose a small study for which healthy volunteers will be offered a luncheon containing 150 gm mola curry which correspond to about 2300 ug vitamin A2 + 380 ug A1 along with rice, dal and vegetable. The lunch will be provided 6 days a week for 6 weeks. We expect that all volunteers will participate at the lunch in the Centres' canteen regularly.

We shall collect 1 ml blood before and another ml after 6 weeks' feeding. During blood collection, there may be little discomfort. We shall ensure all kinds of aseptic measures to prevent any complications.

We expect you to report to us for any sickness and medication during the study period.

We would also like you to refrain from taking any vitamin pill containing vitamin A and fish particularly small fish like mola during the 6 weeks' study period.

We invite you to participate in this study and if you agree, please sign below. Your participation is voluntary and you may withdraw your consent at any stage of the study without any prejudice to your job or status at the Centre. No compensation will be allowed to you for such participation in the study.

Signature of Investigator	Name and signature of Volunteer

PI: M A Wahed (Phone 0189-297010 for any information and clarification)

At the beginning and after the end of feeding, 1 ml venous blood will be collected. Serum will be separated and both A1 and A2 will be measured by HPLC following standard technique. The Nutritional Biochemistry Laboratory is equipped with the instrumentations and similar assay has been conducted before in the laboratory.
Mola fish after being usually cooked was found to have 770ug of All-trans 3,4 Didehydroretinol (A2) per 50 gm curry. We shall offer 150 gm mola curry correspond to 2300 ug A2 to each volunteer. Rice, Dal and Vegetable will be served will be *ad libitum.* Any left over of mola fish curry will be recorded. Each volunteer will act as his/her own control.

Informed consent (form enclosed) will be obtained.

Rationale: Since A1 and A2 are similar at least function wise , if we can demonstrate that substantial A2 is found in blood after mola fish meal, we can still advocate for consumption of mola fish and other vitamin A rich small fish to improve vitamin A irrespective of the fact that A1 in blood after consumption of Mola fish is not increased.

Budget: (USD)

Personnel 1 FRA GS III	820.00
Cook	300.00
Lab cost (A1 and A2 analyses)	484.00
Food cost (Molafish+Rice+Misc)	400.00
Laboratory Supplies	150.00
Stationeries	150.00
Interdepartmental	200.00
Unforeseen	200.00
Total	**2,704.00**

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Cable : Cholera Dhaka

Memorandum

3 December 2001

To : Mr. M. A. Wahed
Laboratory Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub: **Approval of protocol # 2001-025**

This is in reference to your memo of 3 December 2001 with the modified copy of your protocol # 2001-025 entitled "The efficacy of Vitamin A rich small fish in improving Vitamin A status in children in Bangladesh". The modified version of the protocol is hereby approved upon your satisfactory addressing of the issues raised by the ERC considered in its meeting held on 28th November 2001.

I wish you all the success in running the above mentioned study.

Copy: Acting Head
Laboratory Sciences Division

International Centre for Diarrhoeal Disease Research, Bangladesh
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Dec 03,2001

To : Chairman, ERC
From : M A Wahed
Subject : Replies to queries raised by the ERC member regarding Protocol#2001-025

Thank you very much for your careful review and comments. Below are our replies:

1. Your advice to change the title is reasonable. However, please bear with us that if we change the title now, it should have to go to both RRC and the donor for their approval. Since the present title is implied, we would request your consideration to keep the title as it is.
2. The basis of the proposed efficacy trial for 9 weeks was the study carried out by de Pee et al (1998a).
3. You are right that VAC program is on for children upto 59 months. Our proposed age group is 3-7 years. We have observed out of a sample survey that the coverage is not that much. In any case, we shall not include any children have had vitamin A supplementation during last 6 months. We have already mentioned it clearly (Pls see the consent form for feeding trial #3) that under those conditions we shall take out the children from the study and supplement them with Vitamin A.
4. At the end of the study all children will be supplemented with vitamin A. It is mentioned in both the text as well as in the consent form (Feeding Trial #4).
5. Yes, you are right. Our primary aim is the improvement in retinol status and accordingly sample size was calculated. For the other variables, data analysis plan has been described in page 21 of the protocol. Also handling of confounding factors are discussed in page 16.

6. We made it descriptive so that most questions are clear to the consent giver.
7. Please find now the Bangla version of the consent form.
8. It was an inadvertent mistakes. The serial number 10 and 11 would be 8 and 9. A corrected version is enclosed.
9. Thank you very much for pointing out the typo-error and few mistakes. The spelling errors are now corrected. Fresh abstract summary and consent form are also enclosed.

We hope, the protocol may please be approved now.

I have gone through the responses by the investigators. The issues have been addressed and the protocol may be approved.

Dr. Rabb
03/12/2001

ABSTRACT SUMMARY FOR ETHICAL RESEARCH COMMETTE (ERC)

Vitamin A deficiency (VAD) is a public health problem in many developing countries, including Bangladesh. Within food based strategies there has been no focus on the role of small fish in combating vitamin A and micronutrient deficiencies. Few commonly consumed small fish spices have been found to have very high contents of preformed vitamin A. For example, mola (*Amblypharyngodon mola*) contains 2,400-3,000 RE/ 100-g raw edible fish. The *overall aim* of the study is to determine the change in vitamin A status after supplementation of 45 g if vitamin A rich small fish, mola (500 RE) in a daily meal for 9 weeks, to marginally vitamin A deficient Bangladeshi children (3-7 y) in a Dhaka slum, and compare it with the change in vitamin A status obtained after supplementation of 45 g of big fish, rui (*Labeo rohita*) containing no/little vitamin A and 45 g of big fish, rui with added retinyl palmitate (500 RE). Children (180) will be randomly allocated to either of three diet groups. Studies on the efficacy of commonly consumed vitamin A rich small fish on the vitamin A status in children are therefore valuable as a first step towards developing specific agricultural and food based programs. If this study is successful, there is a large scope to increase the production of mola and other small indigenous vitamin A rich fish in combating VAD. The finding of this study will be equally important for other developing countries with habitual fish consumption.

1.**Requirments for the study group and rationale fir using this study group?**

The subject population, children 3-7 y usually have marginal vitamin A deficiency and they are at great risk for such deficiency which may lead to nightblindness or complete blindness if they are not identified and effectively treated. Food based strategies are the best and sustainable. However, fish based food intervention was never tried. We are proposing here to intervene with small fish content of vitamin A to improve the vitamin A status. This age group of children can eat such small.

2. **Describe and assess any potential risk?**

There are no more than minimal risk involved in conducting the study.

Four ml blood sample will be collected and stool sample will be collected from study subjects at *screening.* A second blood sample (4 ml) will be collected at *endpoint* in subjects enrolled in the feeding trial. In a sub-sample, one fourth of the children enrolled in the feeding trial, two blood samples will be taken with an interval of 5 h at *baseline* and at *endpoint* for the Relative Dose Response test. Complications of blood drawing are generally minor but may have mild momentary discomfort.

Children enrolled in the negative control group (no/low vitamin A) will replace only one habitual meal per day by a no/ low vitamin A rich meal, while all other metals will be consumed at home, as normally done. The duration of the study is relatively short, 9 weeks, 6 days per week and no severely vitamin A deficient children (serum retinol concentration <0.35 μmol/L) will be recruited. At the end of the study, the children will receive an oral dose of vitamin A supplementation.

Subjects will not be hospitalized and no other invasive technique will be applied that may cause physical, psychological, social or legal risks.

3. **Procedures for protecting against or minimizing potential risks?**

Disposable syringes and needles will be used for collecting blood for protecting or minimizing any risk. Well experienced personnel will perform blood drawing. Disposable plates will be used daily for serving the test meal

4. **Method for safeguarding confidentiality or protecting anonymity?**

All information collected will be kept strictly confidential. A unique number will be used for data presentation instead of child's name. Other than the investigators, no one will have access to any information of the children. The parents may however, will know the laboratory results, medical conditions and treatment if any.

5.

5 a) Describe how and where informed consent will be obtained?

Singed informed consent will be obtained from the parents or legal guardian of the subjects at household. Contents of the consent from will be read in *Bangla.*

5 b) If information is to be withheld from a subject, justify this course of action?

There is hardly any information to be withheld. However, the subjects will be explained that they are randomized to receive one of the three types of blended fish, *mola, rui and rui with added vitamin* A dishes.

They will be informed that two of the fish dishes will contain vitamin A and one fish dish will contain Low/ no vitamin A. However neither the subjects/ mothers nor the study staff will know which type of fish dish they are going to receive during the feeding trial. This is done in order to eliminate bias.

5 c) Stating whether or not a compensation and / or treatment will be available?

There is no potential risk to the subject or privacy of the individual is involved.

Children who do not fulfill the health examination, show clinical sign of vitamin A deficiency, have serum retinol concentrations < 0.35 μmol- L or show any clinical sign of sickness (high fever, ARI, measles, dysentery etc) during the feeding trial will be not be included in the study and referred to the medical doctor for treatment. All children will be given anti-helminthes treatment before the study. The families of the children will have access to the available services of the medical doctor at the ICDDR, B local Mirpur office. At completion of the feeding trial, the children will be given a recommended oral vitamin A dose and other supplementation and anti-helminthes agents as medically advisable.

6. **Interviews: Describe where and in what context the interview will take place. State approximate length of time required for the interview.**

a) Identification of household and 3-7 yrs. Children to be included in the study – at household levels: once (5 min.)
b) SES information at the household level – once (30 min.)
c) Semi quantitative Dietary assessment of vitamin A rich food at household – once (20 min.)
d) Dietary intake assessment by 24 h. recall fortnightly – 6 times at household (15 min.)
e) Morbidity – baseline at the household and then 5 times more during 9 weeks (10 min.)
f) Child clinical health examination 5 times at the sub- centre (20 min.). Based on needs, children may be examined by the study physicians.

Mothers perception about mola fish will be assessed using both structured and open-ended questionnaire. This may take 10 mints. In addition, focus group discussion with 10-15 mothers at 3-4 sessions at the subcentre can be organized.

Physicians, nurses and RESEARC/HEALTH assistants at the local centre will perform clinical health examination, anthropometry measurement and blood collection at screening, baseline and endpoint (lasting about ½ h each time).

7. **Assess benefits to be gained by the individual subject, the society in general, and how the benefits outweigh the risks**

All potential children will get a free health examination by physician and diagnostic if necessary as well as free deworming medication and vitamin A supplementation. Children will also be identified if they have severe or marginal deficiency based on serum retinol.

All children enrolled in the feeding trail will be served by lunch containing rice, fish curry and vegetable curry each day 6 days a week for 9 weeks. At the completion of the trial, children will be given vitamin A supplementation as medically advised.

The families of the subjects will have access to the available services of the medical doctor at the ICDDR,B local Mirpur office.

If the study is a success, it is valuable as a first step towards improving the production, availability, accessibility and intake of vitamin A rich small fish in the community. Hence, the study may help to improve vitamin A deficiency in people in the community as well as millions of other people who suffer from vitamin A deficiency.

8.**State if the activity requires the use of records (hospital, medical birth, death or other), organs, tissues, body fluids, the fetus or the arbortus?**

The activity does not require use of records. However, blood for measurement of vitamin A, iron status, protein and acute phase protein levels; and stool for presence and load of worms will be collected.

APPENDIX – 9

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Voluntary Consent Form for Feeding Trial

Before recruiting into the study, the study subjects 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 subjects 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 subjects must indicate his/her acceptance of participation by signing or thumb printing on this form

Title of the project:	The efficacy of vitamin A rich small fish in improving vitamin A status in children in Bangladesh
Protocol No.:	2001-025
Investigators:	M. A. Wahed[1], Katja Kongsbak[2], Sakuntala Thilsted[2]
Organisation:	[1] Head, Nutritional Biochemistry, Laboratory Sciences Division, ICDDR,B [2] Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (KVL)

You might remember our previous visit when we informed you on a research study that ICDDR,B is conducting in your community in collaboration with The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Just in case you have forgotten the details, we would like to remind you the purpose and procedures of the study. Vitamin A deficiency is an important health problem in Bangladesh. It may lead to night blindness and other eye changes, and even to complete blindness if the deficiency is not identified and effectively treated. Moreover, vitamin A-deficient individuals cannot fight well against infectious diseases.

The main purpose of our study is to examine ways to improve vitamin A status of children who have marginal deficiency of vitamin A. With your approval, we enrolled your child in the screening phase of the study, and found that she/he has marginal deficiency of vitamin A (serum retinol 0.35-0.70 μmol/L).

In this second "Feeding Phase" of the study, we want to examine if it would be possible to improve vitamin status of children with marginal vitamin A deficiency by providing them with a curry in their diet that contains a small, local fish, called "Mola", which contains good amounts of vitamin A. Since your child has marginal vitamin A deficiency, we would like to enroll her/him in this study. If you allow us to enroll your child, the followings would be done:

1. We would ask you to bring your child to the sub-centre for lunch, 6 days in a week, for 9 weeks, and also to bring her/him in the event she/he develops an illness. In the sub-centre we would provide lunch to all children with a blended curry containing one of the followings: (i) Mola fish, with high vitamin A content (ii) Ruhi fish with low or no vitamin A, and (iii) Ruhi fish with added vitamin A. We would divide all children into three groups and each group would receive one of the above curries during the entire duration of the study by pure chance, and thus your child would have equal chance to fall in any of these groups.

2. Before starting the feeding trial, we would select $1/4^{th}$ of the children in each of the 3 groups by a process similar to lottery. We would determine their Relative Dose Response (RDR), which is considered as a reliable indicator for individual's vitamin A reserve in the liver. This would require collection of 1.0 ml ($1/5^{th}$ of a teaspoonful) of fasting blood in the morning, and another 1.0 ml of blood 5 hours following an oral dose of retinyl palmitate (vitamin A). During this 5-hour period, the study children would stay at the research sub-centre where snacks and drinks, which do not contain vitamin A, would be provided. Your child would have a 25% chance to fall in this group of children. You would also be able to stay at the sub-centre with your child, and snacks and drinks would be provided to you.

3. During the 9-week study period, a study staff would visit your house every fortnight and collect information on foods that your child had taken during the previous 24 hours, and on illness that she/he might have had in the previous fortnight. If your child develops any feature of vitamin A deficiency such as night blindness or eye changes, or if she/he develops an illness that requires routine supplementation of vitamin A such as conditions with measles, pneumonia and diarrhoea, we would pull out your child from the study to safeguard her/his interest and provide her/him with high potency vitamin A as recommended by WHO. Study staff will also enquire and assess your perception about mola fish using open ended questionnaire as well as out of focus group discussion while you are assembled at the subcentre for child's feeding.

4. For the purpose of our study, your child will not be allowed to take any vitamin A or multivitamin or mineral supplements during the study without informing and discussing with our physician. At the end of 9-week trial, we would provide vitamin A to all study children.

5. At the end of the 9-week feeding trials, we would again measure her/his weight, height and mid upper arm circumference. An experienced doctor will collect 4.0 ml (less than a teaspoonful) of blood from a vein in her/his arm for measuring vitamin A, iron status and acute phase reactants. Similar to before, we would select $1/4^{th}$ of the children from each of the 3 feeding groups for Relative Dose Response test. The procedures would be the same as described before, and it would require collection of an additional ml of blood, 5 hours after an oral dose of vitamin A. Your child would have a 25% chance to fall in this group of children.

6. Other than momentary pain from the needle prick, small chance of discolouration of the skin surrounding the puncture site, and rare possibility of infection, collection of a maximum of 6.0 + 5.0 = 11.0 ml of blood by 4/5 different pricks over a 9 week period would cause no other harm. We would use one-time-use, sterile syringes and needles, and also take other precautions to prevent such problems. Possible benefits from participating in the study include identification of vitamin A deficiency and other medical problems, and their appropriate treatment as well as possible improvement in their nutritional status.

7. All information obtained from you/your child will be kept confidential, and none other than the investigators of this study would have an access to those information. We would be happy to provide you information on medical problems, treatment, and results of the laboratory tests of your child. Some laboratory analyses take more time than expected; however, we would try to get the results as soon as possible.

8. Participation in this study is voluntary, and you are the only person to take decision either for or against participation of your child in the study. You would also be able to withdraw your consent at any time during the study. There would be no penalty if you do not agree to our proposal of enrolling your child in the study, and also if you withdraw your consent at a later time.

9. You would be able to ask questions to the doctor and the study staff about our study, health of your child, and the results of the laboratory tests performed on your child. You would also be able to communicate with the principal investigator, M. A. Wahed, at telephone number: 88 11 751

Thank you for your cooperation.

Parent/guardian's name Parent/guardian's signature/LTI Date

Investigator/representative's name Investigator/representative's signature Date:

Witness's name Witness's signature Date:

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র (ICDDR,B)

ঐচ্ছিক সম্মতি পত্র - পরীক্ষামূলক প্রয়োগে অংশগ্রহণ।

গবেষণার বিষয়: বাংলাদেশে শিশুদের রক্তে ভিটামিন-এ র মাত্রা উন্নয়নে ভিটামিন-এ সমৃদ্ধ ছোট মাছের ভূমিকা।

গবেষণার নং: 2001-025

গবেষকবৃন্দ: এম. এ ওয়াহেদ, কাত্জা কংকসব্যাক, শকুন্তলা থিলস্টেড

ঠিকানা: ১. পুষ্টি ও প্রাণরসায়নের প্রধান, আন্তর্জাতিক উদরাময় গবেষণাকেন্দ্র।

2. পুষ্টি গবেষণা বিভাগ, রাজকীয় পশু ও কৃষি ~~শিক্ষা~~ বিশ্ববিদ্যালয় কোপেন হেগেন, ডেনমার্ক।

আপনার হয়তো মনে থাকতে পারে যে ইতিপূর্বে আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র ও কোপেনহেগেনস্থ রয়েল ভেটেনারী ও কৃষি বিদ্যালয়ের একটি যৌথ গবেষণার ব্যাপারে অবগত করা হয়েছিল। আপনি হয়তো ভুলে যেতে পারেন। তাই, আমরা এ গবেষণার উদ্দেশ্য ও পদ্ধতি সমূহ পুনরায় আপনাকে জানাতে চাই। ভিটামিন এ'র অভাব বাংলাদেশের জন্যে একটি গুরুত্বপূর্ণ স্বাস্থ্য সমস্যা। এই ধরনের অভাব রাতকানা; চোখের বিভিন্ন সমস্যা এমন কি পূর্ণ অন্ধত্ব ঘটাতে পারে যদি না ঠিক সময়ে চিহ্নিত করা না হয় ও ফলপ্রসু চিকিৎসা না দেওয়া হয়। এ ধরনের অভাবের কারনে মানুষের সংক্রামক ব্যাধির প্রতিরোধ ক্ষমতা কমে যায়।

আমাদের এই গবেষণার প্রধান উদ্দেশ্য হচ্ছে যে সমস্ত শিশুর শরীরে ভিটামিন এ'র ন্যূনতম স্বল্পতা আছে তাদের ভিটামিন এ'র মান উন্নত করার বিভিন্ন পন্থা নিরূপন করা। ইতিপূর্বে আপনার শিশুর রক্ত পরীক্ষা করা হয়েছে এবং ফলাফল অনুসারে আপনার শিশুর ভিটামিন এ'র ন্যূনতম স্বল্পতা আছে।

আমাদের এই পর্যায়ের গবেষণায় আমরা দেখতে চাই যে, এক ধরনের ছোট মাছ, মলা মাছ যাতে প্রচুর পরিমান ভিটামিন এ আছে, এ ধরনের মাছ দিয়ে রান্না করা খাবার খাইয়ে যেসমস্ত শিশু ন্যূনতম ভিটামিন এ'র স্বল্পতায় ভুগছে তাদের রক্তে ভিটামিন এ'র মান উন্নয়ন করা যায় কিনা। যেহেতু আপনার শিশুর ভিটামিন এ'র ন্যূনতম স্বল্পতা আছে এজন্য আমরা চাই আপনার শিশু গবেষণায় অংশগ্রহণ করুক। আপনি রাজী থাকলে নিম্নলিখিত পদ্ধতি পরিচালনা করতে আপনার সহযোগিতা চাই:

১। আপনি আপনার শিশুকে ৯ সপ্তাহ ধরে সপ্তাহে ৬ দিন দুপুরের খাবারের জন্য আমাদের উপকেন্দ্রে নিয়ে আসবেন। কোনরূপ অসুখ বিসুখ হলেও তাকে উপকেন্দ্রে নিয়ে আসবেন। এই উপকেন্দ্রে আপনার শিশুকে নিম্নবর্ণিত ৩টির যে কোন এক ধরনের রান্না করা মাছ সহ দুপুরের খাবার দেওয়া হবে

ক। মলা মাছ যাতে প্রচুর ভিটামিন 'এ' আছে
খ। রুই মাছ যাতে খুব কম অথবা ভিটামিন 'এ' নেই বললেই চলে।
গ। রুই মাছ ও বাড়তি অংযোজিত ভিটামিন 'এ'

আমরা অনুদয় শিশুকে লটারীর মাধ্যমে ৩টি দলে ভাগ করবো এবং প্রতি দলের শিশুরা উপরে বর্ণিত যে কোন একটি খাবার অবেষনা কালীন সময় খাবে। আপনার শিশু যে কোন দলে অন্তর্ভুক্ত হতে পারে।

২। এই খাবার কার্যক্রম শুরুর আগে প্রতি দল থেকে ৪ ভাগের ১ ভাগ শিশুকে লটারীর মাধ্যমে নির্বাচিত করা হবে। এই নির্বাচিত শিশুদের যকৃতে ভিটামিন 'এ' র পরিমানের নির্দেশক হিসাবে বিবেচিত পরীক্ষা (রিলেটিভ ডোজ রেসপন্স) করা হবে। এই পরীক্ষার জন্য সকালে খালি পেট অবস্থায় বাচ্চার শিরা থেকে ১ মি.লি (১ এক চা চামচ) রক্ত নেওয়া হবে। ১ ডোজ ভিটামিন 'এ' খাওয়ার ৫ ঘন্টা পর আবারো ১ মি:লি. রক্ত নেওয়া হবে। এই ৫ ঘন্টা সময়ে আপনি ও আপনার শিশু আমাদের উপকেন্দ্রে অবস্থান করবেন। আপনার শিশুকে ভিটামিন 'এ' বিহীন খাবার দেওয়া হবে। আপনাকেও জলযোগ করানো হবে। এই পরীক্ষায় আপনার শিশুর অন্তর্ভুক্ত হওয়ার সম্ভাবনা ২৫%।

৩। এই ৯ সপ্তাহ ধরে আমাদের অবেষনা-কর্মী আপনার সাথে আবশ্যকীয় যোগাযোগ রাখবেন। আসে ২ বার আপনার শিশুর পূর্ববর্তী ২৪ ঘন্টার খাদ্য তালিকা, পূর্বকর্তী সময়ে অসুখ বিসুখের তথ্যাদি সংগ্রহ করবে। এই সময় আপনার শিশুর এমন কোন অসুখ যথা রাতকানা, হাম, নিউমোনিয়া, ডায়রিয়া যা ভিটামিন 'এ' স্বাভাবিকভাবে প্রতিরক্ষন হলে উক্ত শিশুকে অবেষনা থেকে প্রত্যাহার করা হতে পারে। বিশেষজ্ঞ চিকিৎসকের সুপারিশ মোতাবেক তাদের উচ্চমাত্রার ভিটামিন 'এ' খাওয়ানো হবে। মলা মাছের ব্যাপারে আপনার অভিজ্ঞতা যাচাই করার জন্য অবেষনাকর্মী আপনার সাক্ষাৎকার গ্রহন করবে এবং আপনি উপকেন্দ্রে থাকার সময় দলবদ্ধ ভাবে আলোচনা করবে।

৪। অবেষনাকালীন সময়ে আমাদের চীফ মেডিকেল অফিসারের পরামর্শ ব্যাতিরেকে আপনার শিশুকে কোনরূপ বাড়তি ভিটামিন 'এ', মালটি ভিটামিন মিরাপ অথবা খনিজ মিরাপ খাওয়াবেন না। ৯ সপ্তাহের অবেষনা শেষে অবশিষ্টদের আমরা ভিটামিন 'এ' খাওয়াবো।

৫। ৯ সপ্তাহ অবেষনা শেষে আমরা পুনরায় শিশুর ওজন, উচ্চতা

বাচ্চার ন্যায় পরিমাপ করবো। রক্তে ভিটামিন 'এ' লৌহ ও প্রদাহ রোগের নির্দেশক আমিষ পরিমাপের জন্যে একজন অভিজ্ঞ ডাক্তার আপনার শিশুর শিরা থেকে ৫.০ মি.লি (১ চা চামুচের কম) রক্ত সংগ্রহ করবে। পূর্বের ন্যায় আবারো প্রতি দল থেকে ১/৪ শিশুর রিলেটিভ ডোজ রেসপন্স টেস্ট করা হবে। পরিমিত ১ ডোজ ভিটামিন 'এ' খাবার ৫ ঘন্টা পর অতিরিক্ত এক মি.লি. রক্ত নেওয়া হবে। আপনার শিশুর এই দলে অর্ন্তভুক্ত হওয়ার সম্ভাবনা ২৫%।

৬। রক্ত নেওয়ার সময় সূচের কারনে সামান্য ব্যথা হতে পারে, সূচ প্রবেশের স্থানে চামড়া সামান্য বিবর্ণ হতে পারে এবং রোগ সংক্রমনের ক্ষীন সম্ভাবনা থাকতে পারে। তাছাড়া ৯ সপ্তাহের মধ্যে ৪-৫ বার সর্বমোট ২১ মি.লি. রক্ত নেওয়াতে কোন ক্ষতির সম্ভাবনা নেই। আমরা জীবাণুমুক্ত এককালীন ব্যবহার যোগ্য সিরিঞ্জ ও সূচ ব্যবহার করবো যাতে এ ধরনের সম্ভাবনা না হয়। এই গবেষণায় অংশ গ্রহণে আপনার শিশুর ভিটামিন 'এ' র অভাব চিহ্নিতকরণ, অন্যান্য রোগ বালাই নির্ধারণ ও চিকিৎসা এবং সম্ভাব্য পুষ্টি উন্নয়ন হতে পারে।

৭। আপনার নিকট থেকে সংগৃহীত যাবতীয় তথ্যাদি গোপন রাখা হবে, শুধু গবেষক বৃন্দ তথ্যসমূহই অবগত থাকবেন। আপনার শিশুর কোন স্বাস্থ্য সমস্যা, চিকিৎসা, রক্ত ও পায়খানা পরীক্ষার ফলাফল সমূহ আপনি জানতে পারবেন। রক্ত পরীক্ষা সময় সাপেক্ষ ব্যাপার তবুও আমরা তাড়াতাড়ি ফলাফল পাওয়ার চেষ্টা করবো।

৮। এই গবেষণায় অংশ গ্রহণ একান্তই আপনার ইচ্ছাধীন। আপনার শিশু অংশগ্রহণ করবে কিনা তার পক্ষে/বিপক্ষে আপনিই একমাত্র সিদ্ধান্ত নেওয়ার মালিক। যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করে নিতে পারেন। আমাদের এই গবেষণায় আপনার শিশুর অংশগ্রহণে যদি রাজী না থাকেন, সম্মতি দেওয়ার পরও তা প্রত্যাহার করে নেন তাতে আপনার কোন ক্ষতি বা জরিমানার সম্ভাবনা নাই।

৯। আমাদের এই গবেষণা সম্পর্কে, আপনার শিশুর স্বাস্থ্য সম্পর্কে, পরীক্ষাগারের ফলাফল সম্পর্কে, আপনি আমাদের চিকিৎসক এবং গবেষণা-কর্মীর নিকট যে কোন বিষয় জানতে চাইতে পারেন।

এই গবেষণার প্রধান গবেষক জনাব এম.এ ওয়াহেদ (টেলিফোন ৮৮-১১৭৫১) এর সংগে আপনি যোগাযোগ করতে পারেন।

আপনার শিশুকে যদি আমাদের এই গবেষণা কার্যক্রমে অংশগ্রহনে রাজী থাকেন তাহলে নীচে স্বাক্ষর করুন অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ দিন।

আপনার সহযোগিতার জন্য ধন্যবাদ।

পিতা-মাতা / অভিভাবকের নাম

পিতা-মাতা / অভিভাবকের সই
বা বাম বৃদ্ধাঙ্গুলীর ছাপ

তারিখ:

গবেষক / প্রতিনিধির নাম

গবেষক / প্রতিনিধির স্বাক্ষর

তারিখ:

স্বাক্ষীর নাম

স্বাক্ষীর স্বাক্ষর

তারিখ:

APPENDIX – 9

আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র (ICDDR,B)

ঐচ্ছিক সম্মতিপত্র - গবেষনার অন্তর্ভুক্তির জন্য বাচ্চাই।

গবেষনার বিষয়: বাংলাদেশে শিশুদের রক্তে ভিটামিন-এ র মাত্রা উন্নয়নে ভিটামিন-এ সমৃদ্ধ ছোট মাছের ভূমিকা।

গবেষনার নং: ২০০২-০২৫

গবেষক বৃন্দ: এম,এ ওয়াহেদ, কাউজা কংকসন্তাক, শকুন্তলা থিলস্টেড

-সংস্থা: ১. পুষ্টি ও প্রানরসায়নের প্রধান, আন্তর্জাতিক উদরাময় গবেষনাকেন্দ্র।

2. পুষ্টি গবেষনা বিভাগ, রাজকিয় পশু ও কৃষি বিশ্ববিদ্যালয় কোপেন হেগেন, ডেনমার্ক।

ভিটামিন 'এ' এর অভাব বাংলাদেশে একটি মারাত্মক খাদ্য সমস্যা। যদি এই অভাব চিহ্নিত করা না হয় এবং তার ফলপ্রসূ চিকিৎসা করা না হয় তবে এর জন্য রাতকানা বা অন্যান্য চক্ষু রোগ এমনকি পূর্ন অন্ধত্বের কারন ও হতে পারে। উপরিন্তু ভিটামিন 'এ' অভাবগ্রস্থ ব্যক্তি সংক্রামক রোগের বিরুদ্ধে প্রকৃত প্রতিরোধ গড়তে পারে না।

আমাদের পূর্ব গবেষনা থেকে আমরা জানি যে, আপনার এলাকার অর্ধেকের ও বেশী স্কুলগামী ছেলে মেয়েরা নূন্যতম ভিটামিন 'এ' এর অভাবে ভুগছে। যদিও আপাত: দৃষ্টিতে এ ধরনের অভাব ধরা পড়ে না। আপনার ৩-৭ বছর বয়সী সন্তানের এ ধরনের অভাব থাকতেও পারে। কোপেনহেগেনস্থ রয়াল ভেটেরিনারী এন্ড এগ্রিকালচারাল বিশ্ববিদ্যালয় এর সাথে আমরা ICDDR,B থেকে যৌথভাবে একটি গবেষনা পরিচালনা করছি।

এই গবেষনার প্রধান উদ্দেশ্য হচ্ছে যে, সব ছেলেমেয়েদের নূন্যতম ভিটামিন 'এ'র অভাব রয়েছে তাদের শরীরের ভিটামিন 'এ'এর পরিমান বাড়ানোর পদ্ধতি পরিক্ষা করা।

৪। আমাদের চিকিৎসকের সংগে ছাড়া-পরামর্শ ব্যতিরেকে আপনার শিশুকে গবেষনাকালীন কোন প্রকার ভিটামিন 'এ' ও খনিজ পদার্থ সমৃদ্ধ খাবার খেতে দিবেন না।

৫। রক্ত পরীক্ষা সময় সাপেক্ষ ব্যাপার। রক্ত পরীক্ষার ফলাফল পাওয়াসাপেই আমরা আপনার নিকট আসবো :

(ক) শিশুর রক্তে ভিটামিন 'এ'র পরিমান কম (০.৩৫ মাইক্রো গ্রাম) থাকলে তাকে উচ্চ মাত্রার ভিটামিন 'এ' দ্বারা চিকিৎসা ব্যবস্থা দেয়া হবে।

(খ) পরিমান নূন্যতম (০.৩৫-০.৭০ মাইক্রো গ্রাম) এ শিশুকে পরবর্তী গবেষনায় অংশগ্রহনের জন্য আপনার অনুমতি চাওয়া হবে।

(গ) পরিমান সন্তোষজনক থাকলে ঐ শিশুর বাড়তি ভিটামিনের প্রয়োজন নাই এবং আমাদের গবেষনায় তাকে অন্তর্ভুক্ত করা হবে না।

৬। আপনার নিকট থেকে সংগৃহীত যাবতীয় তথ্যাদি গোপন রাখা হবে। শুধু গবেষক বৃন্দ তথ্য সমূহ অবগত থাকবেন। আপনার শিশুর কোন স্বাস্থ্য সমস্যা, চিকিৎসা, রক্ত ও পায়খানা পরীক্ষার ফলাফল সমূহ আপনি জানতে পারবেন।

৭। এই গবেষনায় অংশগ্রহনের জন্য আপনার শিশুর কোন ঝুঁকি নাই তবে রক্ত নেওয়ার সময় সামান্য ব্যাথা হতে পারে। এই গবেষনায় অংশগ্রহনে কিছু লাভবান হতে পারে; যেমন রক্তে ভিটামিন 'এ'র অভাব আছে কিনা তার সনাক্ত হওয়া, কৃমি মুক্ত হওয়ার জন্য চিকিৎসা, আনুসাংগিক কোন রোগ সমূহ নির্নয় বা চিকিৎসা এবং চিকিৎসকের উপদেশ পাওয়া।

৮। এই গবেষনায় অংশগ্রহন একান্তই আপনার ইচ্ছাধীন, আপনার শিশু অংশগ্রহন করবে কিনা তার পক্ষে-বিপক্ষে আপনিই একমাত্র সিদ্ধান্ত নেওয়ার মালিক। যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করে নিতে পারেন। আমাদের এই গবেষনায় আপনার শিশুর অংশগ্রহনে যদি

যেহেতু আপনার পরিবারে ৩-৭ বছর বয়সী শিশু রয়েছে, আমরা আমাদের গবেষনায় তার অর্ন্তভুক্তির জন্য আপনার সম্মতি কামনা করছি। যদি আপনি রাজী থাকেন, আমরা নিম্নলিখিত কার্যক্রম পরিচালিত করবো:

১। আমরা আপনার নিকট থেকে আপনার আর্থসামাজিক অবস্থা, আপনার শিশুর খাদ্য তালিকা, খাদ্যে ভিটামিন 'এ' জাতীয় খাবার কতটা থাকে, এবং সম্প্রতি সে কোন রোগে ভুগে থাকলে সে সবের তথ্য সংগ্রহ করবো। আমাদের কর্মী এ ধরনের তথ্যাদি সংগ্রহের জন্য আপনার সাথে আলাপ-আলোচনা করবে এবং তাতে এক ঘন্টারও কম সময় লাগবে।

গবেষনা কর্মী আপনার শিশুর ওজন, উচ্চতা এবং বাহুর মাপ নিবে। আমাদের চিকিৎসক আপনার শিশুর সাম্প্রতিক কোন রোগ-বালাই আছে কিনা তা পরীক্ষা করবেন। যদি কোন রোগ থাকে, তাকে আপনার এলাকায় অবস্থিত আমাদের উপকেন্দ্র থেকে চিকিৎসা দেয়া হবে। অথবা নিকটস্থ কোন স্বাস্থ্য কেন্দ্রে প্রয়োজনীয় চিকিৎসার জন্য উপদেশ দিবেন।

২। আপনার এলাকায় অধিকাংশ শিশুই কৃমি আক্রান্ত। আমরা আপনার শিশুর পায়খানার নমুনা সংগ্রহ করবো। তাতে কৃমি আছে কিনা বা থাকলে কি পরিমান আছে তা পরীক্ষা করবো। আমরা আপনার শিশুকে কৃমি নাশক প্রয়োজনীয় ঔষধ দিব। এবং ঐ ঔষধটি ২ সপ্তাহ পরে আবারো দেয়া হবে। আমরা আশা করি আপনার শিশু এ ধরনের চিকিৎসায় সম্পূর্নভাবে কৃমি মুক্ত হবে।

৩। ভিটামিন 'এ', লৌহ এবং প্রদাহ রোগের নির্দেশক আমিষ পরিমাপের জন্য আমরা কতিপয় ল্যাবরেটরী পরীক্ষা করবো। এ জন্য একজন অভিজ্ঞ চিকিৎসক শিশুর শিরা থেকে ৪.০ মিলি. (এক চা চামচের ও কম) রক্ত সংগ্রহ করবে। রক্ত নেওয়ার সময় সূচের কারনে সামান্য ব্যাথা অনুভূত হতে পারে, সূচ প্রবেশের স্থানে চামড়া সামান্য বিবর্ন হতে পারে, এ ছাড়া এই পরিমান রক্ত নেওয়ার জন্য আপনার শিশুর কোন ক্ষতির আশংকা নাই। এ জাতীয় সমস্যা প্রতিরোধের জন্য আমরা একবারীন ব্যবহার্য জীবানুমুক্ত সিরিঞ্জ ও সূচ ব্যবহার করবো।

রাজী না থাকেন, সম্মতি দেওয়ার পরও তা প্রত্যাহার করে নেন তাতে আপনার কোন ক্ষতি বা জরিমানার সম্ভাবনা নাই।

২। আমাদের এই গবেষনা সম্পর্কে আপনার শিশুর স্বাস্থ্য সম্পর্কে, পরীক্ষাগারের ফলাফল সম্পর্কে আপনি আমাদের চিকিৎসক এবং গবেষনা কর্মীর নিকট যে কোন বিষয় জানতে চাইতে পারেন।

উপরন্তু এই গবেষনার প্রধান গবেষক জনাব ডাঃ ও. ওয়াহেদ (টেলিফোন ৮৮১১৭৫১) এর সংগে আপনি যোগাযোগ করতে পারেন।

আপনার শিশুকে যদি আমাদের এই গবেষনা কার্যক্রমে অংশগ্রহনে রাজী থাকেন তাহলে নীচে স্বাক্ষর করুন অথবা বাম বৃদ্ধাংগুলীর ছাপ দিন।

আপনার সহযোগিতার জন্য ধন্যবাদ।

পিতা মাতা/অভিভাবকের নাম

পিতা মাতা/অভিভাবকের সহি বা বাম বৃদ্ধাংগুলীর ছাপ

তারিখ:

গবেষক/প্রতিনিধির নাম

গবেষক/প্রতিনিধির স্বাক্ষর

তারিখ:

স্বাক্ষীর নাম

স্বাক্ষীর স্বাক্ষর

তারিখ:

CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8826050, 8811568, 8811686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Memorandum

2 December 2001

To : Mr. M. A. Wahed
Laboratory Sciences Division

From: Professor Mahmudur Rahman
Chairman, Ethical Review Committee (ERC)

Sub: **Protocol # 2001-025**

Thank you for your protocol # 2001-025 entitled "The efficacy of Vitamin A rich small fish in improving Vitamin A status in children in Bangladesh", which the ERC considered in its meeting held on 28th November 2001. After review and discussion in the meeting, the Committee made the following observations on the protocol:

a) The study will not use many small fishes; rather a specific kind (Mola fish) will be used. Therefore, the title seems to be confusing. The appropriate title would have been "The efficacy of feeding a small fish .....".

b) The basis of feeding for 9 weeks was not clear.

c) It is assumed that Vitamin A is given normally by the Government VAC programme every six months to all children <six years, and also during the NID, ARI and diarrhoea episodes. Would the investigators deviate from the current Government policy?

d) It was considered that the study should be done in a crossover design. That is those children with no/low Vitamin A diet should get Vitamin A capsules at the end of the study. This should also be clearly mentioned in the consent forms.

e) The protocol has too many objectives and the sample size was calculated only on the basis of serum retinal. The Committee expressed concern that with this sample size, the effect of other variables such as iron status, morbidity, anthropometry, behavioural changes cannot be looked into.

f) The consent form could be made more simple and understandable for the common people. Further, In the Bangla consent form protocol title and PI's name have been mentioned in English. In the consent form (point # 10), it has been mentioned that no penalty will be imposed for withdrawal of from the study, which was considered to be inappropriate. Indeed participation in the study is completely voluntary.

g) There are many spelling mistakes/typos. In the English consent form, (2nd part), points 8 and 9 were found to be missing.

You are, therefore, advised to address the above issues and to submit the modified version of the protocol incorporating the above observations for consideration of the Chair.

Thank you.

Copy: Acting Head
Laboratory Sciences Division

To : Mr B R Saha

From : M A Wahed

Subject: Bangla version of questionnaire

Enclosed pls find the Bangla version of questionnaire. These are awaiting pre-test and after that we shall get these finalized.
I am enclosing these, in case there are points raised by any ERC members.

Thanking you.

সংযোজন-১

আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র বাংলাদেশ

মাছের মাধ্যমে শিশুদের ভিটামিন এ অবস্থা পর্যবেক্ষন প্রকল্প মিরপুর

বাসগৃহ নিবন্ধীকরন ফরম

(যদি বাসগৃহে একজন ও ৩-৭ বছরের শিশু থাকে তবে নিবন্ধীকরন করতে হবে)

১। বাসগৃহের পরিচিতি নং ☐☐☐☐

২। পরিবার প্রধানের নাম

৩। পরিবার প্রধানের পেশা

৪। পূর্ন ঠিকানা

৫। ক্যাপ্স কোড ☐

৬। সাক্ষাৎকারের তারিখ ☐☐☐☐ ২০০১

৭। ১০ বছরের নীচের ছেলে মেয়ের তালিকা

ক্রমিক নং	নাম	লিঙ্গ ১=পুরুষ ২=মহিলা	জন্ম তারিখ ১=আরবী ২=বাংলা ৩=ইং(খৃষ্টী) দিন-মাস-বছর	বয়স বছর	বয়স মাস	মাছ খাবার অভ্যাস কিরূপ কি মাছ খায় ১=ছোট মাছ ২=বড় মাছ ৩=ছোট ও বড় মাছ ৪=না	ভিটামিন এ ক্যাপসুল কি খেয়েছে ১=হ্যাঁ-৬ মাসের মধ্যে ২=হ্যাঁ ৬ মাসের পরে ৩=না	আগামী ভিটামিন খেয়েছি কি? ১=হ্যাঁ ৬ মাসের মধ্যে ২=হ্যাঁ ৬ মাসের পরে ৩=না	বর্তমান রোগের বিবরন (কোড তালিকা দেখুন)	রোগের সময়কাল ৭ দিন	শিশুর পরিচিতি নং

সংযোজন-2

শিশুর স্বাস্থ্য পরীক্ষা ফরম

শিশুর নাম:

প্রাথমিক তথ্য

১। সাক্ষাতের তারিখ ☐☐ ☐☐ ☐☐☐☐ (দিন মাস বছর)

২। শিশুর স্টাডি নং ☐☐☐

৩। শিশুর পরিচিতি নং ☐☐☐

৪। বাসগৃহের পরিচিতি নং ☐☐☐

৫। ক্যাম্পের নাম _____ কোড ☐☐

৬। উত্তরদাতা / দাত্রী ১। মা ২। বাবা ৩। অন্যান্য _____

৭। লিঙ্গ ১। পুরুষ ২। মহিলা

৮। জন্ম তারিখ (বাংলা / ইংরেজী) ☐☐ ☐☐ ☐☐☐☐ (দিন মাস বছর) ৯। জন্ম তারিখের যাচাই ☐ ১ ২ ৩

১০। বয়স ☐☐ (বছর মাস)

১১। উচ্চতা (সে.মি) ☐☐.☐

১২। ওজন (কেজি) ☐☐.☐

১৩। মধ্য বাহুর মাপ (মিমি মিমি) ☐☐ ☐

টিকাদান

১৪। ডিপিটি ১	১ = হ্যাঁ	২ = না
১৫। ডিপিটি ২	১ = হ্যাঁ	২ = না
১৬। ডিপিটি ৩	১ = হ্যাঁ	২ = না
১৭। ওপিভি ১	১ = হ্যাঁ	২ = না
১৮। ওপিভি ২	১ = হ্যাঁ	২ = না
১৯। ওপিভি ৩	১ = হ্যাঁ	২ = না
২০। হাম	১ = হ্যাঁ	২ = না
২১। BCG	১ = হ্যাঁ	২ = না

শিশুর পরিচিতি নং ☐☐☐ শিশুর স্টাডি নং ☐☐☐

অবাবন পরীক্ষা

২২। উপস্থিতির সময়	১= স্বাভাবিক	২ = অসুস্থ	
২৩। ক্যাকাশে ভাব	১ = নাই	২ = আছে	
২৪। চামড়ার অবস্থা	১ = স্বাভাবিক	২ = অস্বাভাবিক	৩ = অন্যান্য

২৫। নাড়ির গতি ☐☐☐ / মিনিট

২৬। তাপমাত্রা |_|_|_| |_| ০ সেন্টিগ্রেড

২৭। শ্বাস-প্রশ্বাস |_|_| / মিনিট

২৮। হাতে-পায়ে পানি

২৯। রোগের বিবরণ ১ = নাই ২ = জটিল ডায়রিয়া ৩ = মারাত্মক ডায়রিয়া ৪ = য

৫ = পুরাতন শ্বাসকষ্ট ৬ = হাঁপানী ৭ = বহুমূত্র ধরন ১

৮ = হেপাটাইটিস বি/জন্ডিস ৯ = ত্রুটিপূর্ণ হৃদযন্ত্র

১০ = হাম ১১ = শ্বাসযন্ত্রের প্রদাহজনিত রোগ

১২ = চর্মরোগ ১৩ = অপুষ্টি (পি ই এম)

১৪ = অন্যান্য

পর্যায়ক্রমিক পরীক্ষা

৩০। হৃদযন্ত্র ( ) ১ = স্বাভাবিক ২ = অস্বাভাবিক শব্দ

৩১। ফুসফুস ১ = পরিষ্কার ২ = বাঁশির শব্দ (এমনিতেই শোনা যায়)

৩ = গরগরানী ৪ = বাঁশির শব্দ (পরীক্ষার মাধ্যমে)

৩২। পেটের আকার ১ = স্বাভাবিক ২ = ফোলা

৩৩। পেটের সংবেদনশীলতা ১ = নাই ২ = আছে

৩৪। চোখের পরীক্ষা : ভিটামিন 'এ' এর অভাবের চিহ্নসমূহ

১ = এক্স এন ২ = এক্স ওয়ান এ ৩ = এক্স ওয়ান বি

৪ = এক্স টু ৫ = এক্স থ্রি এ ৬ = এক্স থ্রি বি

৭ = এক্স এস ৮ = এক্স এফ ৯ = স্বাভাবিক

নমুনা সংগ্রহ

৩৫। মলের নমুনা সংগ্রহণ : ১ = হ্যাঁ ২ = না

৩৬। প্রথম কৃমি নাশক ঔষধ দেওয়া হয়েছে ১ = হ্যাঁ ২ = না

যদি দেওয়া হয়ে থাকে ৩৭। তারিখ |_|_|_| দিন মাস বৎসর

৩৮। ২য় কৃমি নাশক ঔষধ দেওয়া হয়েছে ১ = হ্যাঁ ২ = না

যদি দেওয়া হয়ে থাকে ৩৯। তারিখ |_|_|_| DD MM YY

৪০। রক্তের নমুনা সংগ্রহ করা হয়েছে ১ = হ্যাঁ ২ = না

সংগ্রহ করা হয়ে থাকলে ৪১। তারিখ |_|_|_| দিন মাস বৎসর

৪২। আর টি আর এর জন্য রক্তের নমুনা সংগ্রহ করা হয়েছে ১ = হ্যাঁ ২ = না

হয়ে থাকলে ৪৩। ১ম নমুনা সংগ্রহের সময় |_| | ঘন্টা

৪৪। ২য় নমুনা সংগ্রহের সময় |_| | ঘন্টা

তারিখ _ চিকিৎসক ______________

অনুমোদন- ৩

আন্তর্জাতিক উদরাময় গবেষণা কেন্দ্র, বাংলাদেশ

আদরের স্বাস্থ্যসেবা শিশুদের চিকিৎসাধীন মা'র অবস্থা পর্যবেক্ষণ প্রকল্প, মিরপুর

আংশিক- আনুষ্ঠানিক ফরম-১

১. সাক্ষাৎকার গ্রহণের তারিখ |__|__|__|__|__|__|
দিন মাস বছর

২. বাড়ীর পরিচিতি নং |__|__|__|__|__|

৩. পরিবার প্রধানের নাম _____

৪. পরিবার প্রধানের প্রধান পেশা _____

৫. বিস্তারিত ঠিকানা _____ ক্যাম্প হলে |__|

৬. শিশুর নাম ও পরিচিতি নং _____ / |__|__|__|

শিশুর নাম ও পরিচিতি নং _____ / |__|__|__|

শিশুর নাম ও পরিচিতি নং _____ / |__|__|__|

শিশুর নাম ও পরিচিতি নং _____ / |__|__|__|

আর্থ-সামাজিক জরিপ-2

বাড়ীর পরিচিতি নং ☐☐☐ ক্যাম্প কোড ☐☐

(কোড তালিকা অনুসরন করতে হবে)

ক্রমিক নং	পরিবারের সদস্যের নাম	লিঙ্গ 1 = পুরুষ 2 = মহিলা	বয়স		পরিবার প্রধানের সাথে সম্পর্ক		বৈবাহিক অবস্থা		পেশা		শিক্ষাগত যোগ্যতা		বর্তমানে স্কুলে পড়ে কিনা 1 = হ্যাঁ 2 = না	শিক্ষা- বন্ধ থাকলে সর্বোচ্চ কত বছর পড়াশোনা করেছে
			বছর	মাস		কোড		কোড		কোড		কোড		

3/-

আর্থ সামাজিক ফরম-৩

কোড তালিকা

ক্যাম্প কোড

১ = দুর্গাবল ক্যাউত
2 = বড় টিটি
৩ = ওল্ড বিল্ডিং
৪ = বহুমত

বৈবাহিক অবস্থা

১ = অবিবাহিত
2 = বিবাহিত
৩ = তালাক প্রাপ্ত
৪ = বিচ্ছিন্ন
৫ = বিপত্নীক/বিধবা

শিক্ষাগত অবস্থা

১ = পড়তে পারে
2 = নাম লিখতে পারে
৩ = লিখতে পারে
৪ = লিখতে ও পড়তে পারে
৫ = লিখতে বা পড়তে পারে না

পরিবার প্রধানের সহিত সম্পর্ক

১ = পরিবার প্রধান
2 = ১ম স্ত্রী
৩ = ২য় স্ত্রী
৪ = ৩য় স্ত্রী
৫ = স্বামী
৬ = পুত্র
৭ = কন্যা
৮ = ভাই
৯ = বোন
১০ = ভাতিজা/ভাতিজী
১১ = পিতা
১২ = মাতা
১৩ = চাচা
১৪ = চাচী
১৫ = অন্যান্য

পেশা

0 = শিশু (পাঁচ বছরের নিচে)
১ = ছাত্র
2 = গৃহিনী
৩ = চাকুরীজীবি
৪ = দিনমজুর
৫ = দক্ষ শ্রমিক (মেকানিক রেডিও, টিভি, ফ্যান, কারখানা শ্রমিক)
৬ = রিকসা/ভ্যান/ঠেলা গাড়ী-চালক
৭ = গাড়ী-চালক
৮ = বাস কন্ডাকটর/হেলপার
৯ = ব্যবসায়ী
১০ = বেকার
১১ = শিক্ষক
৯৯ = বলতে পারে না জানে না
৮৮ = অন্যান্য (বিবরণ সহ)

শিক্ষা

0 = কোন শিক্ষা নাই
১-৯ = শ্রেণীগত যোগ্যতা
১০ = মাধ্যমিক
১১ = উচ্চ মাধ্যমিক
১২ = স্নাতক
১৩ = স্নাতকোত্তর

পার্থ আমাড়িকা ফরম - ৪

বাড়ীর পরিচিতি নং   ক্যাম্প কোড

১। ব্যবহৃত পানির উৎস

ক) পানীয়; ১ = পৌরসভা সরবরাহ, ২ = নিজস্ব প্লাস্টিক পাইপ, ৩ = টিউবওয়েল, ৪ = কুয়া, ৫ = অন্যান্য

খ) রান্না

গ) বাসনপত্র

ঘ) গোসল

ঙ) অন্যান্য

২। আপনি কি নিম্নকাজের জন্য পানি সংরক্ষণ করেন?

ক) পানীয়	১ = হ্যাঁ	২ = না
খ) রান্না	১ = হ্যাঁ	২ = না
গ) বাসনপত্র	১ = হ্যাঁ	২ = না
ঘ) গোসল	১ = হ্যাঁ	২ = না
ঙ) অন্যান্য	১ = হ্যাঁ	২ = না

৩ক: পানি সংরক্ষণের জন্য ব্যবহৃত পাত্রের ধরন

১. ছোট বালতি	১ = হ্যাঁ	২ = না
২. মাঝারী বালতি	১ = হ্যাঁ	২ = না
৩. বড় বালতি	১ = হ্যাঁ	২ = না
৪. জগ	১ = হ্যাঁ	২ = না
৫. ছোট পাত্র	১ = হ্যাঁ	২ = না
৬. মাঝারী পাত্র	১ = হ্যাঁ	২ = না
৭. বড় পাত্র	১ = হ্যাঁ	২ = না
৮. অন্যান্য	১ = হ্যাঁ	২ = না

আর্থ-সামাজিক অবস্থা - ৬

বাড়ী পরিচিতি নং; স্যাম্পল কোড

৩খ: ২৪ ঘন্টায় আপনি কতবার পানি সংগ্রহ করেন? (সব ধরনের কাজের জন্য) |_|_|

৪। নিজস্ব তৈয়ারী পানির লাইন কোনটার কাছাকাছি ১= পায়খানা, ২= নর্দমা, ৩= অন্যান্য
৪ = প্রযোজ্য নয়

৫। বয়স অনুপাতে নিম্নের কোন ধরনের পায়খানা ব্যবহার করেন?

ক) পুরুষ (৭ বৎসরের উপরে)	নির্দিষ্ট নয় =১,	খোলা =২,	স্বাস্থ্যসম্মত =৩,	অন্য =৪
খ) মহিলা (" " ")	"	"	"	"
গ) ৫-৭ বৎসরের ছেলেমেয়ে	"	"	"	"
ঘ) ৩-৪ বৎসরের ছেলেমেয়ে	"	"	"	"
ঙ) ৩ বৎসরের নীচের ছেলেমেয়ে	"	"	"	"

৬। পরিবারের সদস্যরা কি নিজস্ব না যৌথ পায়খানা ব্যবহার করেন?
১ = নিজস্ব , ২ = যৌথ

৭। পায়খানায় জুতার ব্যবহার;

ক) তালিকাভুক্ত শিশু কি পায়খানায় জুতা ব্যবহার করে?

শিশুর পরিচিতি নং |_|_|_| হ্যাঁ =১ না=২
শিশুর পরিচিতি নং |_|_|_| হ্যাঁ =১ না=২
শিশুর পরিচিতি নং |_|_|_| হ্যাঁ =১ না=২

খ) পরিবারে মোট সদস্য সংখ্যা : |__|

গ) মোট কতজন সদস্য পায়খানায় জুতা ব্যবহার করেন |_|_|

আর্থ সামাজিক ফরম - ৬

ক্যাম্প কোড

বাড়ীর পরিচিতি নং

৮। হাত ধোয়ার অভ্যাসের ধরন:

উদ্দেশ্য	কি দ্বারা	কিভাবে	পানির উৎস
ক) খাবার তৈয়ারীর পূর্বে			
খ) খাবার গ্রহনের পূর্বে			
গ) পায়খানার পর			
ঘ) বাচ্চাকে শৌচ করানোর পর			
ঙ) অন্যান্য			

কিসের দ্বারা : না = 0, পানি = ১, মাটি = 2, ছাই = ৩, সাবান = ৪

কিভাবে : না = 0, বামহাত = ১, ডানহাত = 2, দুইহাত = ৩,

পানির উৎস : সরকারী সরবরাহকৃত পানি = ১, টিউবওয়েল = ৩, নিজস্ব ব্যবস্থাপনায় প্লাস্টিক পাইপ = 2, কুপ = ৪, অন্যান্য = ৫

৯ক : খাবার পানির পাত্র কি ঢাকা থাকে? হ্যাঁ = ১ না = 2

৯খ : অন্যান্য খাবার কি ঢাকা থাকে? হ্যাঁ = ১ না = 2

৯গ : রান্নাকরা খাবার কি ঢাকা থাকে? হ্যাঁ = ১ না = 2

১০। বাড়ী-ঘরের ধরন :

ক) বেড়া কিসের তৈয়ারী : ইট = ১ বাঁশ = 2, টিন/ঢেউটিন = ৩, হার্ডবোর্ড/পলিথিন = ৪, মাটি = ৫, খড় = ৬.

খ) মেঝে কিসের তৈয়ারী পাকা = ১, বাঁশ = 2 টিন = ৩, হার্ডবোর্ড/পলিথিন = ৪, মাটি = ৫, ইট = ৬,

গ) ছাদ কিসের তৈয়ারী পাকা = ১, বাঁশ = 2, টিন = ৩, হার্ডবোর্ড/পলিথিন = ৪

ঘ) শোবার ঘর ও রান্নাঘর কি একসংগে সংযোজিত? হ্যাঁ = ১, না = 2

আর্থ- সামাজিক অবস্থা-৭

বাড়ীর পরিচিতি নং; ক্যাম্প কোড

১১। বসবাসরত গৃহের জানালা কয়টি?

১২। গৃহের কক্ষ কয়টি?

১৩। গৃহের আশে পাশের খোলা নর্দমার বিবরণ

ক) খোলা নর্দমা কি শয়ন কক্ষের পাশে? হ্যাঁ =১ না = 2

খ) রান্নাঘরের পাশে কি খোলা নর্দমা আছে? হ্যাঁ =১, না = 2

১৪। কি ধরনের গৃহপালিত পশুপাখী আছে?

১ = ছাগল, 2 = মুরগী/হাঁস ৩ = বিড়াল, ৪ = কুকুর ৫ = গরু, ৬ = কবুতর, ৭ = অন্যান্য

১৫। সর্বমোট মাসিক আয় (সব ধরনের আয়) ☐☐☐☐ টাকা

১৬। গৃহের মালিকানাস্বত্ব: ভাড়াবাড়ী =১ কত টাকা ভাড়া ☐☐☐☐ টাকা

নিজস্ব = 2

নিজস্ব বা ভাড়া কোনটাই নয় =৪

১৭। বাসগৃহের জিনিসপত্রের তালিকা: (গোলচিহ্ন দিন)

১) খাট, ২) লেপ, ৩) তোষক, ৪) হারিকেন ৫) ঘড়ি ৬) চেয়ার/টেবিল

৭) আলমারী ৮) রেডিও ৯) টেলিভিশন ১০) সাইকেল ১১) শো-কেস

১২) বিদ্যু ১৩) অন্যান্য (বিবরণসহ)

১৮। কি দ্বারা রান্না করেন?

গ্যাস =১, জ্বালানী কাঠ =2, ঘুঁটে =৩, কেরোসিন = ৪

অন্যান্য = ৫,

সাক্ষাৎকার গ্রহণকারীর স্বাক্ষর কোড ☐

সংযোজন - ৪

২৪ ঘন্টার খাবার গ্রহনের তথ্যাদি

১। খানা পরিচিতি নং ____

২। শিশুর প্রাথমিক পরিচিতি নং ____

৩। শিশুর নাম _____

৪। বাসগৃহের পরিচিতি নং ____

৫। ক্যাম্পের নাম _____ কোড ____

৬। সাক্ষাৎকারের তারিখ ____ (দিন মাস বছর)

৭। উত্তরদাতা/দাত্রী ১। মা ২। বাবা ৩। অন্যান্য

গতকাল আপনি যে খাবার খেয়েছেন সে সম্পর্কে বলুন

৮। আপনি কি গতকাল স্বাভাবিক খাবার খেয়েছেন ?

হ্যাঁ, যথারীতি স্বাভাবিক ---------- ১ ----------→
না স্বাভাবিকের চাইতে একটু বেশী ---------- ২
না স্বাভাবিকের চাইতে কম ---------- ৩
বলতে পারে না ---------- ৯

৮ক। আপনি কেন স্বাভাবিক খাবার খান নাই ?

_____

_____

_____

_____

_____

৯। আপনি গতকাল কি কি ধরনের খাবার, পানীয় খেয়েছেন ?
(সকালে ঘুম থেকে উঠার পরের সময় থেকে তথ্য সংগ্রহ করতে হবে। স্বাভাবিক খাবার ছাড়াও হালকা খাবার পানীয় সবকিছু রেকর্ড করতে হবে)

২৪ ঘন্টার খাবার তালিকা।

সময়	খাদ্য তালিকা	খাদ্য কোড	পরিমান	খাদ্যের পরিমান প্রাপ্ত-পরিমান	পরিমাপের কোড

২০. আপনি কি গতকাল কোন প্রকার ভিটামিন বা ঐ জাতীয় ঔষধ খেয়েছেন ?
হ্যাঁ = ১ না = ২ জানি না = ৯

২০ক। আপনি বলতে পারেন কি ধরনের ভিটামিন খেয়েছেন ?
(উত্তরদাতা/দর্শককে প্যাকেট ছবি বা বোতল দেখানোর অনুরোধ করতে হবে)

২০খ। বিগত দুই সপ্তাহের মধ্যে আপনার কিন্তু কি ভিটামিন জাতীয় ঔষধ খেয়েছে?
হ্যাঁ=১ না = ২ জানি না = ৯

২০গ। যদি খেয়ে থাকে তবে কি আপনি সেগুলোর বর্ণনা দিতে পারবেন ?

২১। গতকাল আপনি রান্নায় কত কাপ বা কত বোতল রান্নার তেল ব্যবহার করেছেন?
বা চা-চামচ
মোট কাপের সংখ্যা |__|__| অথবা মোট কত বোতল |__|__|
জানি না = ৯৯ অথবা মোট কত চা-চামচ |__|__|

২২। কতজন লোক গতকালের রান্না করা খাবার খেয়েছে ?
বয়স্ক (১৮ বছরের উপরে) |__|__|
শিশু (১৮ বছরের নীচে) |__|__|

২৪ ঘন্টায় প্রাপ্ত তথ্যাদি (প্রতিবার মাঠ/বাড়ী পরিদর্শনের পর পূরন করতে হবে)

খাদ্য তালিকা	কোড	পরিমান	পরিমানের পরিমাপের তথ্য	কোড

সাক্ষাৎকার গ্রহনকারীর নাম

কোড

অ্যাপেন্ডিক্স - ৩

১। ক্যাম্প কোড

২। বাড়ীর নম্বর

৩। শিশুর বাছাইকৃত নম্বর

৪। বাচ্চার নাম :

৫। তারিখ

৬। সাক্ষাৎকার গ্রহণকারীর নাম : কোড

৭। উত্তরদাতার নাম : উত্তরদাতার সাথে সম্পর্ক :

খাদ্য তালিকা	খাদ্যের নাম	গত ২৪ ঘন্টায় কতটুকু ব্যবহৃত হয়েছে			আমিষের ব্যবহারের জন্য: ২৪ ঘন্টায়			D = প্রত্যহ M = ১-৬ দিন / সপ্তাহ M = ১-৩ দিন / মাস N = কখনো না
		সকালের নাস্তার পরিমান	দুপুরের পরিমান	রাতের পরিমান	S	M	L	D W M N
	ডাটা শাক							
	লালি শাক							
	পুঁই শাক							
	কলমী শাক							
	মুলা শাক							
	পালং শাক							
	হ্যাংলেচা							
	মিষ্টিকুমড়া শাক							
	লাউ শাক							
	মিষ্টি আলু শাক							
	সরিষার শাক							
	লুনতির শাক							

খাদ্য-তালিকা	নাম	গত 24 ঘন্টায় কতটুকু ব্যবহৃত হয়েছে			আমিষের ব্যবহার জন্য: 24 ঘন্টায় কতবার খেয়া হবে			D = প্রত্যহ M = ১-৩ দিন / সপ্তাহ N = কখনো না / মাস			
	সবজি	সকালের পরিমান	দুপুরের পরিমান	সন্ধ্যার পরিমান	S	M	L	D	W	M	N
	ডাটা-										
	কচুর										
	ঝিঙা										
	লাল ঝিঙা										
	বাঁধা কপি										
	বরবটি										
	পটল										
	করলা/উচ্ছা										
	চিচিঙ্গা										
	মিষ্টি কুমড়া										
	কাকরোল										
	ডুডুস										
*	ঢেঁড়শ										
	কচুর লতি										
	ফুল										
	পাঁকা পেপে										
	আনারস										
*	পাকা ঢেঁড়শ										
	মাছ										
	রুই										
X	রোয়াল মাছ										
	কাতলা										
	কাতল মাছ										

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.

Hypothesis to be Tested

An intake of 45g vitamin A rich small fish (mola, *Amblypharyngodon mola*) improves vitamin A status in Bangladeshi preschool children with marginal vitamin A deficiency.

Research questions

1. How effective is vitamin A rich small fish in improving vitamin A status in Bangladeshi preschool children?

2. Is iron status in these children affected by intake of vitamin A rich small fish?

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).

Overall aim

To determine the change in vitamin A status after supplementation of 45 g of vitamin A rich small fish, mola (500 RE) in a daily meal for 9 weeks, to marginally vitamin A deficient Bangladeshi preschool children (3-7 y), and compare it with the change in vitamin A status obtained after supplementation of 45 g of big fish, rui (*Labeo rohita*) containing no/little vitamin A and 45 g of big fish, rui with added retinyl palmitate (500 RE).

Specific objectives

- To assess the efficacy of vitamin rich small fish, mola in improving vitamin A status (based on serum retinol concentrations, results from the Relative Dose Response (RDR) method, serum retinol binding protein and prealbumin concentrations). This will be done by comparing the increase in vitaminA status after supplementation of mola fish with the increase in vitaminA status after supplementation of non/low vitamin A rich big fish, rui and supplementation of rui with added retinyl palmitate, in a daily meal for 9 weeks to marginally vitamin A deficient children (3-7 y) in a Dhaka slum.

- To investigate the impact of intake of vitamin A rich small fish on iron status (based on haemoglobin, serum ferritin and serum transferrin receptor) in children in comparison with that of non/low vitamin A rich big fish and non/low vitamin A rich big fish with added retinyl palmitate.

- To investigate whether the factors: **a)** nutrient intake during study (based on repeated 24 h recall interview), **b)** initial vitamin A and iron status, **c)** changes in iron status, **d)** markers of infection (based on C-reactive protein (CRP) and α-1 anti-chymotrypsin (ACT)), **e)** morbidity during study (based on incidence of infection such as diarrhoea, acute respiratory infections, fever, measles and night blindness), **f)** marker of protein energy malnutrition (based on prealbumin), **g)** initial and/or change in nutritional status (based on height, weight, MUAC) affect the change in vitamin A status after intake of vitamin A rich small fish in comparison with that of intake of non/low vitamin A rich big fish and non/low vitamin A rich big fish with added retinyl palmitate. In addition, if an effect is seen it will be investigated whether some subgroups benefit more than others from the intervention.

- To investigate qualitatively the mothers' perceptions of the effect of the mola supplementation on the general well being of the children as well as the acceptance of mola for habitual use among the children and their families.

- From the children screened for marginal vitamin A deficiency, assess and study the relationships between vitamin A and iron status indicators and **a)** the degree of parasitic infestation (based on *Ascaris lumbricoides*, *Trichuris trichuria*, hookworms, *Entamoeba histolytica*, *Giardia intestinalis*), **b)** markers of infection, **c)** marker of protein energy malnutrition, **d)** morbidity, **e)** nutritional status as well as **f)** socio-economic data of the households in a Dhaka slum. In addition to that assess and compare two methods of estimating groups at risk of vitamin A deficiency, dietary vitamin A inadequacy (based on a semi-quantitative dietary assessment of vitamin A intake) and serum retinol concentrations.

PROJECT 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: M. A. Wahed

Project Name: **The Efficacy of Vitamin A Rich Small Fish in Improving Vitamin A Status in Children in Bangladesh**

Total Budget: 203,287 US$ Beginning Date: 01.10.2001 Ending Date: 30.09.2004

Vitamin A deficiency (VAD) is a public health problem in many developing countries, including Bangladesh. Due to VAD, it is estimated that 14 million preschool children have eye damage and every year, 350,000 become blind, of whom 60 % die within few months. To reduce or eliminate VAD, food based strategies have received little attention although these are long term, sustainable and culturally accepted. In making use of food based strategies, the content as well as the bioavailability of nutrients in foods consumed must be taken into consideration.

Within food based strategies, there has been a lot of focus on green leafy vegetables and fruits. However, the bioavailability of vitamin A in these foods has been shown to be lower than previously thought and lower than foods rich in preformed vitamin A. There has been no focus on the role of small fish in combating vitamin A and micronutrient deficiencies. Small fish is easily available, relatively cheap and well-liked by most household members, including children. Few commonly consumed small fish species have been found to have extremely high contents of preformed vitamin A. For example, mola (*Amblypharyngodon mola*) contains 2,400 - 3,000 RE/100 g raw edible fish, 5 times higher than the green leafy vegetable, amaranth (*Amaranthus gengeticuss*). An intake of 45 g mola/d meets the RDA (500 RE/d) of vitamin A for 4-6 years old children (taking cooking loss into account). Children can easily consume the above amount of fish and even higher amounts in a single main meal. Small fish is also a rich source of animal protein and minerals such as calcium, iron and zinc.

No study has looked at the efficacy of a daily amount of vitamin A rich small fish on the vitamin A status in humans. The *overall aim* of this study is therefore to determine the change in vitamin A status after supplementation of 45 g (500 RE) of vitamin A rich small fish, mola in a daily meal for 9 weeks, to marginally vitamin A deficient Bangladeshi children (3-7 y) in a Dhaka slum, and compare it with the changes in vitamin A status obtained after supplementation of 45 g of big fish, rui (*Labeo rohita*) containing no/little vitamin A and 45 g of big fish, rui with added retinyl palmitate (500 RE). Children (n=180) will be randomly allocated to either of the three intervention groups. All children will receive one test meal/day (rice, fish curry and vegetable curry), 6 days a week for 9 weeks. Children (n=550) will be health examined, stool will be collected and the children will be dewormed twice prior to *screening* for marginal vitamin A deficiency, 2-6 weeks before the feeding trial begins (at *baseline*). Only children who pass the health examination and have marginal vitamin A deficiency will be enrolled at *baseline*. From the screened children the following will be assessed: degree of parasitic infestation, food intake, the level of risk of dietary vitamin A deficiency, morbidity and antropometry. In addition the socio-economic status of the households will be assessed as baseline information. Blood samples and anthropometry will be taken at *screening* (550 children) and after the feeding trial, *endpoint* (180 children). The blood samples will be analysed for vitamin A and iron status as well as markers of infection and protein energy malnutrition. The Relative Dose Response test will be performed on a subsample of the children (15 in each group) at *baseline* and at *endpoint*. During the study, the total food intake will be assessed using biweekly 24 hour recall interview and from nutrient analyses of duplicate portions of the test meals. Morbidity data will be registered biweekly. At completion of the feeding trial, the children will be given a recommended oral vitamin A dose, other supplementation and anti-helminthic agents as medically advisable. The additional aims to be addressed in the study are:

a) assess the efficacy of intake of vitamin A rich small fish on iron status in children in comparison with that of non/low vitamin A rich big fish and non/low vitamin A rich big fish with added retinyl palmitate; b) to investigate qualitatively the mothers' perceptions of the effect of the mola supplementation on the general well being of the children as well as the acceptance of the mola meal for habitual use among the children and their families; c) from the screened children assess and study the relationships between vitamin A status and iron status, the degree of parasitic infestation, morbidity and nutritional status among children (3-7 y) in a Dhaka slum. In addition, assess and compare two methods of estimating groups at risk of vitamin A deficiency, i.e. using dietary vitamin A inadequacy and serum retinol concentrations.

Studies on the efficacy of commonly consumed vitamin A rich small fish on the vitamin A status in children are valuable as a first step towards developing specific agricultural and food based programmes. If this study is successful, there is a large scope to increase the production and consumption of mola and other small indigenous vitamin A rich fish for combating VAD. The findings of this study will be equally important for other developing countries with habitual fish consumption.

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

Name	Professional Discipline/ Specialty	Role in the Project
1. M. A. Wahed	Nutrition & biochemistry/ Vitamin A & trace elements	PI, overall supervision and monitoring, blood and food analyses
2. Ph.D. scholar Katja Kongsbak (Ms.)	Food science & technology/ Nutrient bioavailability	Co-PI, daily running of the project, data analyses and publication of results
3. Dr. Shakuntala H. Thilsted (Ms.)	Nutrition/ Nutrition in Developing Countries	Co-investigator, supervision and follow up of the study and policy implications
4. Dr. Rashidul Haque	Parasitology	Consultant
5. Dr. Abbas Bhuiyan	Social Science	Consultant
6. Dr. D S Alam	Food Consumption	Consultant

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[X] Check here if appendix is included

Yes/No

X ☐ ☐ Is the proposal funded?

If yes, sponsor Name:
1) Thrasher Research Fund, USA
2) Council for Development Research, Danish International Development Assistance (Danida), Ministry of Foreign Affairs, Denmark (partial funding)
3) Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Denmark (partial funding)
4) International Center for Living Aquatic Resources Management (ICLARM), Malaysia and United States of America Agency for International Development (USAID) (partial funding for pilot study completed)

Yes/No

☐ ☐ X Is the proposal being submitted for funding ?

If yes, name of funding agency:

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? NO

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

Dates of Proposed Period of Support	**Cost Required for the Budget Period ($)**			
(Day, Month, Year - DD/MM/YY)	***1st Year***	***2nd Year***	***3rd Year***	***Other years***
Beginning date 01.10.2001	190,357 US$	0	0	
End date 30.09.2004	*b. Direct Cost:* 190,357US$	*Total Cost :* 203,287 *US$*		

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.

Dr. GB Nair, Laboratory Sciences Division — Nov 9, 2001

Name of the Division Director — Signature — 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 M. A. Wahed

Date: 04.11.2001

Name of Contact Person (if applicable)

M A Wahed

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

- ☐ Case Control study
- ☐ Community based trial / intervention **X**
- ☐ 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) **X**
- ☐ Bangalee
- ☐ Tribal groups
- ☐ Expatriates
- ☐ Immigrants
- ☐ Refugee

Consent Process *(Check all that apply)*:

- ☐ Written **X**
- ☐ Oral **X**
- ☐ None
- X ☐ Bengali language
- X ☐ English language

Proposed Sample size: Total sample size: 550 ☐X

Sub-group ***Screening:*** 550 children ☐ X ***Feeding trial:*** 3 intervention groups of 60 children each ☐X
Relative Dose Response (RDR) method: 15 children in each of the 3 intervention groups
☐ X

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
- X ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☐ New treatment regime

Yes/No

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

☐ ☐X 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:

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

☐ ☐X 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
- X ☐ 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".

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

FOR OFFICE USE ONLY

RESEARCH PROTOCOL

Protocol No.: 2001-025

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title:

The Efficacy of Vitamin A Rich Small Fish in Improving Vitamin A Status in Children in Bangladesh

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

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

Key words: human nutrition, vitamin A deficiency, vitamin A rich small fish, iron, children, intervention study

Principal Investigator: M. A. Wahed **Division:** Laboratory Sciences Division **Phone:** 8811751-60
Address: ICDDR,B **Email:** wahed@icddrb.org

Co-Principal Investigator(s):
Ph.D. scholar Ms. Katja Kongsbak, Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark
(phone: + 45 35 28 25 39; fax +45 35 28 24 83, email: kak@kvl.dk)

Co-Investigator(s):
Dr. Shakuntala Haraksingh Thilsted, Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark
(phone: + 45 35 28 24 97; fax +45 35 28 24 83, email: sht@kvl.dk)

Student Investigator/Intern:

Collaborating Institute(s):
Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark (phone: + 45 35 28 24 97; fax +45 35 28 24 83)

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

Gender
- ☐ Male **X**
- ☐ Females **X**

Age
- ☐ 0 – 5 years **X**
- ☐ 5 – 9 years **X**
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
- ☐ CSW
- X ☐ Others (specify Children , 3-7 y)
- ☐ Animal

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

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

They will be informed that two of the fish dishes will contain vitamin A and one fish dish will contain Low/no vitamin A. However neither the subjects/mothers nor the study staff will know which type of fish dish they are going to receive during the feeding trail. This is done in order to eliminate bias

5c) Stating whether or not a compensation and/or treatment will be available?

There is no potential risk to the subjects or privacy of the individual is involved.

Children who do not fulfil the health examination, show clinical sign of vitamin A deficiency, have serum retinol concentrations < 0.35mmol/L or show any clinical sign of sickness (high fever, ARI, measles, dysentery etc) during the feeding trial will be not be included in the study and referred to the medical doctor for treatment. All children will be given anti-helminthic treatment before the study.

The families of the children will have access to the available services of the medical doctor at the ICDDR, B local Mirpur office. At completion of the feeding trial, the children will be given a recommended oral vitamin A dose and other supplementation and anti-helminthic agents as medically advisable.

6. Interviews: Describe where and in what context the interview will take place. State approximate length of time required for the interview.

a) Identification of household and 3-7 yrs. children be included in the study – at household levels: once (5 min.)

b) SES information at the household level – once (30 min.)

c) Semi quantitative Dietary assessment of vitamin A rich food at household – once (20 min.)

d) Dietary intake assessment by 24 h. recall fortnightly – 6 times at households (15 min.)

e) Morbidity – baseline at the household and then 5 times more during 9 weeks (10 min.)

f) Child clinical health examination 5 times at the sub-centre (20 min). Based on needs, children may be examined by the study physicians

Mothers perception about mola fish will be assessed using both structured and open-ended questionnaire. This may take 10 mints. In addition, focus group discussion with 10-15 mothers at 3-4 sessions at the subcentre can be organised.

Physicians, nurses and local assistants at the local centre will perform clinical health examination, anthropometry and blood collection at screening, baseline and endpoint (lasting about ½ h each time).

7. Assess benefits to be gained by the individual subject, the society in general, and how the benefits outweigh the risks

All potential children will get a free health examination by physician and diagnostic if necessary as well as free deworming medication and vitamin A supplementation. Children will also be identified if they have severe or marginal deficiency based on serum retinol.

All children enrolled in the feeding trail will be served by lunch containing rice, fish curry and vegetable curry each day 6 days a week for 9 weeks. At the completion of the trial, children will be given Vitamin A supplementation as medically advised.

The families of the subjects will have access to the available services of the medical doctor at the ICDDR, B local Mirpur office.

If the study is a success, it is valuable as a first step towards improving the production, availability, accessibility and intake of vitamin A rich small fish in the community. Hence, the study may help to improve vitamin A deficiency in people in the community as well as millions of other people who suffer form vitamin A deficiency.

8. State if the activity requires the use of records (hospital, medical birth, death or other), organs, tissues, body fluids, the fetus or the arbortus?

The activity does not require use of records. However, blood for measurement of vitamin A, iron status, protein and acute phase protein levels; and stool for presence and load of worms will be collected.

ABSTRACT SUMMARY FOR ETHICAL REVIEW COMMETTEE (ERC):

Vitamin A deficiency (VAD) is a public health problem in many developing countries, including Bangladesh. Within food based strategies there has been no focus on the role of small fish in combating vitamin A and micronutrient deficiencies. Few commonly consumed small fish species have been found to have extremely high contents of preformed vitamin A. For example, mola (*Amblypharyngodon mola*) contains 2,400 - 3,000 RE/100 g raw edible fish. The *overall aim* of the study is to determine the change in vitamin A status after supplementation of 45 g of vitamin A rich small fish, mola (500 RE) in a daily meal for 9 weeks, to marginally vitamin A deficient Bangladeshi children (3-7 y) in a Dhaka slum, and compare it with the change in vitamin A status obtained after supplementation of 45 g of big fish, rui (*Labeo rohita*) containing no/little vitamin A and 45 g of big fish, rui with added retinyl palmitate (500 RE). Children (180) will be randomly allocated to either of the three diet groups. Studies on the efficacy of commonly consumed vitamin A rich small fish on the vitamin A status in children are therefore valuable as a first step towards developing specific agricultural and food based programs. If this study is successful, there is a large scope to increase the production of mola and other small indigenous vitamin A rich fish in combating VAD. The findings of this study will be equally important for other developing countries with habitual fish consumption.

1. Requirements for the study group and rationale for using this study group?
The subject population, children 3 - 7 y usually have marginal vitamin A deficiency and they are at great risk for such deficiency which may lead to nightblindness or complete blindness if they are not identified and effectively treated. Food based strategies are the best and sustainable. However, fish based food intervention was never tried. We are proposing here to intervene with small fish having high content of vitamin A to improve the vitamin A status. This age group of children can eat such small fish.

2 . Describe and assess any potential risk?
There are no more than minimal risk involved in conducting the study.
Four ml blood will be collected and stool sample will be collected from study subjects at *screening*. A second blood sample (4 ml) will be collected at *endpoint* in subjects enrolled in the feeding trail. In a subsample, one forth of the children enrolled in the feeding trail, two blood samples will be taken with an interval of 5 h at *baseline* and at *endpoint* for the Relative Dose Response method. Complications of blood drawing are generally minor but may have mild momentary discomfort.
Children enrolled in the negative control group (no/low vitamin A) will replace only one habitual meal per day by a no/low vitamin A rich meal, while all other meals will be consumed at home, as normally done. The duration of the study is relatively short, 9 weeks, 6 days per week and no severely vitamin A deficient children (serum retinol concentration <0.35mmol/L) will be recruited. At the end of the study, the children will receive an oral dose of vitamin A supplementation.
Subjects will not be hospitalised and no other invasive technique will be applied that may cause physical, psychological, social or legal risks.

3. Procedures for protecting against or minimising potential risks?
Disposable syringes and needles will be used for collecting blood for protecting or minimising any risk. Well-experienced personnel will perform blood sampling. Disposable plates will be used daily for serving the test meal.

4. Method for safeguarding confidentiality or protecting anonymity?
All information collected will be kept strictly confidential. A unique number will be used for data presentation instead of child's name. Other than the investigators no one will have access to any information of the children. The parents may however, will know the laboratory results, medical conditions and treatment if any.

5.
5a) Describe how and where informed consent will be obtained?
Signed informed consent will be obtained from the parents or legal guardian of the subjects at household. Contents of the consent form will be read in *Bangla.*
5b) If information is to be withheld from a subject, justify this course of action?
There is hardly any information to be withheld. However, the subjects will be explained that they are randomised to receive one of the three types of blended fish , *mola, rui and rui with added vitamin A* dishes.

Attachment I

Date: 20.11.2001

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: M. A. WAHED

Application No. 2001-025

Title of Study: The efficacy of vitamin A rich small fish in improving Vitamin A status in Children in Bangladesh

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) Thrashers

Project Status: _____

[✓] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
   - (a) Ill subjects — Yes / No ✓
   - (b) Non-ill subjects — Yes ✓ / No
   - (c) Minor or persons under guardianship — Yes ✓ / No

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes / No ✓
   - (b) Social risk — Yes / No ✓
   - (c) Psychological risks to subjects — Yes / No ✓
   - (d) Discomfort to subjects — Yes ✓ / No
   - (e) Invasion of privacy — Yes / No ✓
   - (f) Disclosure of information damaging to subject or others — Yes / No ✓

3. Does the Study Involve:
   - (a) Use of records (hospital, medical, death or other) — Yes / No ✓
   - (b) Use of fetal tissue or abortus — Yes / No ✓
   - (c) Use of organs or body fluids — Yes ✓ / No

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — Yes ✓ / No
   - (b) Procedures to be followed including alternatives used — NA — Yes / No
   - (c) Physical risk — Yes ✓ / No
   - (d) Sensitive questions — Yes ✓ / No
   - (e) Benefits to be derived — Yes ✓ / No
   - (f) Right to refuse to participate or to withdraw from study — Yes ✓ / No
   - (g) Confidential handling of data. — Yes ✓ / No
   - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — Yes ✓ / No

5. Will Signed Consent Form be Required:
   - (a) From subjects — Yes / No ✓
   - (b) From parents or guardian (if subjects are minor) — Yes ✓ / No

6. Will precautions be taken to protect anonymity of subjects — Yes ✓ / No

7. Check documents being submitted herewith to Committee:
   - ___ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required
   - ✓ Informed consent form for subjects
   - ✓ Informed consent form for parent or guardian
   - ✓ Procedure for maintaining confidentiality
   - ✓ Questionnaire or interview schedule*

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
   2. Example of the type of specific questions to be asked in the sensitive areas
   3. An indication as to when the questionnaire will be presented to the Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

M. A. Wahed
Principal Investigator

_____
Trainee

১৭ক. যদি হ্যাঁ হয় তাহলে কেন?

১৭খ. যদি না হয় তাহলে কেন না?

১৮. মলার তরকারী-তে আপনি অন্য কোন ধ্বাদ অনুভব করেন?
১=হ্যাঁ ২=না

১৮ক. যদি হ্যাঁ হয় তাহলে কোনগুলি

১৮খ. যদি না হয় তাহলে কেন না?

তারিখ..... সাক্ষাৎকার গ্রহনকারীর স্বাক্ষর..... ফোন ___

২৫. যদি আমরা বলি মলা বাচ্চার চোখের জন্য ধাতুর জন্য ভাল তাহলে কিভাবে আপনার বাচ্চাকে মলা মাছ রান্না করে দিবেন?

১. মাথা সহ
২. মাথা ছাড়া
৩. মাথা সহ এবং মাথা ছাড়া উভয়েই
৪. ভর্তা
৫. সিদ্ধ
৬. চটকিয়ে
৭. অন্যান্য _____

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২৬. বাজারে মলা মাছের দাম ১ কিলো ১০০ টাকা, আপনি কি এই দামে কিনবেন?

১= হ্যাঁ ২ = না

২৬ক. যদি হ্যাঁ হয় তাহলে কতবার মলা মাছ কিনবেন?

১. কখনও না
২. প্রতিদিন
৩. সপ্তাহে ১-২ বার
৪. সপ্তাহে ৩-৪ বার
৫. সপ্তাহে ৫-৬ বার
৬. মাসে ১-৩ বার
৭. মাসে ১ বারেরও কম

২৬খ. যদি হ্যাঁ হয় তাহলে কত কেজী দাম পর্যন্ত মলা কিনতে রাজী আছেন?

|_|_|_|_| টাকা/কেজি

২৬গ. যদি না, তাহলে কত কম দামে কিনতে রাজী আছেন?

|_|_|_|_|

বিভাগ বি : বাচ্চাদের জন্য প্রশ্ন

২৭. আপনি কি মলা মাছের তরকারী খেতে পছন্দ করেন?

১ = হ্যাঁ ২= না

২২গ. যদি না হয় তাহলে কেন ?

১. বাচ্চারা গলা পছন্দ করে না
২. ডুখাছ না
৩. কাটা
৪. পরিবারের কিছু সদস্য গলা পছন্দ করে না
৫. গলার দাম বেশী
৬. অন্যান্য _____

২২ঘ. বাচ্চা কি খাবে এটা পরিবারের কে সিদ্ধান্ত নেয় ?

১. মা
২. বাবা
৩. বাচ্চা
৪. অন্যান্য _____

২৩. যদি বলা হয় গলা বাচ্চার স্বাস্থ্যের জন্য ভাল তাহলে কতবার আপনার বাচ্চাকে গলা খেতে দিবেন ?

১. কখনও না
২. প্রতিদিন
৩. সপ্তাহে ১-২ বার
৪. সপ্তাহে ৩-৪ বার
৫. সপ্তাহে ৫-৬ বার
৬. মাসে ১-৩ বার
৭. মাসে ১ বারেরও কম
৮. অন্যান্য _____

২৪. যদি বলা হয় গলা মাথা ও চোখ সহ খাওয়ালে বাচ্চার বৃদ্ধি ও স্বাস্থ্যের জন্য ভাল তাহলে কি আপনি গলা মাছ চোখ ও মাথা সহ রান্না করবেন ?

১ = হ্যাঁ ২ = না

২৪ক. যদি না হয় তাহলে কেন?

২১. মলার তরকারী বাচ্চার স্বাস্থ্যের জন্য কি কোন ক্ষতি করে

১=হ্যাঁ ২=না

২১ ক. যদি হ্যাঁ হয়, তাহলে কি কারন ?

২২. যদি আপনার শিশুকে প্রতিদিন মলার তরকারী দিতে বলা হয় তাহলে আপনি কি দিবেন ?

১ = হ্যাঁ ২= না

২২ক. যদি হ্যাঁ হয় তাহলে কেন ?

১. বাচ্চার স্বাস্থ্যের জন্য ভাল
২. বাচ্চা মলা পছন্দ করে
৩. সুস্বাদু
৪. ভিটামিন ও খনিজ পদার্থ সমৃদ্ধ
৫. মলা সস্তা
৬. অন্যান্য _____

_____

_____

২২খ. যদি হ্যাঁ হয় তাহলে কিভাবে চালিয়ে যাবেন ?

১. কখনও না
২. প্রতিদিন
৩. সপ্তাহে ১-২ বার
৪. সপ্তাহে ৩-৪ বার
৫. সপ্তাহে ৫-৬ বার
৬. মাসে ১-৩ বার
৭. মাসে ১ বারের কম
৮. অন্যান্য _____

_____

৮। গবেষনা চলাকালীন আপনার বাচ্চা কি প্রতিদিন গলার তরকারী খেতে পছন্দ করতো ?

১ = হ্যাঁ ২ = না

৮ক. যদি হ্যাঁ হয়, তাহলে

১. সুস্বাদু
২. পেট ভরে
৩. কম অসুস্থতা
৪. খুশী হয়ে
৫. বেশী শক্তি পায়
৬. ভাল লাগে
৭. অন্যান্য _____

৮খ. যদি না হয় তাহলে

১. স্বাদ ভাল নয়
২. প্রতিদিন গলার তরকারী খেতে পছন্দ করে না
৩. অন্যান্য _____

৯. আপনি কি মনে করেন গলার তরকারী আপনার বাচ্চার স্বাস্থ্যের জন্য উপকারী

১ = হ্যাঁ ২ = না ৩ = জানি না

১০. যদি হ্যাঁ, তাহলে কিভাবে গলার তরকারী আপনার বাচ্চার স্বাস্থ্যের উপকার করে থাকে ?

ক. পেট ভরে
খ. কম অসুস্থতা
গ. খুশী
ঘ. বেশী শক্তি পায়
ঙ. হাড়ের সহায়ক
চ. চোখের জন্য ভাল
ছ. দুশ্চিন্তামুক্ত
জ. অন্যান্য _____

সংযোজন-৭

আন্তর্জাতিক উদ্ভিদ গবেষণা কেন্দ্র বাংলাদেশ
মাছের মাধ্যমে শিশুদের ভিটামিন এ-র অবস্থা পর্যবেক্ষন প্রকল্প, মিরপুর

গলার তথ্যাদি

১। স্টাডি নাম্বার |__|__|__|
২। শিশুর প্রাথমিক পরিচিতি নং |__|__|__|__|
৩। বাসগৃহের পরিচিতি নং |__|__|__|__|
৪। ক্যাম্পের নাম _____ কোড |__|__|
৫। সাক্ষাৎকারের তারিখ |__|__|__|__|
৬। উত্তর দাতার নাম ১= মা ২ = বাবা
৩= অন্যান্য (শিশুর সাথে সম্পর্ক) _____

ভূমিকা : এই প্রকল্প চলাকালীন সময়ে বাচ্চাদের মাছের তরকারী খাওয়ানো হবে। সেটা তৈরী হবে গলা অথবা কই মাছ দিয়ে। আপনাদের বাচ্চাকে এই প্রকল্প চলাকালীন প্রতিদিন এই মাছের তরকারী দেওয়া হবে। আমরা আপনাকে এবং আপনার বাচ্চাকে যারা এ প্রকল্পে অংশগ্রহন করছে তাদের গলা এবং গলার তরকারী সম্পর্কে কিছু প্রশ্ন করবো।

বিভাগ এ : মায়েদের জন্য প্রশ্ন

প্রথমত আমি আপনাকে গলা সম্পর্কে সবিধন কিছু প্রশ্ন করবো

৭। গলা মাছ খাওয়ার উপকারীতা সম্পর্কে আপনার ধারনা কি?

ক. ভিটামিনে পরিপূর্ন
খ. ভিটামিন এ -তে পরিপূর্ন
গ. চোখের জন্য ভাল
ঘ. পুষ্টিসমৃদ্ধ
ঙ. স্বাস্থ্যের জন্য ভাল
চ. হাড়ের জন্য ভাল
ছ. রক্তের পরিমান বাড়ায়
জ. সুস্বাদু
ঝ. অন্যান্য _____

সংযোজন-৬

অসুখ-বিসুখের ইতিহাস

১. স্টাডি # |_|_|_|_|

২. বাচ্চার পরিচিতি নম্বর |_|_|_|_|

৩. ঘর পরিচিতি নম্বর |_|_|_|_|

৪. ক্যাম্পের নাম ..... ক্যাম্প কোড |_|_|

৫. বাচ্চার নাম .....

৬. তারিখ |_|_|_|_|_|_| সাপ্তাহিক পরিদর্শন: ১।২।৩।৪।৫।৬।৭।৮।৯

৭. উত্তরদানকারী ১= মা, ২= বাবা, ৩= অন্যান্য (বাচ্চার সাথে অসম্পর্ক)

৮. ডায়রিয়া: ১ = হ্যাঁ ২ = না

৯. যদি হ্যাঁ তাহলে কতদিন ____ দিন

১০. আমাশয় ১ = হ্যাঁ ২ = না

১১. যদি হ্যাঁ তাহলে কতদিন |_____| দিন

জটিল শ্বাস প্রশ্বাসের সমস্যা:

১২. নাক দিয়া পানি পড়া ১ = হ্যাঁ ২ = না

১৩. যদি হ্যাঁ, তাহলে কতদিন |_____| দিন

১৪. গলা ব্যথা ১ = হ্যাঁ ২ = না

১৫. যদি হ্যাঁ, তাহলে কতদিন |_____| দিন

১৬. কাশি ১ = হ্যাঁ ২ = না

১৭. যদি হ্যাঁ, তাহলে কত দিন |_____| দিন

১৮. টনসিল ১ = হ্যাঁ ২ = না

১৯. যদি হ্যাঁ, তাহলে কতদিন |_____| দিন

ALRI (নিউমোনিয়া):

20. তরল কিছু পানি অক্ষমতা ১ = হ্যাঁ ২ = না

২১. বুকের পাঁজর বসে যাওয়া ১ = হ্যাঁ ২ = না

২২. যদি হ্যাঁ, তাহলে কতদিন |_____| দিন

২৩. দ্রুত শ্বাস-প্রশ্বাস ১ = হ্যাঁ ২ = না

সংযোজন-৬

24. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

25. হাসপাতালাইজড ১ = হ্যাঁ ২ = না

26. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

27. জ্বর ১ = হ্যাঁ ২ = না

28. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

29. হাম ১ = হ্যাঁ ২ = না

30. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

31. রাতকানা ১ = হ্যাঁ ২ = না

32. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

33. বাঘি ১ = হ্যা ২ = না

34. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

35. কান কথা / পাঁকা ১ = হ্যাঁ ২ = না ৩ = একটা কান ৪ = দুই কান

36. যদি হ্যাঁ তাহলে কতদিন [ ] দিন

37. চোখ উঠা ১ = হ্যাঁ ২ = না ৩ = একটা চোখ ৪ = দুই চোখ

38. যদি হ্যাঁ, তাহলে কতদিন [ ] দিন

39. হেপাটাইটিস ১ = হ্যাঁ ২ = না

40. যদি হ্যাঁ তাহলে কতদিন [ ] দিন

41. অন্য হাসপাতাল বা ডাক্তারের কাছে গিয়েছিল কিনা

১ = হ্যাঁ ২ = না

42. অন্য ডাক্তার বা হাসপাতালে কি কারনে নেয়া হয়েছিল :

43. কি ধরনের চিকিৎসা দেয়া হয়েছে

তারিখ :

সাক্ষাৎকারী অনুসন্ধানকারীর নাম:

খাদ্য-তালিকা-	নাম	গত 24 ঘন্টায় কতটুকু ব্যবহৃত হয়েছে			অধিকতর ব্যবহারের জন্য: 24 ঘন্টায় কতবার চাহিয়াছে।						
		সকালের পরিমান	দুপুরের পরিমান	সন্ধ্যার পরিমান	S	M	L	D	W	M	N
	লাল মাছ										
	মাগুড়										
	মলা মাছ										
	ম্যাসএব;										
	ডিম										
	গরুর ম্যাস										
	ডিম										
	দুধ বা দুগ্ধজাতীয় খাবার										
	গরুর দুধ										
	ঘী										
	অন্যান্য খাবার										

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

Vitamin A deficiency is still a public health problem in many developing countries, including Bangladesh, despite the goal set at the World Summit for Children in 1990 and the International Conference on Nutrition in 1992 to eliminate vitamin A deficiency as a public health problem by the end of 2000. Worldwide, 230 million children are at risk of clinical/subclinical vitamin A deficiency. As a result of vitamin A deficiency, 14 million preschool children have eye damage and every year, 350,000 become partially or totally blind, of whom 60 % die within few months. It is estimated that over one million vitamin A associated childhood deaths occur annually (WHO, 2000).

Actions are still needed in order to reduce and eliminate vitamin A deficiency and thus prevent blindness and improve the growth, development and survival of children (UNICEF, 1997). Improving vitamin A status among deficient children increases their chances of survival by more than 23 %, reduces the severity of infectious illness, especially measles and chronic diarrhoea (Sommer and West, 1996; Beaton *et al*, 1993) and may improve iron status in children suffering from iron deficiency anaemia, (Wolde-Gebriel *et al*, 1993; Suharno *et al*, 1992; Bloem *et al*, 1989; Mejia *et al*, 1977) another major public health problem in many developing countries (Bloem, 1995).

In Bangladesh, vitamin A deficiency has been recognised as a major public health problem since 1960s. Vitamin A programmes such as high potency vitamin A capsule supplementation to preschool children and an increase in the production and consumption of vitamin A rich foods have been strengthened over the past 15 years. A recent evaluation of these programmes in rural Bangladesh revealed that vitamin A deficiency is still a major problem although the magnitude has been reduced. In preschool children (6-59 months), 74 % had serum retinol < 1.05 μmol/L, 22 % < 0.7 μmol/L and 1.8 % < 0.35 μmol/L, indicating a public health problem according to WHO (HKI, 1999) and more than 60% had iron deficiency anaemia (haemoglobin levels < 110 g/L) (HKI, 2000). A study of preschool children living in a slum of Dhaka, Bangladesh showed that 57 % were vitamin A deficient (serum retinol < 0.7 μmol/L) of whom 7 % were severely deficient (serum retinol < 0.35 μmol/L) (Wahed *et al*, 2001), even though the area is covered by the national biannual vitamin A supplementation programme.

Supplementation, food fortification, dietary modification and plant breeding are different strategies used in alleviating and preventing vitamin A deficiency. Food based strategies (which has received little attention) are long term, sustainable and culturally accepted ways of controlling and preventing vitamin A deficiency. While vitamin A supplementation is often only targeted at preschool children and food fortification is not universal and sustainable in many areas, food based strategies reach all household members and also contribute with other nutrients. In making use of food based strategies, the content as well as the bioavailability of nutrients in foods consumed (the proportion of total nutrients that is absorbed and utilised by the body) must be taken into consideration.

One of the most well known food based strategies is to increase the production and consumption of green leafy vegetables and fruits rich in provitamin A carotenoids, especially β-carotene in rural population through home gardening programmes. In Bangladesh, it is estimated that 90 % of the total vitamin A intake stems from plant foods (Ahmad and Hassan, 1983). Evaluation of home gardening programmes in Bangladesh showed increase intake of vegetables, reduction of night blindness in children and increase in household incomes (Bloem *et al*, 1993; Talukder *et al*, 1994; Bloem *et al*, 1996). However, the evidence of the effect of vegetables and fruits in improving vitamin A status in experimental studies has been subject to debate (de Pee *et al*, 1995a; de Pee *et al*, 1995b; Reddy 1995; Underwood, 1995; de Pee and West, 1996; de Pee *et al*, 1998a, de Pee *et al*, 1998b). A recent intervention study conducted in Indonesia by de Pee and colleagues (1998a) suggests that the bioavailability of provitamin A carotenoids from green leafy vegetables and fruits is less than previously thought and varies in different vegetables and fruits. This means that the general acceptable conversion factors of 6:1 and 12:1 in converting dietary β-

carotene and other provitamin A carotenoids, respectively to retinol equivalent (RE) (FAO/WHO, 1967) needs to be revised, taking into consideration the different types of food.

Preformed vitamin A, which is found in foods of animal origin, is more efficiently absorbed than most carotenoids and more effective in improving vitamin A status (National Research Council, 1989). However, these foods are considered as not being easily available to and affordable for poor households. Within food based strategies, there has been no focus on the role that small fish can play in preventing and combating micronutrient deficiencies. Small fish is a commonly consumed food in many developing countries and are easily available, relatively cheap and well liked by most household members, including children. In Bangladesh, small fish rank third with respect to amount of raw food intake after rice and vegetables in many communities for large parts of the year (Thilsted *et al*, 1997). Some commonly consumed small fish species have been found to have extremely high contents of preformed vitamin A (Roos, 2001). For example, mola contains 2,400 - 3,000 RE/100 g raw edible fish (Roos, 2001), 5 times higher than the green leafy vegetable, amaranth (*Amaranthus gengeticuss*) (Varming, 1996). Few other indigenous small fish species in Bangladesh have been identified to have very high contents of vitamin A, chanda (*Parambassis baculis*) (> 1500 RE/100 g raw edible fish); dhela (*Osteobrama cotio cotio*) and darkina (*Esomus danricus*) (500-1,500 RE/100 g raw edible fish) while several other species have been found to have medium contents of vitamin A (100-500 RE/100 g raw edible fish) (Roos, 2001). However, the bioavailability of vitamin A from these small fish still needs to be established. An intake of 45 g mola/d (2,400 RE/100 g raw edible fish) meets the full daily vitamin A recommended dietary allowance of 4-6 years old children (500 RE/d) (National Research Council, 1989), taking a cooking loss of about 50 % into consideration. A pilot trial has shown that children can easily consume 45 g of fish (given as 60 g of fish curry) and even higher amounts, 70 g of fish (given as 90 g of fish curry) from a single main meal (own observations). No study has looked at the efficacy of a daily amount of vitamin A rich small fish on the vitamin A status in humans.

In addition to being an excellent source of animal protein, small fish also contribute greatly to the intakes of other nutrients such as calcium, iron and zinc (Thilsted and Roos, 1999; Roos, 2001). It has recently been shown in studies with both humans and rats that the bioavailability of calcium from whole small fish, mola is as high as that from milk (Hansen *et al*, 1998; Larsen *et al*, 2000). Fish protein is also a potent enhancer of iron and zinc absorption in humans (Aung-Than-Batu *et al*, 1976; Sandström *et al*, 1989).

Small fish is an essential and often irreplaceable food for the poor in Bangladesh and other developing countries with inland water bodies, including ponds and rice fields. Small fish, including mola dominate the fish intake in rural Bangladesh, contributing 84% by weight (Roos, 2001). The perceptions of rural women in Bangladesh with respect to small fish being a valuable source of vitamin A have been shown in a study in which 45% women considered mola as being good for/protect the eyes. In addition, mola is considered to be very tasty and is a well-liked fish (Thilsted and Roos, 1999).

Studies on the efficacy of commonly consumed vitamin A rich small fish on the vitamin A status in children are therefore valuable as a first step towards developing specific agricultural and food based programmes, which can play a central role in preventing and reducing vitamin A deficiency among millions of children in Bangladesh and other developing countries. If the proposed study shows that mola has a positive impact on children's vitamin A status and thereby child health and well being, actions need to be taken to improve the production, availability, accessibility and intake of vitamin A rich small fish in adequate quantities to reach at risk children and adults, both in rural and urban areas. This can be achieved through policies, programmes and projects within food production, fisheries, health and nutrition implemented by government institutions, bilateral and United Nations agencies and non governmental organisations.

Organisations such as Danish International Development Assistance (Danida), International Center for Living Aquatic Resources Management (ICLARM) and CARE are already implementing these production strategies in Bangladesh. In addition, Danida is funding a project in the Mekong delta (Cambodia) in which vitamin A and mineral rich commonly consumed fish will be identified for the purpose of promoting and enhancing their production and consumption. Based on the results of the above-mentioned studies, the Bangladesh Integrated Nutrition Project (BINP), funded by The World Bank is currently planning to implement a fisheries component pilot study in which mola together with carps will be produced in small seasonal ponds belonging to 100 poor households. This is expected to lead to an increased household income from the sale of fish as well as an increased intake of fish, especially mola. Also, the present nutrition education component of BINP will include messages on

the nutritional benefits of consuming mola, especially among children. Based on the experience from this pilot study, the fisheries component in BINP will be expanded.

In many developing countries, for example in South East Asia with water resources where fish is a commonly consumed, international organisations (for example ICLARM), bilateral organisations (for example Danida) and non governmental organisations (for example CARE), in their efforts to increase fish production, availability and consumption have focused on large fast growing fish, such as carps. Little attention has been given to the nutrient dense small indigenous fish, commonly consumed by the poor. The proposed intervention study (small vs. large fish) may therefore be applicable in many regions.

It has been shown that it is technologically feasible to increase the production of mola within the fisheries and agriculture sector in Bangladesh. Studies have shown that mola can be produced successfully in small ponds, together with carps (big fish), without hampering the production of carps (Roos *et al*, 1999). Mola breeds in ponds and other water bodies and must be harvested continually, thus making it suitable for regular consumption. Large fish are stocked and are harvested all at once, leading to sale of these fish. Studies in rural Bangladesh have shown that with increased production of fish, large fish and small fish, the household sell most of the large fish and some of the small fish while consuming a large portion of the small fish (Roos, 2001). Also, the restoration and management of inland fish habitats have shown to increase the production and consumption of small indigenous fish, including mola (Center for Natural Resource Studies, 1996).

If this intervention study is successful, there is a large scope to increase the production of mola and other small indigenous vitamin A rich fish in inland water bodies (including ponds and rice fields). This study can therefore play a significant role in making progress towards preventing and combating vitamin A deficiency among millions of people in Bangladesh, including preschool children who consume the family diet. In addition, the findings of this study will be equally important for other developing countries with water resources and habitual fish consumption.

Preliminary Observations

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B): Centre for Health and Population Research (ICDDR,B) and the principal investigator have been conducting different scientific research in the proposed study area, Mirpur slum in Bangladesh for the past 7 years. Mirpur slum is a suburb of Dhaka, which is 10 km from ICDDR,B. The short distance from Mirpur slum to ICDDR,B facilities makes it feasible to carry out field studies in the area. In addition, ICDDR,B has a local office in the Mirpur slum, which employ both ICDDR, B professionals and local community people. There are good infrastructure, records of the area and households as well as a good rapport and collaboration with the community leaders and inhabitants in the area. Based on previous ICDDR,B studies, the population in this area suffer from various micronutrient deficiencies such as vitamin A, iron and zinc deficiency.

A pilot study was conducted at the local office in the Mirpur slum in October 2000 to develop and test the recipes to be used in the study as well as the procedures and the logistics, in terms of physical set up and manpower of carrying out the study.

The test meals in the 3 different intervention groups have been designed and will consist of rice, a blended fish curry and a vegetable curry. The dishes developed were cooked and served to 20 children (4-11 yrs age) for 5 days. Cooked rice (200-450g on various days), and cooked vegetables 200g were offered and eaten by the children. Cooked and blended *mola, rui fish* (60 g on day 1-3 and 90 g rest days) were eaten by the children.
Based on their feedback, adjustments were made with respect to ingredients in each recipe and amount of each dish to be served in the test meal. Based on the children's behaviour and reactions, it was observed that they needed motivation to eat the test meals, at least initially, but at the same time, they liked the dishes, expressing that they were very tasty.

Samples of raw mola and rui and the corresponding cooked fish curries were taken to determine the cooking loss of vitamin A. Samples of the cooked vegetable curries were also taken for determination of vitamin A but these have not yet been analysed. We assumed that the cooking loss of vitamin A could be maximum 50%.

Amounts of each food needed for the whole study period were determined. Contacts and arrangement have been established for the supply of big and small fish (a fish farm), rice (Bangladesh Rice Research Institute (BRRI)) as well as vegetables (a local wholesale vendor).

A centrally located building in the Mirpur slum will be rented for 6 months for conducting the study, including food storage and cleaning, preparation of test meals, cooking and feeding of test meals to 180 children conducting the health examination of the children and blood collection.

Further description of the developed test meals, set-up and manpower for carrying out the study, which has been based on the pilot study, is seen in the next section "Research Design and Methods" under subsections: "Test meal" and "Test meal preparation and distribution".

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).

Reference is made to the "Time schedule" at the end of this section "Research Design and Methods".

Study population and subjects

Preschool children, age 3-7 y of both sexes will be selected from the urban slum in Mirpur, a suburb of Dhaka which is 10 km from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B): Centre for Health and Population Research. Mirpur slum is subdivided into camps covers a large area with a very high population density and poor housing. It is estimated that more than 3,000 children between 3 and 7 y live in the study area. Both the subjects and the study area are suitable for this study. Preschool children are known to suffer from the major health effects of vitamin A deficiency. Data from 2 merged studies have shown that children 3-6 y in Mirpur slum have a high prevalence of vitamin A deficiency, 53% (n=286) had serum retinol concentrations below < 0.70 μmol/L, even though there is a national vitamin A programme of biannual capsule supplementation. The prevalence of vitamin A deficiency (serum retinol concentrations below < 0.70 μmol/L) subdivided into age groups was: 3-4 y, *49 %* (n=145); 4-5 y, *58 %* (n=128); and 5-6 y, *56 %* (n=13), respectively (Wahed *et al.*, 1998; Wahed *et al.*, 2001).

Mola is not eaten frequently in the study area and assembling this age group in one place in order to serve one meal per day is considered most appropriate. The study site and population are already familiar to ICDDR,B, being used for different health research studies which have been conducted or are presently being carried out. ICDDR,B has a local office in the Mirpur slum, which employs both ICDDR, B professionals and local community people. There are good infrastructure, records of the area and households as well as a good rapport and collaboration with the community leaders and inhabitants. The short distance from the study area to the ICDDR,B facilities (with electricity, laboratory, storage and freezer) is crucial for carrying out the study successfully.

The selected children will be randomly across age range and gender and the children enrolled in the study will be randomly allocated to three intervention groups. Differences in age and gender are not expected to effect the output of the study. However, the effect of these variables will be taken into consideration in the statistical analysis.

In Bangladesh, there are three distinct seasons with respect to food availability - winter, summer and monsoon. The study will be conducted in winter for a period of 9 weeks, in which the habitual food pattern will be similar.

Design and intervention groups

A house to house survey will be conducted in about 4 camps purposely selected in order to achieve proposed target children (inclusions criteria in points 1-4 on the next page and **Appendix 1:** Household mapping form).

For the intervention study only marginal vitamin A deficient children (based on serum retinol concentrations 0.35-0.70 μmol/L) will be selected, since only in these children is the intervention expected to have an increase in serum retinol concentration. Expecting that one out of three children (33%) has marginal vitamin A deficiency, approximately 550 children who meet the inclusion criteria listed in points 1-7 below, will be ***screened*** to determine their serum retinol concentration.

Children who meet all of the following inclusion criteria will be selected for *screening*:

1. Preschool children of both sexes between 3 and 7 years of age from families of lower socio-economic status
2. Children who eat fish
3. Taking no vitamin A supplementation and multivitamin in the preceding 6 months
4. Not suffering form serious illness like: persistent or chronic diarrhoea, tuberculoses, chronic pneumonia disease, asthma, diabetes type 1, hepatitis B, cardiac malformation, measles, respiratory tract infections, acute diarrhoea and skin infections
5. Parents and children who give their consent to participate in the study
6. Children who pass the clinical health examination (Appendix 2: Child clinical health examination form): For example having no clinical signs of vitamin A deficiency, chronic diseases, measles, respiratory tract infections, acute diarrhoea, skin infections and sever malnutrition
7. On the time of blood drawing, have no fever and show no clinical sign of acute infections, including diarrhoea

Children (n=180) identified as marginal vitamin A deficient (based on serum retinol concentrations 0.35-0.70 µmol/L), will be randomly allocated to either of the following three intervention groups and enrolled in the feeding trial at ***baseline***. In each intervention group, there will be 60 children, receiving the same basic meal and either:

1) small fish, mola (vitamin A rich group) or
2) big fish, rui (negative control group, no/low vitamin A) or
3) rui and purified vitamin A, retinyl palmitate (positive control group)

All children will receive one meal per day, replacing one of the meals at home for 6 days a week for 9 weeks.

The negative control group is included because changes in serum retinol over time may be related to factors other than the small fish, such as seasonal variation and infection. The positive control group, receiving pure retinol is included in order to determine the maximal change in serum retinol.

Methods

Before screening for vitamin A deficiency

Children (n=550) will be clinically health examined (Appendix 2: Child clinical health examination form), stool will be collected (for later assessment of the degree of parasitic infestation) and the children will be given anti-helminthic treatment (albendazole tablets, 400 mg). All children will be given anti-helminthic treatment, as it is common in the study area that children have worms. It has been found that >90% children are infected in Mirpur slum (Hall *et al*, 1992). Two weeks after the first deworming a second deworming (albendazole tablets, 400 mg) will be given in order to ensure that all the children is free of worms. Deworming will be beneficial for the children's health. In addition, deworming of the children prior to the feeding trail may maximise the efficacy of the intervention, since helminths have shown to reduce vitamin A absorption.

At screening

Ten days after the second deworming, children will be clinically health examined, anthropometry will be measured and one blood sample will be drawn (Appendix 2: Child clinical health examination form). The feeding trial will begin (at *baseline*) after the *screening*. The *screening* including the analysis of serum retinol concentration will last for about 6 weeks.

Severely vitamin A deficient children (serum retinol concentration <0.35mmol/L) will not be enrolled in the feeding trial, but referred to a medical doctor for necessary treatment including vitamin A supplementation.

The values of the biochemical indicators from blood collected at *screening* will be used as the *baseline values* for children enrolled in the feeding trial. This will be done in order to reduce the discomfort of the child in connection with blood drawing and the cost of analyses. The biochemical indicators in the blood are expected to be unchanged from *screening* to *baseline* or any changes are expected equally distributed across intervention groups.

Collection of baseline information of the households and the screened children (n=550):

In addition to the above, the following baseline information will be collected from the households and *screened* children in a period of about 5 weeks, starting 6 weeks before *baseline*:

a) socio-economic data of the households (Appendix 3: Household socio-economic form), **b)** the risk of dietary vitamin A deficiency (based on one interview per child, - Appendix 4: Semi-quantitative dietary assessment of vitamin A), **c)** food and nutrient intake (based on one 24 h recall interview per child, - Appendix 5: Dietary intake assessment by 24 h recall) and **d)** morbidity data (based on one registration of incidence within the last 30 days, - Appendix 6: Morbidity form).

At baseline
Marginal vitamin A deficient children (n=180), who have been randomly allocated to either of the three intervention groups will be clinically health examined and anthropometry will be measured (Appendix 2: Child clinical health examination form). In addition, on a subsample of children (15) in each of the 3 intervention groups the RDR test will be conducted. The RDR test is thought to be a reliable test for individuals liver reserve.

Between baseline and endpoint (feeding trial)
During the feeding trial, all children will receive one meal per day, replacing one of the meals at home for 6 days a week for 9 weeks. The children will eat the other meals and snacks at home as usual. Twenty-four (24 h) dietary recall interview (Appendix 5: Dietary intake assessment by 24 h recall) and morbidity data (Appendix 6: Morbidity form) of all children will be collected biweekly. In addition, duplicate portions of dishes will be collected for nutrient analyses.

Endpoint
At *endpoint*, children will be clinically health examined and anthropometry will be recorded (Appendix 2: Child clinical health examination form). One blood sample will be collected from all children. An additional blood sample will be collected for the RDR test. This will be done on a subsample of children (15) in each of the 3 intervention groups.
At the completion of the feeding trial, all children will be supplemented with vitamin A.

Mothers' perceptions of the effect of the mola supplementation
At *endpoint* a qualitative questionnaire (Appendix 7: Perception of mola form) will be conducted (in the mola fish group) to evaluate the effect of the mola supplementation on the general well being of the children as well as the acceptance of the proposed mola meal for habitual use among the children and their families.

The mothers will be asked how the children felt after eating the test meal, how well they like the test meals and what benefits they think the test meal have on the nutritional status of their children. The mothers will also be asked if they will prepare the mola test meal at home and which resources they need to do so as well as which constraints they may face and how they can overcome these constraints.

This data from this questionnaire are important for large scale implementation of the results of this study, taking into account factors such as socio-economic status of the household and specific constraints for consuming mola.

Both structured and open ended questionnaire will be used to assess the perception of the mothers about mola fish. A focus group discussion with 15 mothers at 3-4 sessions will be conducted to assess the perception.

Calculation of sample size

Using retinol rich foods, fruit or vegetable, de Pee *et al* (1998a) found an increase in serum retinol of 0.23 µmol/L, 0.12 µmol/L, 0.07 µmol/L respectively from a baseline of approximately 0.70 µmol/L in a 9 weeks' intervention study in children 7-11 y. A dose of 650 RE/d given in two meals daily was used. The maximum standard deviation, 0.17 µmol/L in the change in serum retinol from baseline to the end of the study was found in the fruit group.

The expected increase in serum retinol in the mola group in this study, 0.15 µmol/L is based on an expected increase which is lower than that found in the group given the retinol rich food but higher than that in the group given fruit. Children in the positive control group might show a similar or a larger increase in serum retinol concentration than the mola group.

Assuming similar prevalence and distribution of serum retinol concentration at baseline in Bangladeshis as in the Indonesian children, about 0.70 µmol/L and the expected increase amounts to a 20% increase from the baseline value.

The number of participants in each group, n is calculated from power calculations based on the following formula (Armitage & Berry, 1987):

$$n = [(Z_\alpha + Z_\beta)^2 * (sd_1^2 + sd_2^2)] / d^2$$

Z_α= 1.96 for $\alpha = 0.05$, Z_β= 1.28 for $\beta = 0.10$ (power of 0.90)

sd_1, sd_2 = standard deviation of the change in serum retinol from baseline to the end of the study for group 1 and group 2 $sd_1 = sd_2 = 0.20$ µmol/L

d = difference in change in serum retinol between group 1 and group 2, d= 0.15 µmol/L

The sample size required in each group is 37. Assuming a maximum possible drop out of 40%, the total number of children in each intervention group is calculated to be approximately 60. The total number of children to be used in this study is 180.

Test meals

A pilot study was conducted in the Mirpur slum in October 2000 to develop and test the recipes to be used in the study as well as the procedures and the logistics, in terms of physical set up and manpower of carrying out the study. The dishes developed were cooked and served to 20 children for 5 days. Based on their feedback, adjustments were made. Samples of raw mola and rui and the corresponding cooked fish curries were taken to determine cooking loss of vitamin A. Samples of the cooked vegetable curries were also taken for determination of vitamin A.

The test meals in the different intervention groups have been designed in such a way that they appear and taste as similar as possible. The test meals will consist of rice, a fish curry and a vegetable curry. All foods given in the test meals other than mola are non/low vitamin A rich foods. Rice will be given *ad libitum* in order to adjust for the differences in energy needs due to different ages. Subsequently, all children are given the same fixed amount of rice, the few children who ask for more will be given more. The fish curry to be used in the intervention groups will differ only with respect to the fish species either small fish, mola or big fish, rui. The other ingredients of the fish curry will be onion, garlic, ginger, turmeric, chilli seed, iodised salt and soybean oil. The amount of cooked fish curry used in the intervention groups is fixed to correspond to either 45 g raw cleaned mola or rui/child/meal. The cooked mola curry will be homogenised (blended) so that the vitamin A in mola is uniformly distributed throughout the whole amount. The rui curry will also be homogenised. It was observed that the two fish curries differed in colour, the mola curry being dark green and the rui curry had a yellow tinge. The same vegetable curry will be used in all intervention groups during the study and will be served in a fix amount. The vegetable curry will consist of bottle gourd in addition to potato, cucumber, onion, mug dal, garlic, ginger, turmeric, chilli seeds, iodised salt and soybean oil.
The recipe for 200 children are shown in appendix 14. However, we shall offer cooked rice 400g, vegetables 200g and blended fish (mola/rui) curry 90 g.

In the positive intervention group, retinyl palmitate solution will be added to the meal of each child at serving. In order to ensure that the vitamin A recommended intake is met in the vitamin A rich and positive control groups, the daily test meal will contain approximately 500 RE/d.

Mola (300 kg) and rui (1100 kg) will be caught over a period of 4-6 months (starting at the end of 2001 and completing in the beginning of 2002). The fish will be cleaned, packed, weighed in daily portions and stored frozen at the Mymensingh Aquaculture Extension Project until transport to Mirpur. The rice (2400 kg) will be purchased from the Bangladesh Rice Research Institute. The vegetables (6500 kg) will be supplied by a local wholesale vendor.

Test meal preparation and distribution

The test meals will be prepared at a centrally located building in the Mirpur slum. The building, which will be rented for 6 months, will be used to conduct the health examination of the children, blood drawing, food storage and cleaning, cooking and feeding of test meals to 180 children. Four head cooks, 24 local kitchen helpers and 7 research assistants will be in charge of preparing and distributing the meals.

The head cooks will be in charge of cooking all dishes, while the local kitchen helpers are needed, especially for the peeling, cleaning and cutting of the vegetables. On a daily basis, four dishes (rice, mola fish curry, rui fish curry and vegetable curry) will be prepared; approximately 130 kg boiled rice, 70 kg vegetable curry, 10 kg mola curry and 15 kg rui curry. In addition, the local kitchen helpers will help in bringing the children to the building, make the children feel welcome, prepare them for eating (for example seeing that they wash their hands), arrange them in intervention groups, and in preparation for receiving the different test meals. They will also assist with meal distribution, encourage and motivate the children to eat the test meal, especially all of the fish curry. In order to ensure that the children eat all the fish curry served, they will first be served the fish curry along with the rice and after finishing the fish curry, they will be served the vegetable curry. The test meals will be served at lunchtime (1300 - 1400 h).

The research assistants will be responsible for instructing and overview the kitchen helpers, supervising that the amounts and procedures described in the recipes are followed by the cooks, weigh the amounts of raw foods to be cooked in each dish, weigh the dishes and serve the test meals. Rice, vegetable curry and fish curry will be weighed and served separately to each child in disposable plastic containers. Disposable plastic containers will be used for hygienic purposes, ease of weighing separate portions as well as ease of administration. Drinking water will be given ad libitum. The containers will be marked with the child's identification number. Leftovers of each dish will be weighed to calculate total intake.
We shall have 3 floors of a building to accommodate 3 groups of children.
For the nutrient analyses of dishes, duplicate samples will be collected in labelled plastic boxes and stored frozen. One sample each of cooked rice (n=5) and vegetable curry (n=5) will be taken biweekly. For the three fish dishes, one sample each will be taken every week (n=9), in total 27.The duplicate samples will be used for chemical analyses of dry matter, vitamin A, energy, protein, fat, iron, calcium and zinc.

In order to motivate the children to comply with the study procedure, each child will be given a notebook, a pen and an identification card that can be hung around his/her neck. The identification card will be colour coded (each intervention group having a different colour) and include the child's name, ID number, parents' names, camp name, dates of study and the child's picture.

The research assistants will record all data collected daily with respect to attendance of the children, leftover dishes and duplicate samples and enter them in a computer programme.

Biochemical indicators of vitamin A and iron status

The following biochemical indicators for assessing vitamin A and iron status will be measured at *screening* (550 children) and at *endpoint* (180 children) from 4 ml blood drawn by venepuncture in the morning: retinol, retinol binding protein, prealbumin, haemoglobin, ferritin, transferrin receptor. Changes in vitamin A and iron status will be calculated by subtracting the values at *screening* from those at *endpoint*.

In addition, as an indirect measure of liver vitamin A stores the RDR test will be performed on two different subsamples of children (15 children per intervention group, in total 45 children) at *baseline* and at *endpoint*. The RDR method is included in order to confirm low vitamin A status at *screening* and improvement by the intervention in terms of body stores, since serum retinol levels can be artificially lowered by concurrent infection.
RDR will be conducted by administering 3.5 μmol (1000μg) retinol equivalent as retinyl palmitate orally. Venous blood will be obtained at 0 h (4 ml blood) and 5 h (1 ml blood) after administering the retinyl palmitate dose. The RDR value will be calculated as [(retinol 5h - retinol 0h/retinol 5h)] * 100. Values ≥ 20% are considered abnormal (Flores *et al*, 1984). The 0 h blood sample will also be used for the analysis of vitamin A and iron status indicators as described above.

Indicators of iron metabolism are included due to the interdependence between vitamin A and iron (Wolde-Gebriel *et al*, 1993; Suharno *et al* 1992; Bloem *et al* 1989; Mejia *et al* 1977). The ratio between ferritin and transferrin receptor will be used as an indicator of iron status as suggested at the INACG (International Anaemia Consultative Group) meeting held in Hanoi in February 2001.

Biochemical markers of infection and protein energy malnutrition

CRP and ACT (acute phase proteins) will be measured at *screening* (550 children), *baseline* (45 children participating in the RDR test) and at *endpoint* (180 children) as indicators of acute phase response due to infection. In addition, prealbumin will be measured as an indicator of protein nutritional status as these may effect the biochemical indicators of vitamin A and iron status. Serum prealbumin < 10-40 mg/dL is considered as an indicator for protein energy malnutrition. CRP > 5 mg/L and ACT > 0.4 g/L indicate underlying inflammatory disease. Blood analyses will be carried out at Nutritional Biochemistry Laboratory, ICDDR,B, which has extensive in-house and external quality control system.

Parasitic infestation

Parasitic infestation will be measured from stool collected from the screened children (n=550) before the 1st deworming. The intensity of infestation (eggs per gram) will be assessed for helminths *(Ascaris lumbricoides and Trichuris trichuria)* and hookworms. Protozoa cysts (*Entamoeba histolytica, Giardia intestinalis* and other protozoa cysts) will be verified (as zero, few, moderate or heavy). The data will be used for the assessment of infestation and relationships between outcome parameters.
The faecal samples will be examined microscopically by the quantitative ether sedimentation technique (Hall, 1981). Stool samples will be analysed at the Parasitology Laboratory at ICCDR,B.

Confounding factors

Since the outcome of the intervention study may be affected by factors other than the fish consumed, the confounding variables markers of infection and protein energy malnutrition as well as anthropometry, food intake, morbidity as described below will be measured during study and adjusted for in the statistical analysis.

Anthropometry

Age, sex, height, weight and upper arm circumference (MUAC) will be recorded in the selection of potential subject. Height, weight and MUAC will be measured at *screening* (550 children), at *baseline* (180 children) and at *endpoint* (180 children).

Antropometry will be measured with the following accuracy: Height will be measured to the nearest 2 mm (using a stadiometer), weight to the nearest 0.1 kg (using an electronic scale) and MUAC to the nearest 1 mm (using MUAC strips). Inter-, intra- individual and instrument variability will be determined before the study.

Changes in weight, height and MUAC will be calculated as well as weight for age, weight for height and height for age. The anthropometric measurements will be compared with the standard values of the National Center for Health Statistics and expressed as Z-scores (NCHS, 1976).

Food intake

One 24 h dietary recall of all 550 children will be conducted as baseline information in order to assess habitual food and nutrient intakes at the group level. In addition, a semi-quantitative dietary assessment of vitamin A intake will be done in order to assess the risk of inadequate dietary vitamin A intake at the group level.

During the feeding trial, between *baseline* and *endpoint*, intakes of nutrients (vitamin A as well as energy, carbohydrate, protein, fat, iron, zinc and calcium) from foods not provided in the test meals will be calculated from biweekly food consumption surveys of the 180 children, using the 24 h recall method.
The results will be used to calculate the total daily intakes of nutrients in each treatment group during the feeding trial based on the following:
1) data from the food consumption surveys conducted between *baseline* and *endpoint* (mean of 4-5 dietary recalls) and 2) the amount of food given in the dishes (corrected for leftovers) and from the chemical analyses of duplicate samples of the dishes (please see below).
The total daily intakes of nutrients during feeding trial will be compared between treatment groups as well as with habitual food and nutrient intake.

The conduction of the 24 h recall method will be based on the method described by Gibson, 1990. The questionnaire is enclosed as Appendix 5.
The method used for conducting the semi-quantitative dietary assessment of vitamin A intake will be based on the method proposed by the International Vitamin A Consultative Group (IVACG) (Underwood *et al*, 1989). The questionnaire is enclosed as Appendix 4.

The health assistants will be trained, the questionnaires will be pre-tested, the health assistants will be checked in the field by supervisors and the filled out questionnaires will be checked daily.

A random check of 5% of the total number will be conducted in order to test the reliability of the 24 h dietary recalls, semi-quantitative assessment of vitamin A intake and morbidity questionnaires. This means that another person repeats the visit in the same household on the same day for 5% of the households. A quick comparison of the field assistant's questionnaire and the supervisor's questionnaire is done on the same day. At *baseline*, the field system may be adjusted to correct the faults.

Socio-economic data, semi-quantitative questionnaire on vitamin A intake, 24 h dietary recall and morbidity data will be conducted by the 9 health assistants and 2 supervisors.

Nutrient analysis of dishes
For the nutrient analysis of dishes, duplicate samples will be taken in labelled plastic boxes and stored frozen. One sample each of cooked rice (n=5) and vegetable curry (n=5) will be taken biweekly. For the three fish dishes, one sample each will be taken every week (n=9), in total 27.The duplicate samples will be used for chemical analyses of dry matter, vitamin A, energy, protein, fat, iron, calcium and zinc.

Morbidity
The incidence of infection: diarrhoea, dysentery, upper acute respiratory infection, pneumonia, fever, measles, night blindness, vomiting, ear and eye infections, within the last 30 days will be recorded once per screened child, in connection with the collection of *baseline* information (550 children) (Appendix 6). The data will be used for the assessment and relationships between outcome parameters.
For the children (n=180) enrolled in the feeding trial, morbidity (incidence within the last 2 weeks) will be registered biweekly between *baseline* and *endpoint*. The results will be used to calculate mean morbidity (based on 4-5 registrations) in each intervention group during the feeding trial.
Below are description of common morbidity:

URI	Presence of cough and or nasal discharge
ALRI (Pneumonia)	Presence of cough and tachypnoea (>40 resp/min) with or without chest indrawing
Diarrhoea	3 or more loose stools in 24 hours
Dysentery	Loose stools with blood or blood with mucus
Fever	$>38^{0c}$ body temp.

Compliance
The children and their families will be carefully informed about the importance of eating all of the test meals, not changing their normal diet as well as not taking any nutrient supplements and deworming medication between *screening* and *baseline* as well as during the feeding trial. A physician will be available for any health problems of the children.

Good rapport between the community leaders, parents and ICDDR, B will ensure compliance. Regular visits of study staff to the households as well as daily contact with the children and mothers when consuming the test meals will be used to motivate the children and their families as well as reinforce the messages regarding compliance. During biweekly visits to the households, for collection of morbidity and food intake data the mothers will be asked if their children have been taking nutrient supplements and/or deworming medication.

During previous studies, we have found compliance of follow up was nearly 90%. Child who will eat 80% of the assigned meal will be considered as successful compliance. In this study, we expect atleast 80% compliance to come to feeding at the subcentre although according to sample size calculation if 37 children would complete 6X9 meals would be enough to test the hypothesis of this efficacy trial. In case of drop out, we may carry out stratified analysis for less number of intake.

Time schedule

Fall 2000	Pilot study: Composition and testing of test meals (including some food analyses). Funded by the Council for Development Research, Ministry of Foreign Affairs, Denmark, International Center for Living Aquatic Resources Management, Bangladesh and USAID. Already completed.
Fall 2001	Submission of research project for approval by the Research Review Committee (RRC) and Ethical Review Committee (ERC) of ICDDR,B. Design of forms and questionnaires. Selection of camps and identification of households and target children (inclusion criteria points 1-4, page 12)
Fall 2001 – beginning of January 2002	**Setting up the survey (2 months):** ➢ Organisation of study facilities and development of forms and Questionnaires ➢ Recruitment of field staff and training as well as pilot testing of forms and ➢ Questionnaires, procedures and equipment ➢ Obtaining informed consent from parents (inclusion criteria points 5, page 11)
Mid December – beginning of February 2002	**Before screening for vitamin A deficiency:** ➢ Clinical health examination Children fulfilling inclusion criteria listed in points 1-6, page 11: ➢ Stool collection and 1st deworming medication,- stool analysis (n≈550) ➢ 2nd deworming (2 weeks after the 1st deworming)
Mid January 2002 - mid February	**At screening (n≈550):** ➢ 10 days after the 2nd deworming: Clinical health examination, anthropometry (age, height, weight and MUAC), blood collection of children, fulfilling inclusion criteria point 7, page 12 ➢ Analysis of serum retinol from blood: During a period of 6 weeks, the concentration of serum retinol will be analysed from blood of the 550 children of which 180 with marginal vitamin A deficiency (0.35–0.70 µmol/L) will be randomly selected
End January 2002 - mid February	**Collection of <u>baseline information</u> of the households and the screened children (n≈550):** - socio-economic data - habitual food and nutrient intake (one 24 h recall interview per child) - risk of dietary vitamin A deficiency (one interview per child) - morbidity data (incidence within the last 30 days, one per child) - data entry ➢ Training of staff for cooking and feeding Continued on the next page!

Time schedule (continued)

End February 2002	**Baseline (n=180):** ➢ Selection of 180 marginal vitamin A deficient children ➢ Randomisation of the children to the 3 different intervention groups ➢ Clinical health examination, anthropometry (age, weight, height and MUAC), blood collection for the RDR method on a subsample of children (15 in each of The 3 intervention groups)
Mid March 2002	**Feeding period (9 weeks) (n=180)**: ➢ Feeding lunch, 6 days a week for 9 weeks to 180 children ➢ -Biweekly registration of food intake, beside test meal provided, one 24 h recall interview per child -Biweekly registration of morbidity ➢ Collection of duplicate portions of test meals
Mid May 2002	**Endpoint (n≈ 180):** ➢ Clinical health examination, anthropometry (age, height, weight and MUAC), Blood collection of children, RDR test on a subsample of children (15 in each of the 3 intervention groups)
June 2002 to October 2002	**After completion of the feeding trial** ➢ Chemical analyses of blood for assessing vitamin A and iron status, CRP, ACT, prealbumin, analyses of nutrient composition of dishes and data entry
November 2002 to end 2003	➢ Data entry and statistical analyses of data ➢ Writing articles for publication in international journals ➢ Reports to sponsors and institutions in Bangladesh ➢ Presentation of results to organisations/institutions which plan and implement Policies, strategies, programmes and projects for the prevention and reduction of vitamin A deficiency in children in developing countries, especially Bangladesh

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 Mirpur slum, Dhaka in a house purposely rented for the study .The building will be used e.g. to conduct the health examination of the children, blood drawing, food storage, cleaning, and preparation, cooking and feeding of test meals to 180 children. In the same building the families of the children will have access to the services of a medical doctor, free of charge during study.

The laboratories at Laboratory Sciences Division, ICDDR,B have the required expertise to perform assays of the study.

The following chemical analyses of blood will be done by Laboratory Sciences Division, ICDDR,B:

Biochemical Indicator	Device and reference
Serum retinol	HPLC, Bieri JG *et al.*, 1979
Serum retinol binding protein	Single radial imunodiffusion technique using commercial partigen plates (SRID)
Haemoglobin (whole blood)	Colorimetric, Von Klein *et al.*, 1965
Serum ferritin	Enzyme Immunoassay, Dubois S *et al*, 1988
Serum transferrin receptor	Enzyme Immunoassay, Flowers CH *et al*, 1989
CRP	Immunoturbidimetric (Roche, commercial kit)
ACT	Immunoturbidimetric (Roche, commercial kit)

The following chemical analyses of dish samples will be done by Laboratory Sciences Division, ICDDR,B and in Denmark:

Nutrient	Device and reference	Place of analysis
Dry matter	Freeze-drying	ICDDR,B
Vitamin A	HPLC, Ball GFM 1988	ICDDR,B
Energy	Bomb Calorimetric, British standard	ICDDR,B
Protein	Micro Kjeldhal, Horwitz ED, 1975	ICDDR,B
Fat	Titration van Dekamer, Van de Kamer JH, 1958	ICDDR,B
Ash	Muffle Furnace	Denmark
Iron, calcium, zinc	Atomic absorption spectrophotometer. AOAC official methods of analysis, 1990	Denmark

The following stool analyses will be done at the Parasitology Laboratory, ICDDR,B:

Type of parasitic infestation	Type of examination
Helminths, *Ascaris lumbricoides and Trichuris trichuria*	Intensity of infestation (mean eggs/g faeces)
Hookworms	Intensity of infestation (mean eggs/g faeces)
Protozoa cysts, *Entamoeba histolytica, Giardia intestinalis* and other protozoa cysts	Verification as zero, few, moderate or heavy

The faecal samples will be examined microscopically by the quantitative ether sedimentation technique (Hall, 1981).

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 first, second and third specific objectives refer to the results from the feeding trial (n=180, one test meal for 9 weeks). The fifth specific objective refers to the results for health, food intake, morbidity and nutritional status from the screening (pre-feeding trial) (n=550).

The data from the feeding trial is longitudinal as in each individual, each indicator is measured more than once over time. For indicators for changes in vitamin A and iron status, analysis of covariance will be used, testing group effect as a factor with nutrient intake during study, initial vitamin A and iron status, changes in iron status, markers of infection, morbidity during study, marker of protein energy malnutrition, initial and/or change in antropometry as covariates. Thus, comparisons of changes in the indicators between the three groups will be analysed (specific objectives one and two). In addition, the effect of the above mention variables on changes in the indicators for vitamin A and iron status will be explored. If an effect is found, sub group analysis will be performed (specific objective three).

In order to study the relationship between the indicators for vitamin A and iron status, parasites, infection, morbidity, food and nutrients intake and anthropometry, (multi)-analysis of variance will be used (specific objective five).

Variance homogeneity of residual variation will be investigated through plots of standardised residuals against predicted values. Normal distribution of data will be investigated graphically and by Wilk-Shapiro's test for normal distribution. If variance homogeneity does not exist or the data are not normally distributed, suitable mathematical transformations will be tried before nonparametric statistical analysis is performed.

The effect of sample size on the results obtained will be explored.

The children (n=550) are given a unique number at *screening*, numbers **300-850**.
Children (n=180) who are selected for participating in the feeding trial are randomly assigned to the 3 treatment groups and subsequently given another number; group 1: **001-060**, group 2: **061-120** and group 3: **121-180**. If more than one measurement is done on the same child of a parameter, the child will be labelled with the above given number followed by another number starting with 1, i.e. **child number - 1, child number- 2 ..** etc.
The blood analysis will be done without knowledge, of which group the children are assigned, since every blood analysis is given a unique number. The data analysis will be done without knowledge of which intervention group the children are assigned. The groups will be decoded at the completion of the analysis.

The co-principal investigator Ph.D. scholar Ms. Katja Kongsbak will do the major statistical analysis. During the statistical analysis of data, a qualified statistician from The Royal Veterinary and Agricultural University, Denmark will give appropriate assistance. A statistician has been involved in the planning of the study design, and the details of the statistical analysis are being developed as the study plan and implementation progress.

The statistical analysis will be performed with the Statistical Analysis System software (SAS Institute, Inc., Cary, NC, USA).

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.

Vitamin A deficiency is still a major public health problem in the developing countries. Previous studies in the proposed site indicated that > 50 % preschool children have subclinical deficiency based on serum retinol.
The children and their parents will be informed and explained orally and in writing about the purpose and procedures of the study. Parents of children participating will give their written consent. Children who do not fulfil the health examination, show clinical sign of vitamin A deficiency, have serum retinol concentrations < 0.35mmol/L or show any clinical sign of sickness during the feeding trial will be referred to the medical doctor for treatment.

The following ethical considerations have been taken into account with respect to enrolling children in a negative control group (receiving big fish). The children will replace only one habitual meal per day by a no/low vitamin A rich meal, while all other meals will be consumed at home, as usual. The study will be conducted for a relatively short period, 9 weeks, 6 days per week. No severely vitamin A deficient children (serum retinol concentration <0.35mmol/L) will be recruited.
At completion of the feeding trial, the children will be given a recommended oral vitamin A dose and other supplementation and anti-helminthic agents as medically advisable.

The families of the children will have access to the available services of the medical doctor and treatment at the ICDDR, B local Mirpur office.
All children will be given anti-helminthic treatment before the study. In the study area, it has been found that >90% children are infected.

For conducting the RDR method extra effort and motivation will be done to keep the mother along with the child for extra 5 h during. During this time the child and the mother will be served beverage.

Strict confidentiality will be observed regarding all information. Unique number will be used for data presentation instead of child's name. The parents may have access to laboratory results and medical conditions of their child. There is hardly any risk to participate in the study other than little discomfort during blood draw. Trained physician, following all aseptic conditions will draw blood.

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

Animals are not used.

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.

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de Pee S, West CV, Muhilal, Karyadi D and Hautvast JGA (1995b). Vitamin A status and dark green leafy vegetables. Letter to the editor. Lancet 346: 1635-1636.

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Roos N, Islam MM, Thilsted SH, Ashrafuddin M, Mursheduzzaman M, Mohsin DM and Shamsuddin ABM (1999). Culture of mola (*Amblypharyngodon mola*) in polyculture with carps - experience from a field trial in Bangladesh. NAGA, ICLARM Quarterly 22, 2:16-19.

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Underwood BA, Chavez M, Hankin J, Kusin JA, Omololu A, Ronchi-Proja F, Butrum R, Ohata S (1989). Guidelines for the development of a simplified dietary assessment to identify groups at risk for inadequate intake of vitamin A. IVACG, International Life Science Institute-Nutrition Foundation, Washington, D.C..

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Wahed MA, Haque R, Rahman H et al.(1998). Biochemical evidence of selected micronutrient deficiences in children living in urban slums. The FASEB J. vol.12 (4) March 17, p A212.

Wahed MA, Haque R, Rahman HASM, Ahmed T and Albert JM (2001). Effect of β-carotene supplementation and anti-helminthic therapy on vitamin A status in preschool children in Bangladesh. The FASAB J Vol 15 (5), March 18, p A732.

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Wolde-Gebriel Z, West CE, Gebru H, Tadesse AS, Fisseha T, Gabre P, Aboye C, Ayana G and Hautvast JGAJ (1993). Interrelationship between vitamin A, iodine and iron status in schoolchildren in Shoa Region, Central Ethiopia. Br J Nutr 70: 593-607.

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 immediate output of the study is to establish whether a supplementation of vitamin A rich small fish to marginally vitamin A deficient children improves their vitamin A status and to what extent. If vitamin A rich small fish has a positive impact on children's vitamin A status, actions will be suggested to improve the production, availability, accessibility and intake of vitamin A rich small fish. This can be achieved though policies, programmes (for example the National Nutrition Programme funded by the World Bank) and projects within food production, fisheries, health and nutrition implemented by government institutions, bilateral and United Nations agencies and non governmental organisations.

The data of this project are for the Ph.d. programme of Ms. Katja Kongsbak. For other publications, authorship will be on the basis of input, effort, expertise and consent among all involved partners. Katja Kongsbak, M.A. Wahed and Shakuntala Haraksingh Thilsted will be co-authors on all publications from this study.

The results will be published in international journals and presented at international and local conferences and workshops. Reports will be written to the sponsors and institutions in Bangladesh.

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)**

1. This research project will be carried out by researchers of ICDDR,B and The Royal Veterinary and Agricultural University, Denmark. Research collaboration between these two institutions has been formalised as detailed in the Memorandum of Understanding (MoU) which is included (Appendix 8). The MoU gives researchers from both institutions the opportunity to collaborate on research projects, without having to seek additional approval by the institutions.

2. ICDDR,B has conducted numerous research projects and has ongoing research projects in Mirpur slum, the study site of this research project. ICDDR,B does not require endorsement by the Government of Bangladesh to carry out research projects in Bangladesh

3. ICDDR,B will be accountable to the Thrasher Research Fund for the use of all funds. Funds spent at the The Royal Veterinary and Agricultural University, Denmark will be transferred from ICDDR,B to The Royal Veterinary and Agricultural University, Denmark. The Royal Veterinary and Agricultural University, Denmark will be accountable to ICDDR,B for the use of funds.

4. ICDDR,B will be responsible for all administrative procedures and reporting to the Thrasher Research Fund. The Royal Veterinary and Agricultural University, Denmark will report to ICDDR,B regarding administrative procedures.

The PI, co-PI and co- investigators have worked closely together in the pilot phase of the project in October 2000.

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.

Name	Position	Date of Birth
MD. Abdul Wahed **January, 1947**	**Associate Scientist**	**1th**

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

Institution and Location	Degree	Year	Field of Study
Wageningen Agrilcultural University, The Netherlands.	Graduate Course	Oct 1998	International Graduate Course on: Production and Use of Food Composition Data in Nutrition
Asia Pacific Food Analyses network, Brisbane, Australia.	Training	Nov 1997	Training Workshop: Food analyses
Iowa State University, USA	Training	Aug 1992	Assessment of Human Vitamin A status
DUNN-MRC Nutrition Lab, Cambridge	Training	Apr 1988	Nutrition Biochemistry Methodologies and techniques
Bangladesh Management Development Centre	Course	Feb 1985	Industrial Quality Contro
Biochemistry Departmnent of Royal Perth Hospital, University of Western Australia	Stay	Feb1984/Sep 1985	Clinical Biochemistry
United Nations University, INMU, Bangkok, Thailand	Postgraduate	1982	Nutritional Biochemistry
University of Rajshahi	Bachelor	1966	Chemistry,Physics, Mathematics

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).**

Employment:

Present	Associate Scientist and Head, Nutritional Biochemistry Laboratory Sciences Division, International Centre for Diarrhoeal Disease Research (ICDDR,B), Bangladesh
June 2000	Consultant, Department of Paediatrics, Aga Khan University, Karachi, Pakistan. Vitamin A estimation by HPLC
May-June, 1992	Project Consultant, Department of Public Health Science, University of Alabama, Birmingham, USA - "Collaborative Vitamin A" project
1990	Consultant, Aga Khan University, Karachi, Pakistan (to set up Nutrition Biochemistry lab methods)
1988	Consultant - Evaluation of laboratory methods & procedures to support ORS study in Kenya (Collaboration between Kenya Med Res Council and ICDDR,B)
November, 1983-February 1984	Biochemist, Diarrhoea Control Centre, Dammam, Saudi Arabia (Collaborative Project of ICDDR,B)
February,1979-81	Guest Faculty in Clinical Biochemistry at the Institute of Medical Laboratory Technology, Dhaka

Current responsibilities:

a) Investigators on different research projects related to nutritional biochemistry
b) Coordination with scientist in view of proposal writing, data analyses, report writing etc
c) Training to junior staff and non-ICDDR,B trainees
d) Overall administration of the Nutritional Biochemistry Laboratory as head of the programme.
e) Supervision of students (Postgraduate and PhD levels)
f) Collaboration with institutions both at home and overseas

Professional association:

1) Senior Vice President, Nutrition Society of Bangladesh
2) Member, Australian Association of Clinical Biochemists
3) Member, International Association for Trace Element Research in Man and Animal
4) Fellow of the Institute of Research into Science and Technology, UK

Publications (Last 6 yrs and only regarding vitamin A and trace elements):

1. **Wahed** MA, Alvarez JO, Khaled MA et al. Comparison of the MRDR and RDR in the assessment of vitamin A status in malnourished children. Am J Clin Nutr 1995; 61:1253-1256.
2. Mitra AK, Rahman MM, Mahalanabis D, Patra FC, **Wahed MA**. Evaluation of an energy-dense meal liquefied with amylase of germinated wheat in children with acute watery diarrhoea: a randomized controlled clinical trial. Nut Res 1995; 15:939-951.
3. Rahman MM, Mitra AK, Mahalanabis D, **Wahed MA**, Khatun M, Majid M. Absorption of macronutrients from an energy dense diet liquefied with amylase from germinated wheat in infants with acute diarrhoea. J Pediatr Gastroenterol Nutr 1996; 24:119-123.
4. Rahman M, Mahalanabis D, **Wahed MA** et al. Conjunctival impression cytology failed to detect subclinical vitamin A deficiency in young children. J Nutr 1995; 125:1869-1874.
5. Rahman M, Mahalanabis D, **Wahed MA** et al. Administration of 25,000 IU vitamin A doses at routine immunization in young infants. Eur J Clin Nutr 1995; 49(6):439-445.
6. Shoda R, Mahalanabis, Islam KN, **Wahed MA**, Albert MJ. Effect of vitamin A supplementation on lectin induced diarrhoea and bacterial translocation in rats. Nutr Res 1996; 16(3):459-465.
7. Hussain M, **Wahed MA**, Haque ATM, Jahan F. Zinc concentration of breast milk and its diurnal variation in Bangladeshi mothers. BMRC Bull 1996; 22(2): 7-73.
8. Rahman MM, Mahalanabis M, Alvarez JO, **Wahed MA** etal. Acute respiratory infections prevent improvement of vitamin A status in young infants supplementaed with vitamin A. J Nutr 1996, 126(3):628-33.
9. Van Loon FP, Banik AK, Nath SK, Patra FC, **Wahed MA** et al. The effect of L-glutamin on salt and water absorption: a jejunal perfusion study in cholera in humans. Eur J Gastroenterol Hepatol. 1996,8(5);443-8
10. **Wahed MA**, Alvarez JO, Rahman MM, Hussain M, Jahan F, Habte D. Subclinical vitamin A deficiency among young infants in Bangladesh. Nutr Res 1997; 17(4):591-598
11. Rahman MM, Mitra AK, Mahalanabis D, **Wahed MA et al**. Absorption of nutrients from an energy dense diet liqufied with amylase from germinated wheat in infants with acute diarrhoea. J Gastroenterol Nutr. 1997,24(2);119-**23**
12. Haskell MJ, Handleman GJ, **Wahed MA** et al. Assessment of vitamin A status by the deuterated retinol dilution technique and comparison with hepatic retinol concentration. Am J Clin Nutr 1997; 66:67-74.
13. Mazumder RN, Hoque SS, Ashraf H, Kabir I, **Wahed MA**. Early feeding of an energy-dense diet during acute shigellosis enhances growth in malnourished children. N Nutr 1997; 127(1):51-54.
14. Rahman MM, Alvarez JO, **Wahed MA**, Islam MA, Habte D. Effect of early vitamin A supplementation on cell-mediated immunity in infants younger than 6 months. Am J Clin Nutr 1997; 65:144-148.
15. Rahman MM, Mahalanabis D, Ali M, Mazumder RN, **Wahed MA**, Fuchs GJ. Absorption of macronutrients and nitrogen balance in children with dysentery : an amylase treated energy dense porridge. Acta Pediatr. 1997,86(12);1312-6.
16. Haskell MJ, Islam MA, Handleman GJ, Peerson JM, **Wahed MA** et al. Plasma kinetics of an oral dose of [2H4] retinyl acetate in human subjects with estimated low or high total body stores of vitamin A. Am J Clin Nutr. 1998;67:1-6
17. Mitra AK, Alvarez JO, **Wahed MA**, Fuchs GJ, Stephensen CB. Predictors of serum retinol in children with shigellosis. Am J Clin Nutr 1998 Nov; 68(5):1088-94.
18. Rice AL, Stoltzfus RJ, de Francisco A, Chakraborty J, Kholhede CL, **Wahed MA**. Maaternal vitamin A or beta-carotene supplementation in lactating bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency. J Nutr 1999 Feb; 129(2):356-65.
19. Mitra AK, Alvarez JO, Guay-Woodford L, Fuchs GJ, **Wahed MA**, Stephensen CB. Urinary retinol excretion and kidney function in children with shigellosis. Am J Clin Nutr 1998 Nov, 68(5):1095-103.
20. Haskell MJ, Mazumder RN, Peerson JM, Jones AD, **Wahed MA**, Mahalanabis D. Use of the deuterated retinol dilution technique to assess total body vitamin A stores of adult volunteers consuming different amounts of vitamin A. AM J Clin Nutr 1999;70(5);874-80.
21. **Wahed MA** ,Mitra AK, AK Azad, Fuchs GJ. Retinol concentration in liver and serum among children who died in a diarrheal hospital Nutr. Res;1999,19(12):1719-29
22. Rahman MM, Mahalanabis D, Hossain S, **Wahed MA**, Alvarez JO, Siber GR et al. Simultaneous vitamin A administration at routine immunization contact enhanced antibody response to diptheria vaccine in infants younger than six months. J Nutr.1999,129(12):2192-5.
23. Osendarp SJ, van Raaij JM, Arifeen SE, **Wahed MA**, Baqui AH, Fuchs GJ. A randomized placebo control trial of the effect of zinc supplementation during pregnancy on pregnancy outcome in Bangladeshi urban poor. AM J Clin Nutr.2000,71(1):114-9
24. **Wahed MA** et al. (2000). Vitamin A and zinc deficiency among urban poor children in Bangladesh. The FASEB Journal, A536
25. **Wahed MA**, Haque R, Rahman HASM, Ahmed T and Albert JM (2001). Effect of β-carotene supplementation and anti-helminthic therapy on vitamin A status in preschool children in Bangladesh. Presented at: Experimental Biology 2001, 31st March – 4th April 2001, Orlando, Florida, U.S.A.

Chapters in books:

1. **Wahed MA**, Molla AM, Sarker SA, Rahaman MM, Greenough WB III. Hypernatremic dehydration in Bangladesh. In: An Annotated Bibliography: Oral Rehydration Therapy.World Health Organization.2nd ed. Washington: Pan American Health Organization,1983:131.
2. Rahaman MM, **Wahed MA**. Direct nutrient loss and diarrhoea. In: Diarrhoea and Malnutrition ed: Chen & Scrimshaw. 1983: 55-160.
3. Rahman MM, **Wahed MA**, Mahalanabis D, Sack RB. Preparing and preserving green leafy vegetables for poor communities in Bangladesh. In: Wasantwisut E, Attig GA, editors. Empowering Vitamin A Foods: A Food-Based Process for the Asia and Pacific Region. Salaya: Institute of Nutrition, Mahidol University, 1995: 61-67.

Scientific papers in preparation:

1. Rahman MM, **Wahed MA**, Fuchs GJ, Baqui AH, Vermund SH, Alvarez JO. Effect of simultaneous zinc and vitamin A supplementation on diarrhea and acute respiratory infection in Bangladeshi children.
2. Rahman MM, Tofail F, **Wahed MA**, Fuchs GJ, Baqui AH, Alvarez JO. Short term supplementation with zinc and vitamin A: no effect on the growth of undernourished Bangladeshi children.
3. Rahman MM, **Wahed MA**, Fuchs GJ, Baqui AH, Alvarez JO. Synergistic effect of vitamin A and zinc on the biochemical indices of vitamin A nutrition in children.

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.

Name	Position	Date of Birth
Shakuntala Haraksingh Thilsted (Ms., Dr.)	**Associate Professor**	**29th October, 1949**

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

Institution and Location	Degree	Year	Field of Study
Department of Animal Science, The Royal Veterinary and Agricultural University (RVAU), Denmark	Ph.D.	1980	Physiology of Nutrition
Veterinary Faculty for FAO Fellows, RVAU, Denmark	Postgraduate Course	1976	Postgraduate Course in Physiology of Animal Nutrition
University of the West Indies, Trinidad	B.Sc.	1971	B.Sc. Tropical Agriculture, Upper Second Class Honours (Animal Production Option)

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).**

Employment (last 15 years):

Sep 1992 - present	Associate Professor, The Research Department of Human Nutrition, RVAU, Denmark
Dec 1996 and Apr - May 1997	Nutrition Consultant, International Fund for Agricultural Development (IFAD), Italy
Nov 1995 - Jan 1996	Nutrition Consultant, UNICEF, Arab Republic of Egypt
Oct - Dec 1994	Nutrition Adviser Central Laboratory for Food and Feed, Agricultural Research Centre, Ministry of Agriculture, Land Reclamation, Fisheries and Animal Wealth, Arab Republic of Egypt
Jan 1991 - Aug 1992	Associate Professor, Department of Production Physiology and Human Nutrition, RVAU, Denmark
Dec 1989 - Dec 1990	Coordinator, Child Health Programme, International Centre for Diarrhoeal Disease Research (ICDDR,B), Bangladesh
Jan 1988 - Nov 1989	Nutrition Coordinator, Child Health Programme, ICDDR,B, Bangladesh
Jul - Dec 1987	Nutrition Coordinator, Urban Volunteer Programme, ICDDR,B, Bangladesh
Jan 1985 - Jun 1987	Associate Professor, Department of Animal Physiology, RVAU, Denmark

Posts held:

1999 - present	Member of the Steering Committee, Danish Network for Poultry Production and Health in Developing Countries
1997 - present	International Coordinator, RVAU, Denmark
1997 - present	Coordinator, Interuniversity Conference for Agricultural and Related Sciences in Europe, White Paper Project
1996 - present	Member, Research and Ph.D. Committee, Research Department of Human Nutrition, RVAU, Denmark
1996 - present	Member of the Steering Committee of the European Forum for International Nutrition
1995 - present	Coordinator of the DANIDA (Danish International Development Assistance) ENRECA (The Bilateral Programme for Enhancement of Research Capacity in Developing Countries) project "Food and Nutrition Security in Bangladesh: Energy and Micronutrient Availability in Rice-based Diets"
1994 - present	Member, The ENRECA Health Network, Denmark

Posts held (continued):

1992 - present	Member of the Steering Committee of the Nordic Network for International Nutrition
1992 - present	Coordinator of the European Union (EU) NECTAR (Natura European Community Training programme for Agricultural universities in southern Regions)/NATURA (Network of European Agricultural (Tropical and subtropical oriented) Universities and scientific complexes Related with Agricultural development) programme "Food and Nutrition Sciences"
1993 - 1994	Member of the Board of Department, Research Department of Human Nutrition, RVAU, Denmark
1992 - 1995	Member of the Education Committee, Research Department of Human Nutrition, RVAU, Denmark
Apr 1991 - Aug 1992	Member of the Board of Institute, Institute of Anatomy and Physiology, RVAU, Denmark
1990	Member of the Consultative Group organized by UNICEF headquarters, New York, for preparing a video film "Figuring the Problem: Surveying Nutrition Status" by the British Broadcasting Cooperation (BBC) for national statistical bureaux in developing countries. Bangladesh
1989 - 1990	Member of the Consultative Group organized by the National Nutrition Council and UNICEF, Bangladesh for designing and testing a modified growth chart for Bangladesh
12 - 26 Oct 1989	Member of the Consultative Group for the Pre-appraisal of The Village Milk Production Project, Bangladesh and Collaboration between The Bangladesh Livestock Research Institute and The Department of Animal Science and Animal Health, RVAU, Denmark
1989	Member of the Committee for the National Campaign for the Protection and Promotion of Breastfeeding organized by UNICEF, Bangladesh
1989	Member of a Working Group on Breastfeeding and Weaning Diets, organized by UNICEF, Bangladesh
Jan 1988 - Dec 1989	Nutrition Consultant, Urban Volunteer Programme, ICDDR,B, Bangladesh
Mar - Nov 1986	Head of Department, Department of Animal Physiology, RVAU, Denmark
Mar 1985 - Mar1986	Assistant Head of Department, Department of Animal Physiology, RVAU, Denmark

Recent publications related to developing countries:

1. Larsen HN, Rasmussen OW, Rasmussen PH, Alstrup KK, Biswas SK, Tetens I, Thilsted SH, Hermansen K (2000). Glycaemic index of parboiled rice depends on the severity of processing: study in type 2 diabetic subjects. European Journal of Clinical Nutrition, 54: 380-385.

2. Larsen T, Thilsted SH, Kongsbak K, Hansen M (2000). Whole small fish as a rich calcium source. British Journal of Nutrition, 83: 191-
196.

3. Thilsted SH (1999). Importance of diet diversity for improved nutrition. *In* Proceedings of the National Workshop on Food-based Strategies for Improving Nutrition in Bangladesh. Bangladesh Agricultural Research Council, Dhaka, Bangladesh. p. 33-34.

4. Thilsted SH, Roos N (1997). Policy issues on fisheries in relation to food and nutrition security. p.61-67. *In* Ahmed M, Delgado C, Svedrup-Jensen S, Santos RAV (eds.) Fisheries policy research in developing countries: issues, priorities and needs. ICLARM Conf. Proc. 60, 112 p.

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6. Nielsen BB, Hedegaard M, Thilsted SH, Joseph A, Liljestrand J (1998). Does ante natal care influence postpartum health behaviour? Evidence from a community based cross sectional study in rural Tamil Nadu, South India. British Journal of Obstetrics and Gynaecology, 105: 697-703.

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fish. Journal of Trace Elements in Medicine and Biology, 12:148-154.

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9. Nielsen BB, Lijestrand J, Hedegaard M, Thilsted SH, Joseph A (1997). Reproductive pattern, perinatal mortality, and sex preference in rural Tamil Nadu, South India: community based cross sectional study. British Medical Journal, 314, 1521-1524.

10. Tetens I, Biswas S, Glitso LV, Kabir KA, Thilsted SH, Choudhury NH (1997). Physico-chemical characteristics as indicators of starch availability from milled rice. Journal of Cereal Science, 26, 355-361.

11. Thilsted SH, Roos N, Hassan N (1997). The role of small indigenous fish species in food and nutrition security in Bangladesh. NAGA Supplement July - December 1997, p. 13-15.

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.

Name	Position	Date of Birth
Katja Kongsbak (Ms.)	**Ph.D. scholar**	**12th June, 1968**

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

Institution and Location	Degree	Year	Field of Study
Research Department of Human Nutrition (**RDHN**), The Royal Veterinary and Agricultural University (**RVAU**), Rolighedsvej 30, 1958 Frederiksberg C. Denmark	Msc Nutrition, Food Science and Technology	Mach 1997	Micronutrient malnutrition in developing countires.Thesis: Calcium bioavailability of small fish evaluated in rats and humans.
RDHN, RVAU, Denmark	Ph.D. course	February 1998	Ph.D. course in human Nutrition: "Research Methods in Human Nutrition"
RDHN, RVAU, Denmark	Ph.D. scholar	June 2000	Human nutrition. Micronutrient malnutrition in developing countries, especially vitamin A deficiency.
Department of Mathematics and Physics, RVAU, Denmark	Ph.D. course	February 1998	Ph. D. course in statistic: "Multivariate Analysis"

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).**

Employment:

June 2000	Ph.D. scholar
November - December 1999	Technical assistant, RDHN, RVAU
November 1998 - October 1999	Research assistant, RDHN, RVAU
September - October 1998	Research assistant, Danish Institute of Agricultural Sciences (**DIAS**), Denmark
August 1997 - August 1998	Research assistant, RDHN, RVAU
May - July 1997	Research assistant, DIAS

Teaching Experience:

January 1999	Assistant and teacher in "Food and Nutrition Security in Developing Countries" a 3 weeks' M.Sc.course, RDHN, RVAU
January 1998	Assistant and teacher in "Food and Nutrition Security in Developing Countries" a 3 weeks'M.Sc. course, RDHN, RVAU
November - December 1997	Participated in development of the M.Sc.teaching module "Food Habits and Communication: Actions and Strategies" for African universities. Funded and published by the EU (European Union) financed NATURA/NECTAR programme: Network of european Agricultural (Tropically and subtropically oriented) Universities and scientific complexes Related with Agricultural development / Natura European Community Training Programme for Agricultural Universities in Southern Regions programme

National and International Conferences:

August/September 1999	8th Asian Congress of Nutrition, Seoul, Korea (abstract, poster and oral presentation)
April 1998	The National Workshop "National Strategies for Improving Nutrition in Bangladesh" organized by Bangladesh Agricultural Research Council (BARC), Dhaka, Bangladesh.
August 1996	The Annual Meeting of the European Academy of Nutritional Sciences, Copenhagen, Denmark (abstract and poster)

Stays Abroad:

August-November 2000	Conducted a pilot study on "The effectiveness of vitamin A rich small fish in improving vitamin A status in children in Bangladesh" in Mirpur slum, Dhaka, Bangladesh
April - June 1999	Field study in Bangladesh. Conducting the food consumption study "Validation of 1 d observed food weighing and 24 h recall methods in rural Bangladesh" with Professor Nazmul Hassan, Institute of Nutrition and Food Science, University of Dhaka, Bangladesh
April 1998	Field visit in Bangladesh
1998/1999	Participated in international meetings in connection with preparation of the EU-financed project Interuniversity Conference for Agricultural and Related Sciences in Europa)
November - December 1997	Wageningen University, The Netherlands. Development of the M.Sc. teaching module "Food Habits and Communication: Actions and Strategies" together with African and Dutch colleagues financed by NATURA/NECTAR programme

Other Activities:

April - December 1999	Member of the board of the department, RDHN, RVAU

Publications:

1. Hansen M, Thilsted SH, Sandström B, Kongsbak K, Larsen T, Jensen M, Sørensen SS (1998). Calcium absorption from small soft-boned fish. Journal of Trace Elements in Medicine and Biology; 12: 148-154.

2. Kongsbak K, Thilsted SH, Hansen M, Larsen T (1996). Calcium bioavailability of Bangladeshi small fish and amaranth leaves evaluated in rats (Abstract). European Academy of Nutritional Sciences, Annual Meeting: The role of trace elements for health promotion and disease prevention, Denmark.

3. Kongsbak K, Hassan, N, Thilsted SH (1999). Validation of 1 d observed food weighing and 24 h recall methods in rural Bangladesh (abstract). 8thAsian Congress of Nutrition. 29th August - 2nd September, Seoul, Korea.

4. Larsen T, Thilsted SH, Kongsbak K, Hansen M (2000). Small fish as a rich calcium source. British Journal of Nutrition; 83: 191-196.

Detailed Budget for New Proposal

Project Title: "The Efficacy of Vitamin A Rich Small Fish in Improving Vitamin A Status in Children in Bangladesh"

Name of PI: M. A. Wahed

Protocol Number: Name of Division: Laboratory Sciences Division

Funding Source: Thrasher Research Fund Amount Funded (direct): 190,357 US$ Total: 203,287 US$ Overhead (%): 7% of the total grant minus equipment: 12,930 US$

Starting Date: 01-10 -2001 Closing Date: 30-09 -2004

Strategic Plan Priority Code(s):

Sl. No	Account Description	Salary Support			US $ Amount Requested		
	Personnel	**Position**	**Effort%**	**Salary**	**1st Yr**	**2nd Yr**	**3rd Yr**
1	M A Wahed, Principal Investigator	Man month 9	20	1480.00	2,664		
2	Physicians (3) (No. of persons)	12	100	600.00	7,200		
3	Nurses (2)	12	100	400.00	4,800		
4	Scientific officers (2)	12	100	550.00	6,600		
5	Supervisors (2)	12	100	433.00	5,196		
6	Logistic support ass. (1)	9	100	200.00	1,800		
7	Field research officer (1)	3	100	333.00	1,000		
8	Research assistants (7)	39	100	300.00	11,700		
9	Dietician (1)	6	100	300.00	1,800		
10	Health Assistants (9)	60	100	250.00	15,000		
11	Cooks (4)	12	100	140.00	1,680		
12	Kitchen helpers and attendants (24)	84	100	120.00	10,080		
13	Field assistants (3)	21	100	180.00	3,780		
14	Data analyst (1)	3	100	650.00	1,950		
15	Data entry tech. (2)	9	100	400.00	3,600		
16	Security guards (3)	21	100	120.00	2,520		
	Sub Total				**81,370**		
17	**Consultants**(3),social science, parasitololgy, food consumption	3		1000.00	3,000		
	Local Travel				0		
	International Travel				0		
	Sub Total				**3,000**		
	Supplies and Materials (Description of Items)						
18	Food for test meals etc.				19,500		
19	Medicines etc.				1,200		
20	Utensils, scales, blenders etc.				4,435		
21	Furniture/fittings etc.				1,600		
22	Syringes, needles, tubes, containers etc.				2,400		
23	Children's books, children's photos, ID cards, papers etc.				1,800		
24	Stationers, printing etc.				1,500		

25	Transport of items	900		
	Sub Totals	**33,335**		
	Equipment			
26	Freezer and refrigerator	1,150		
27	Generator and stabiliser	1,000		
28	Electronic scales, MUAC strips etc.	700		
29	Field centrifuge	800		
30	Computer	2,002		
	Sub Totals	**5,652**		

	Other Contractual Services			
	Repair and Maintenance			
31	Rent, Communications, Utilities	3,600		
	Training Workshop, Seminars			
	Printing and Publication			
	Staff Development			
32	Other expenses	1,000		
	Sub Total	**4,600**		

	Interdepartmental Services	**1st Yr**	**2nd Yr**	**3rd Yr**
	Computer Charges			
	Pathological Tests			
	Microbiological tests			
	Biochemistry Tests			
	X-Rays			
	Patients Study			
	Research Animals			
33	Biochemistry and Nutrition (blood: Retinol, RBP, prealbumin, APP, iron status, RDR etc.) (food: energy, fat, protein, vit. A etc.)	48,200		
34	Transport	2,000		
	Xerox, Mimeographs etc.			
35	Parasitology Laboratory	6,000		
36	Food anal.(Fe/Ca/Zn), blood: ACT and external validation at KVL, Denmark	6,200		
	Sub Total	**62,400**		
	Other Operating Costs			
	Capital Expenditure			

TOTAL DIRECT COST **190,358 $**

(Overhead (7%) 12,929 $)

NOTE:
In the "Time Schedule"(page 18), it is shown that the intervention trial, field work and laboratory analyses will be conducted in the first year (October 2001 - October 2002). In the following period, until October 2004, the activities will be supported by co-funding sources, please see the next section "Budget Justification". Thus the "Detailed Budget for New Proposal" on the previous page show the expected funds from the Thrasher Research Fund, which will be utilised in the first year only.

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.

1) Specific functions of all personnel and consultants (all values are based on a pilot study conducted in October 2000):

M A Wahed, Principal investigator, 20 % work load during 9 m. Overall supervision and responsible for biochemical analyses.

3 Physicians, 1 for 6 m for health examination and selection of children and together with 2 for 3 m each clinical health examination/blood drawing of potential participants (550 children) at screening, baseline (45 children) and endpoint (180 children). Routine health care and examination of children and mothers.

2 Nurses, 6 m each, assisting during clinical health examination and/or blood drawing of potential participants (550 children) at screening, baseline (45 children) and endpoint (180 children) and blood handling in the field (centrifugation of blood, separation of serum and distribution of each serum sample into storage tubes).

2 Scientific officers, 6 m each, development and testing of questionnaires and forms, training of research/health assistants, overall supervision/checking of cook, kitchen helpers, research/health assistants, quality control of recorded data, assisting the research/health assistants.

2 Supervisor, 1 for 3 m to supervise the baseline survey of 550 households and 1 for 9 m to supervise field activities, data collection etc.

1 Logistic support ass., 9 m. Office work, liaison with field and ICDDR,B. Local purchasing help.

1 Field research officer, 3 m for the baseline survey of 550 households.

7 Research assistants, 1 for 3 m for the mapping of potential participants and 6 for 6 m each. See "Research Design and Methods" - section "Test meal preparation and distribution" for further description. In addition, recording of data and assisting in the health examination, anthropometry, stool collection and qualitative study.

1 Dietician, 6 m, planning and overall supervision of test meals preparation and feeding.

9 Health assistants, 2 for 9 m each. Identification and mapping of target children, and together with 7 health assistant 6 m each, collection of baseline information of 550 children: socio-economic, morbidity, 24 h dietary recall and semi quantitative questionnaire on vitamin A food and during feeding trial: 24 h dietary recall and morbidity of 180 children as well as perception of mola.

4 Cooks, 3 m each, preparation of raw foods and cooking of all test meals, see "Research Design and Methods" – "Test meal preparation and distribution" for further description.

24 Kitchen helpers and attendants, 2 for 9 m each to work along with health assistant for mapping etc. 22 for 3 m each for cleaning of all raw foods, preparation of all raw foods etc, see "Research Design and Methods" - section "Test meal preparation and distribution" for further description. In addition, bring the child to the feeding place.

3 Field assistants, 1 for 9 m for mapping and field support activities and 2 for 3 m each for fish collection. Purchasing and collection as well supervising cleaning of all fish for test meals. See also "Research Design and Methods" - section "Test meals".

1 Data analyst, 3 m, assisting in designing programmes for entry of data collected and data analysis.

2 Data entry tech., 1 for 3 m and 1 for 6 m for data entry and cleaning.

Budget Justifications (continued)

3 Security guards, 1 for 9 m and 2 for 6 m each. Security guard (24 h) for the rented building for conducting the field trial in the slum. See "Research Design and Methods", section "Test meal preparation and distribution".

3 Consultants, 1 m each for specific scientific issues: parasitology, social science and food consumption surveys.

2) Justification for equipment to be purchased:

1 Freezer, short-term storage of fish and duplicate food samples in Mirpur
1 Refrigerator, short term cooling/thawing of foods and samples
1 Generator, due to irregular electricity supply
1 Stabiliser, due to irregular electricity supply
2 Electronic weighing scales, weighing of children
1 Centrifuge at the field office. To spin the blood and for stool preparation.
1 Computer at the field office for data entry: logbook of study children with address and administrative support.

3) Funds received by co-funding institutions:

1. International Centre for Living Aquatic Resources Management (ICLARM), Malaysia and United States of America Agency for International Development (USAID). (Pilot study already conducted)
2. Council for Development Research, Danish International Development Assistance (Danida), Ministry of Foreign Affairs, Denmark
3. Research Department of Human Nutrition, The Royal Veterinary and Agricultural University (RVAU), Denmark

Total amount of funds received from co-funding institutions: 144,474 $

Personnel

Ms Katja Kongsbak, Ph.D. scholar, Research Department of Human Nutrition, RVAU, Rolighedsvej 30, 1958 Frederiksberg C, Denmark (phone: + 45 35 28 24 97; fax +45 35 28 24 83, email: kak@kvl.dk) will be responsible for the daily running of the project, data analyses and publication of results. Funded by the Council for Development Research, Danida, Ministry of Foreign Affairs, Denmark, (2 ½ years' salary)

Dr. Shakuntala H. Thilsted, Research Department of Human Nutrition, RVAU, Rolighedsvej 30, 1958 Frederiksberg C, Denmark (phone: + 45 35 28 24 97; fax +45 35 28 24 83, email: sht@kvl.dk) is responsible for the overall implementation, monitoring and co supervision of the study as well as follow up of the study and policy implications. (3 m salary)

Transport, accommodation and allowances for co supervisor/co principal investigator

Airplane tickets (3 trips) Denmark-Bangladesh return for Ph.D. scholar Katja Kongsbak (Funded by the Council for Development Research, Danida, Ministry of Foreign Affairs, Denmark)

Airplane tickets (2 trips) Denmark-Bangladesh return for Dr. Shakuntala H. Thilsted for co supervisor (Funded by Danida, Ministry of Foreign Affairs, Denmark)

Stay in Bangladesh for Ph.D. scholar Katja Kongsbak (including accommodation and allowances)

Stay in Bangladesh for Dr. Shakuntala H. Thilsted co supervisor (including accommodation and allowances)

Transport (in Bangladesh) for Ph.D. scholar Katja Kongsbak and partial for study personnel during study (Funded by the Council for Development Research, Danida, Ministry of Foreign Affairs, Denmark)

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)

M. A. Wahed, Principal investigator:

Project title: Causes of low birth weight: effect and efficacy of micronutrients supplements
Function: Co investigator; laboratory analyses; training of personnel
Funding period: May 2001 - December 2003
Source of funding: UNICEF
Yearly amounts: US $ 463,000.00
Annual work load: 10 %

Project title: Nutritional Biochemistry Laboratory
Function: Administrative Head
Funding period: on going
Source of funding: ICDDR,B/USAID
Yearly amounts: US $ 40,000.00
Annual work load: 45 %

Project title: Size at birth and biochemical indicators
Function: Co investigator; laboratory analyses
Funding period: June 2001 - April 2002
Source of funding: The World Bank
Yearly amounts: US $ 79,000.00
Annual work load: 5 %

Project title: Arsenic exposure, health outcome and mitigation
Function: Co investigator; laboratory analyses; sample collection; liaison with collaborators
Funding period: October 2001 - September 2003
Source of funding: SIDA (Swedish International Development Agency), WHO
Yearly amounts: US $ 200,000.00
Annual work load: 20 %

Other Support (continued)

Shakuntala Haraksingh Thilsted, Co investigator:

Project title:	"Food and Nutrition Security in Bangladesh: Energy and Micronutrients Availability in Rice-based Diets" The Bilateral Programme for Enhancement of Research Capacity in Developing Countries (ENRECA)
Function:	Overall coordinator of the project
Funding period:	February 1999 - January 2003
Source of funding:	Danida (Danish International Development Assistance), Ministry of Foreign Affairs, Denmark Yearly amounts budgeted to The Royal Veterinary and Agricultural University, Denmark: *Year 1* - US $ 100,00.00 *Year 2* - US $ 125,000.00 *Year 3* - US $ 187,500.00 *Year 4* - US $ 208,000.00
Annual work load:	3 months per year

Project title:	Content and bioavailability of vitamin A, iron and zinc in commonly consumed foods in developing countries
Function:	Component coordinator
Funding period:	2001 - 2004
Source of funding:	Danida (Danish International Development Assistance), Ministry of Foreign Affairs, Denmark Yearly amounts budgeted to The Royal Veterinary and Agricultural University, Denmark: *Year 1* - US $ 72,00.00 *Year 2* - US $ 79,000.00 *Year 3* - US $ 34,000.00 *Year 4* - US $ 14,000.00
Annual work load:	1 months per year

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:

From :

Subject :

Appendix 1
International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Household Mapping Form

If the household has at least one child 3-7 years the following questions will be asked

1. **Household mapping ID#:** |___|___|___|___|
2. **Name of head of household:** _____
3. **Occupation of head of household:** _____
4. **Address/location** _____
5. **Camp** _____ Code |___|
6. **Date of interview:** |___|___|___|___| - 2001
7. **List all children in the household less than 10 years:**

SERIAL NO.	NAME	SEX 1=Male 2=Female	DATE OF BIRTH 1= Arabic 2=Bangali 3=English DD.MM.YY	AGE YY	 MM	FISH EATING HABIT DOES THE CHILD EAT FISH? 1 = Small Fish 2 = Big fish 3 = Small and big fish 4=No	VITAMIN A CAPSULE TAKEN 1 = Yes, within the last 6 m 2 = Yes, within the last 3 m 3 = No	MULTI-VITAMIN TAKEN 1 = Yes, within the last 6 m 2 = Yes, within the last 3 m 3 = No	SUFFERING FROM ILLNESS Only current illness (See code list)	DURATION OF ILLNESS (Days)	CHILD'S MAPPING ID# (0300 – 1XXX)
1											
2											
3											
4											
5											

Name of interviewer: _____ Code |___|

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Household Mapping Form

"Suffering from illness" – CODE LIST

Chronic Diseases:

1 = Persistent or chronic diarrhoea
2 = Tuberculoses
3 = Chronic pneumonia disease
4 = Asthma
5 = Diabetes Type 1
6 = Hepatitis B / Jundice
7 = Cardiac malformation

Acute Diseases:

8 = Measles
9 = Respiratory tract infections
10 = Acute diarrhoea
11 = Skin infections
12 = Typhoid
13 = Dysentery
14 =
15 =

Appendix 2

International Centre for Diarrhoeal Disease Research, Bangladesh

Fish intervention on vitamin A status in children – a study in Mirpur

Child Clinical Health Examination Form

Name of child |_____|

Basic data

1. Date of interview |__|__|__| DD MM YY
2. Child study ID# |__|__|__|__|
3. Child mapping ID# |__|__|__|__|
4. Mapping household ID# |__|__|__|__|
5. Camp _____ code |__|__|
6. Respondent 1= Mother 2= Father 3= Other_____
7. Sex 1= Male 2= Female
8. Date of birth (Bengali/Urdu calendar) |__|__|__| DD MM YY
9. Date of birth (English calendar) |__|__|__| DD MM YY
10. Date of birth (Arabic calendar) |__|__|__| DD MM YY
11. Age |__|__|__|__| Y Y M M

Anthropometry

12. Height (cm) |__|__|__|.|__|
13. Weight (kg) |__|__|.|__|__|
14. MUAC (mm) |__|__|.|__|

Immunization

15. DPT1 1= Yes 2= No
16. DPT2 1= Yes 2= No
17. DPT3 1= Yes 2= No
18. OPV1 1= Yes 2= No
19. OPV2 1= Yes 2= No
20. OPV3 1= Yes 2= No
21. Measles 1= Yes 2= No
22. BCG 1= Yes 2= No

Child mapping ID# |__|__|__|__| Child study ID# |__|__|__|__|

General Examination

23. Appearance 1= Normal 2= Ill looking

24. Pallor 1= Absent 2= Present

25. Skin condition 1= Normal 2= Rash 3= Others_____

26. Pulse |__|__|__| / m

27. Temperature |__|__| . |__| °C

28. Respiration |__|__| / Rate/min

29. Oedema 1= Yes 2= No

30. Presence of illness 1= No 2= Acute diarrhoea 3= Persistent or chronic diarrhoea
(If necessary, circle more than one) 4= Tuberculoses 5= Chronic pneumonia disease
6= Asthma 7= Diabetes Type 1
8= Hepatitis B/ Jundice 9= Cardiac malformation
10= Measles 11= Respiratory tract infections
12= Skin infections 13= PEM
14= Others_____

Systemic Examination

31. Heart (auscultation) 1= Normal 2= Added sound

32. Lungs 1= Clear 2= Wheeze 3= Crepitating 4= Rhonce

33. Abdomen, shape 1= Normal 2= Distended

34. Abdomen, tenderness 1= Absent 2= Present

35. Eye examination, signs of vitamin A deficiency:

1= XN 2= X1A 3= X1B 4= X2 5= X3A 6= X3B
7= XS 8= XF 9= Normal

Sample Collection

36. Stool sample collected 1= Yes 2= No

37. 1st deworming given 1= Yes 2= No
If yes, 38. Date of 1st deworming |__|__|__|
DD MM YY

39. 2nd deworming given 1= Yes 2= No
If yes, 40. Date of 2nd deworming |__|__|__|
DD MM YY

41. Blood sample collected 1= Yes 2= No
If yes, 42. Date of collection |__|__|__|
DD MM YY

43. Blood sample collected for RDR 1= Yes 2= No
If yes, 44. First blood (0 h) - time of collection |__|__| h

45. Second blood (5 h) - time of collection |__|__| h

Date..... Physician.....

Appendix 3
International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Household Socio-economic Form (1)

1. **Date of interview** |__|__|__|__|__|__|
   D D M M Y Y

2. **Household mapping ID#** |__|__|__|__|

3. **Name of head of household** _____

4. **Primary occupation of head of household** _____

5. **Address/location/camp** _____ **Camp code** |__|

6. **Name of child / Child mapping ID#** _____ / |__|__|__|__|

   Name of child / Child mapping ID# _____ / |__|__|__|__|

   Name of child / Child mapping ID# _____ / |__|__|__|__|

   Name of child / Child mapping ID# _____ / |__|__|__|__|

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Household Socio-economic Form (2)

Household mapping ID# |____|____|____|____| **Camp code** |_____|

Please see the attached code list!

SE ID #	Name of HH members	Sex 1 = Male 2 = Fcmal	Age YY	MM	Relation to head of HH code		Marital status code		Occupation code		Literacy status code		Current enrolled in school 1=yes 2=no	Education (Years completed)	
01															
02															
03															
04															
05															
06															
07															
08															
09															
10															
11															
12															
13															
14															
15															

International Centre for Diarrhoeal Disease Research, Bangladesh

Fish intervention on vitamin A status in children – a study in Mirpur

Household Socio-economic Form (3)

CODE LIST

Camp code:
1 = Football Ground Old
2 = ?
3 = ?
4 = ?

Marital status:
1 = Unmarried
2 = Married
3 = Divorced
4 = Separated / abandoned
5 = Widow / widower

Literacy status
1 = Can read only
2 = Can sign only
3 = Can write only
4 = Can write & read
5 = Cannot write & read

Relation to head of HH code:
1 = Head of household
2 = 1th wife
3 = 2nd wife
4 = 3rd wife
5 = Husband
6 = Son
7 = Daughter
8 = Brother
9 = Sister
10 = Nephew
11 = Father
12 = Mother
13 = Uncle
14 = Aunt
15 = Other

Occupation:
0 = Dependent (< 5 y)
1 = Student
2 = Housewife
3 = Office worker
4 = Day labour
5 = Skilled labour (radio, TV, fan, industry worker)
6 = Rickshaw / push car puller
7 = Driver
8 = Conductor / Helper
9 = Merchant
10 = Unemployed
11 = Teacher
88 = Do not know
99 = Other (specify)

Education
0 = No. formal education
01-09 = No. of class passed
10 = S.S.C. passed
11 = H.S.C. passed
12 = B. A. /B.Sc./ B. Com passed
13 = M.A. / M.Sc./ M. Com passed

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Household Socio-economic Form (4)

Household mapping ID# |___|___|___|___| **Camp code** |___|

1. Which source of water is used for:

a. Drinking	Municipality supply = 1 Tube well = 3	Own arrangement by plastic pipe = 2 Well = 4	Others = 5_____
b. Cooking	Municipality supply = 1 Tube well = 3	Own arrangement by plastic pipe = 2 Well = 4	Others = 5_____
c. Washing utensils	Municipality supply = 1 Tube well = 3	Own arrangement by plastic pipe = 2 Well = 4	Others = 5_____
d. Bathing	Municipality supply = 1 Tube well = 3	Own arrangement by plastic pipe = 2 Well = 4	Others = 5_____
e. Others	Municipality supply = 1 Tube well = 3	Own arrangement by plastic pipe = 2 Well = 4	Others = 5_____

2. Do you store water for each of the following purposes:

a. Drinking	Yes = 1	No = 2
b. Cooking	Yes = 1	No = 2
c. Washing utensils	Yes = 1	No = 2
d. Bathing	Yes = 1	No = 2
e. Others	Yes = 1	No = 2

3A. Which type of pot is used to store the water:

1. Small bucket	Yes = 1	No = 2	**5. Small container**	Yes = 1	No = 2
2. Medium bucket	Yes = 1	No = 2	**6. Medium container**	Yes = 1	No = 2
3. Big bucket	Yes = 1	No = 2	**7. Big container**	Yes = 1	No = 2
4. Jug	Yes = 1	No = 2	**8. Other_____**	Yes = 1	No = 2

Household Socio-economic Form (5)

Household mapping ID# |___|___|___|___| **Camp code** |___|

3B. How many times during 24 hours is <u>water</u> being collected (for all purposes)

|___|___|

4. What is the self-made <u>waterline</u> near to:

Latrine = 1 Drain = 2 Other = 3 N.A. = 4

5. Which type of <u>latrine</u> is used for each of the following age groups:

a.	**Males (>7 years)**	No fixed place = 1	Open = 2	Sanitary = 3	Pit = 4
b.	**Females (>7 years)**	No fixed place = 1	Open = 2	Sanitary = 3	Pit = 4
c.	5-7 years children	No fixed place = 1	Open = 2	Sanitary = 3	Pit = 4
d.	3-4 years children	No fixed place = 1	Open = 2	Sanitary = 3	Pit = 4
e.	< 3 years children	No fixed place = 1	Open = 2	Sanitary = 3	Pit = 4

6. Is the household using an individual or shared <u>latrine</u>:

Individual = 1 Shared = 2

7. Using slippers to the <u>latrine</u>:

a. **Does the study child use slippers to the latrine**

Study child ID# |___|___|___| Yes = 1 No = 2

Study child ID# |___|___|___| Yes = 1 No = 2

Study child ID# |___|___|___| Yes = 1 No = 2

b. **Total members in the household** |___|

c. **Number of members who use slippers to the latrine** |___|

Household Socio-economic Form (6)

Household mapping ID# |___|___|___|___| **Camp code** |____|

8. What kind of <u>hand washing practice</u> is being used for each of the following purposes:

Purpose	Type of agents*	How*	Source of water*
a. Before food preparation			
b. Before eating			
c. After defecating			
d. Cleaning child's bottom			
e. Other			

***Type of agent** No = 0 Water = 1 Mud = 2 Ash = 3 Soap = 4
Other = 5_____

***How** No = 0 Left hand = 1 Right hand = 2 Both hand = 3

***Source** Municipality supply = 1 Own arrangement by plastic pipe = 2
Tube well = 3 Well = 4
Others = 5_____

9A. Is the <u>drinking water</u> container being covered Yes = 1 No = 2

9B. Is the <u>raw</u> food being covered Yes = 1 No = 2

9C. Is the <u>cooked</u> food/ curries being covered Yes = 1 No = 2

10. Construction of the house:

a. **What is the <u>wall</u> made of**
Pacca = 1 Bamboo = 2 Tin = 3 Hardboard/Polythene=4
Katcha/Mud = 5 Brick made = 6 Thatched = 7 Corrugated tin = 8

b. **What is the <u>floor</u> made of**
Pacca = 1 Bamboo = 2 Tin = 3 Hardboard/Polythene=4
Katcha/Mud = 5 Brick made = 6 Thatched = 7 Corrugated tin = 8

c. **What is the <u>roof</u> made of**
Pacca = 1 Bamboo = 2 Tin = 3 Hardboard/Polythene=4
Katcha/Mud = 5 Brick made = 6 Thatched = 7 Corrugated tin = 8

d. **Is the bedroom and kitchen attached?** Yes = 1 No = 2

Household Socio-economic Form (7)

Household mapping ID#: |___|___|___|___| Camp code: |____|

11. Number of windows in dwelling house: |___|___|

12. Number of rooms in dwelling house: |___|___|

13. Open drain around the house:

a. **Is there an open drain beside the bedroom** Yes = 1 No = 2

b. **Is there an open drain beside the kitchen** Yes = 1 No = 2

14. Are domestic/wild animals presence in the household:

Goat = 1 Chicken = 2 Cat = 3 Dog = 4

15. Total monthly household income (including all sources of income):

|__|__|__|__|__| taka

16. House possession :

House rented = 1, If yes |__|__|__|__| Taka

House owned = 2

Not owned, not rented and do not pay rent = 3

17. Which of the following items does the household own:

(Please circle the answer)

a. Khat	b. Lep	c. Tosak	d. Hurricane
e. Watch/clock	f. Chair/table	g. Almirah	h. Radio
i. Television	j. Bike	k. Showcase	l. Cow
m. Goat	n. Electricity		

18. Form of fuel used for cooking: Gas = 1 Firewood = 2 Cow dung = 3 Kerosene = 4 Other = 5_____

Signature of the interviewer: _____ **Code:** |____|

Study ID |___|___|___|___|

Appendix 4

International Centre for Diarrhoeal Disease Research, Bangladesh

Fish intervention on vitamin A status in children – a study in Mirpur

Dietary Intake Assessment by 24 h recall

1. Study ID#: |___|___|___|
2. Child Mapping ID#: |___|___|___|___|
3. Name of child: |_____|
4. Household mapping ID#: |___|___|___|___|
5. Camp _____ Camp code |___|___|
6. Date of interview: |____|____|____| DD MM YY
7. Respondent(s): 1= mother 2= father 3= others (relation to the child)_____

I would like to talk now specifically about what you ate yesterday.

8. Did you eat the normal amount of food yesterday?

Yes, same as usual	1 (Go to 9)
No, more than usual	2
No, less than usual	3
Don't know	9 (Go to 9)

8a. Why didn't you eat the normal amount of food? **[MORE ANSWERS POSSIBLE]**

_____ |___|___|

_____

_____

_____

9. Now I would like you to remember exactly what you did and what foods you ate and any drinks you drunk yesterday. We will start with the first thing you did when you woke up yesterday. What time did you first eat something after you woke up yesterday?
What did you eat? **[PROBE FOR MAIN INGREDIENTS OF BHAJJIS, CURRIES, COMPOSITE DISHES]** When did you eat after that? etc. **[PROBE FOR SNACKS BETWEEN MEALS, PROBE FOR DRINKS (TEA WITH/WITHOUT MILK AND SUGAR, ALCOHOLIC DRINKS)] PROBE FOR BINP NUTRITION SUPPLEMENT AND ASK FOR FRACTIONS CONSUMED!!!**

24H-RECALL

Hour	Food (List ingredients of composite dishes separate if possible)	Food code	Amount #	Household measure	HH measure code

Study ID |___|___|___|___|

Hour	Food (List ingredients of composite dishes separate if possible)	Food code	Amount #	Household measure	HH measure code

10. Did you take any other vitamin/mineral tablets yesterday?

Yes 1
No.....2 (Go to 11)
Don't know.....9 (Go to 11)

10a. Could you describe the vitamin/mineral tablets **[PROBE vitamin A, multivitamin; ask respondent to show package; show pictures/bottles to respondent]**

_____|___|___|

_____

_____

_____

10b. D*uring the last 2 weeks did the child take any* vitamin/mineral tablets?

Yes..... 1
No.....2 (Go to 11)
Don't know.....9 (Go to 11)

10c. Could you describe the vitamin/mineral tablets **[PROBE vitamin A, multivitamin; ask respondent to show package; show pictures/bottles to respondent]**

_____|___|___|

_____

_____

_____

Finally I would like to ask you some questions about the oil you normally use for cooking in the household.

11. How many cups or small bottles of oil were used **by the entire family** for cooking purposes yesterday?

Number of cups |___|___| **OR** Number of bottles |___|___|

[IF RESPONDENT DOESN'T KNOW THEN FILL IN "99"; FRACTIONS ARE ALLOWED]

12. How many people were eating in the household yesterday?

Number of adults (< 18 y) |___|___|

Number of children (< 18 y) |___|___|

24H-RECALL CODES: (to be filled in after each field visit)

Food	Code	Amount	Amount unit	Code

Name of interviewer: _____ Code |___|

Appendix 5

International Centre for Diarrhoeal Disease Research, Bangladesh

Fish intervention on vitamin A status in children – a study in Mirpur

Semi-quantitative Dietary Assessment of Vitamin A

1. Child Mapping ID# |__|__|__|__|
2. Name of child |_____|
3.
4. Household mapping ID# |__|__|__|__|
5. Camp _____ Camp code |__|__|
6. Date of interview |__|__|__|__| DD MM YY
7. Respondent(s) 1= mother 2= father 3= others _____

FOOD CODE	FOOD NAME	CONSUMPTION DURING THE LAST 24 HOURS			OFFICE USE: FREQ/24 H SERVING SIZE			USUAL PATTERN: D = DAILY W= 1-6 DAYS/WEEK M= 1-3 DAYS/MONTHS N = NEVER			
	Bengali name	Morning Amount	Afternoon Amount	Evening Amount	S	M	L	D	W	M	N
	GREEN LEAFY VEGETABLES:										
	Data shak										
	Lal shak										
	Pui shak										
	Kolmee shak										
	Mula shak										
	Palong shak										
	Helencha										
	Misti kumra shak										
	Lau shak										
	Misti allo shak										
	Sharisha leaves										
	Kanta naute										

Child's screening no. |__|__|__|

FOOD CODE	FOOD NAME	CONSUMPTION DURING THE LAST 24 HOURS			OFFICE USE: FREQ/24 H SERVING SIZE			USUAL PATTERN: D = DAILY W= 1-6 DAYS/WEEK M= 1-3 DAYS/MONTHS N = NEVER			
	Bengali name	Morning Amount	Afternoon Amount	Evening Amount	S	M	L	D	W	M	N
	VEGETABLES:										
	Data										
	Begun										
	Shim										
	Lal shim										
	Badha kopi										
	Borboti										
	Potol										
	Korola/Uchche										
	Chichinga										
	Misti kumra										
	Kakrol										
	Dherosh										
	Tomato (Kacha)										
	Kochu data/Lati										
	FRUITS:										
	Paka Pepe										
	Anarosh										
	Paka Tomato										

Child's screening no. |__|__|__|

FOOD CODE	FOOD NAME	CONSUMPTION DURING THE LAST 24 HOURS			OFFICE USE: FREQ/24 H SERVING SIZE			USUAL PATTERN: D = DAILY W= 1-6 DAYS/WEEK M= 1-3 DAYS/MONTHS N = NEVER			
	Bengali name	Morning Amount	Afternoon Amount	Evening Amount	S	M	L	D	W	M	N
	FISH:										
	Boal										
	Katla										
	Mohashole										
	Magur										
	Mola Mach										
	Dhela										
	MEAT and EGG:										
	Gorur Mangshaw										
	Murgee										
	Dim										
	MILK and MILK PRODUCT:										
	Gorur Dudh										
	Ghee										
	Other foods?										

Name of interviewer: _____ Code |__|

Appendix 6

International Centre for Diarrhoeal Disease Research, Bangladesh

Fish intervention on vitamin A status in children – a study in Mirpur

Morbidity Form

1. **Study ID#** |___|___|___|
2. **Child Mapping ID#** |___|___|___|___|
3. **Household mapping ID#** |___|___|___|___|
4. **Camp** _____ **Camp code** |___|
5. **Name of child** |_____|
6. **Date of interview** |____|____|____| **Week of visit:** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
   DD MM YY
7. **Respondent** 1=mother 2=father 3=others (relation to the child)_____

8. Diarrhoea: 1 = Yes 2 = No
9. If yes, duration in days: ☐ Days

10. Dysentery: 1 = Yes 2 = No
11. If yes, duration in days: ☐ Days

Acute Respiratory Infection

UARI:

12. Nasal discharge: 1 = Yes 2 = No
13. If yes, duration in days: ☐ Days
14. Sore throat: 1 = Yes 2 = No
15. If yes, duration in days: ☐ Days
16. Cough: 1 = Yes 2 = No
17. If yes, duration in days: ☐ Days

18. Cold: 1 = Yes 2 = No

19. If yes, duration in days: ☐ Days

ALRI (Pneumonia):

20. Able to drink: 1 = Yes 2 = No

21. Chest indrawing: 1 = Yes 2 = No

22. If yes, duration in days: ☐ Days

23. Fast breathing 1 = Yes 2 = No

24. If yes, duration in days: ☐ Days

25. Central cyanosis 1 = Yes 2 = No

26. If yes, duration in days: ☐ Days

27. Fever: 1 = Yes 2 = No

28. If yes, duration in days: ☐ Days

29. Measles: 1 = Yes 2 = No

30. If yes, duration in days: ☐ Days

31. Night blindness: 1 = Yes 2 = No

32. If yes, duration in days: ☐ Days

33. Vomiting: 1 = Yes 2 = No

34. If yes, duration in days: ☐ Days

Child Mapping ID#|__|__|__|__| **Study ID#** |__|__|__|

35. Ear discharge: | 1 = Yes | 2 = No | 3 = one ears | 4 = both ears |

36. If yes, duration in days: ☐ Days

37. Eye infection (conjunctivitis): | 1 = Yes | 2 = No | 3 = one eye | 4 = both eyes |

38. If yes, duration in days: ☐ Days

39. Angular stomatis: | 1 = Yes | 2 = No |

40. If yes, duration in days: ☐ Days

41. Referred / hospital visit required: | 1 = Yes | 2 = No |

42. Diagnosis for which referred /hospital required: _____

_____

43. Treatment given: _____

_____

_____

Date ..... **Name of interviewer:** ..... |__|__|

Appendix 7
International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Perception of Mola Form

1.	**Study ID#**	|__|__|__|
2.	**Child Mapping ID#**	|__|__|__|__|
3.	**Household mapping ID#**	|__|__|__|__|
4.	**Camp**	_____ **Camp code** |__|
5.	**Date of interview**	|__|__|__|__| **- 2002**
6.	**Respondent**	1= Mother 2=Father
		3= Other (relation to child)_____

Introduction:
All children were given a fish curry during the study. The fish curry was made either with mola or with rui. Your child was given the mola curry every day during the study. We will like to ask you and your child who took part in the study some questions regarding mola and the mola curry.

Section A: Questions for the Mother

First I will like to ask you a question about mola in general:

7. What do you consider as the beneficial effects of eating mola?
a. Full of vitamins
b. Full of vitamin A
c. Good for/protect eyes
d. Nutritious
e. Good for health
f. Good for growth
g. Increases blood volume
h. Tasty
j. Others_____
List other options during pretesting:_____
_____

Perception of Mola Form

Study ID# |___|___|___|

8. Did your child like to eat the mola curry each day during the study?
1 = Yes 2 = No

8A. If yes, why:
a. Tasty
b. Felt full
c. Less sick
d. Happier
e. Got more energy
f. Grow better
g. Others_____
List other options during pretesting:_____

8B. If no, why not:
a. Did not taste good
b. Did not like to eat mola curry every day
List other options during pretesting:_____

---

9. Do you think that the mola curry had a beneficial effect on your child's health?

1 =Yes 2=No 3=Do not know

10. If yes, how did the mola curry benefit your child's health?
a. Felt full
b. Less sick
c. Happier
d. Got more energy
e. Grow better
f. Good for/protect eyes
g. Nutritious
i. Others_____
List other options during pretesting:_____

---

11. Did the mola curry have a <u>negative effect</u> on your child's health?
1 = Yes 2 = No

11A. If yes, what are the reasons?
List other options during pretesting:_____

Perception of Mola Form

Study ID# |___|___|___|

12. If we ask you to give mola regularly could you continue to give your child mola?
1 = Yes 2 = No

12A. If yes, why:
a. Good for the child's health
b. The child likes mola
c. Tasty
d. Contain vitamin and minerals
f. Mola is cheap
g. Others_____
List other options during pretesting:_____

_____

12B. If yes, how often could you continue:
a. Never
b. Every day
c. 1- 2 times per week
d. 3 - 4 times per week
e. 5 - 6 times per week
f. 1-3 times per mo
g. < 1 times per mo
List other options during pretesting:_____

_____

12C. If no, why:
a. Child does not like mola
b. Not tasty
c. Bones
d. Some family members do not like
e. Mola is costly
f. Others

List other options during pretesting:_____

_____

12D. Who decide in the household what the child should eat?
a. Mother
b. Father
c. Child
List other options during pretesting:_____

_____

Perception of Mola Form

Study ID# |___|___|___|

13. If we tell you that mola is good for your child's health how frequent do you wish to give mola to your child?

a. Never
b. Every day
c. 1- 2 times per week
d. 3 - 4 times per week
e. 5 - 6 times per week
f. 1-3 times per mo
g. < 1 times per mo
List other options during pretesting:_____

---

14. If we tell you that mola must be eaten with the head and eyes to benefit your child's eyes (vision), growth, health and wellbeing, will you always prepare the mola with the head and eyes?

1 = Yes 2 = No

14A. If No, why?
List other options during pretesting:_____

---

15. If we tell you that mola is good for your child's eyes (vision), growth, health and wellbeing, in which way will you prepare mola for your child?

a. With the head
b. Without head
c. Both with the head and without head
d. Fried
e. Boil
f. Smashed
List other options during pretesting:_____

---

16. The present price of mola in the market is xx taka per xxx per kilo. Will you buy mola for this price?

1 = Yes 2 = No

Perception of Mola Form

Study ID# |___|___|___|

16A. If yes, how often will you buy mola?

a. Never
b. Every day
c. 1- 2 times per week
d. 3 - 4 times per week
e. 5 - 6 times per week
f. 1-3 times per mo
g. < 1 times per mo

16B. If yes, how high a price are you prepared to pay? |__|__|__| taka/kg

16C. If no, how low a price are you prepared to pay? |__|__|__| taka/kg

Section B: Questions to the Child

17. Did you enjoy eating the mola curry?
1 = Yes 2 = No

17A. If yes, why?
List other options during pretesting:_____
_____

17B. If no, why not?
List other options during pretesting:_____
_____

18. Did the mola curry make you feel different?
1 = Yes 2 = No

18A. If yes, how?
List other options during pretesting:_____
_____

18B. If no, why not?
List other options during the pretesting:_____
_____

Date..... Signature of interviewer..... Code |___|

THE ROYAL VETERINARY AND AGRICULTURAL UNIVERSITY
Registrar's Office
Secretariat for Developing Countries

KVL

Appendix 8

International Centre for Diarrhoeal Disease Research
GPO Box 128
Dhaka-1000, Bangladesh
Att. Dr. Wahed

J.nr. 834/171-9
MIJ
Phone +45 3528 2857
February 2, 2001

Memorandum of Understanding

I am pleased to forward you your copy of the Memorandum of Understanding (MoU), duly signed by the Rector of KVL Mr Bent Schmidt-Nielsen.

Please let me take this opportunity to thank you for the collaboration on this matter and I hope that the MoU can develop further the collaboration between our institutions.

Yours sincerely

Mikael Jørgensen
Head of Section

1/4

Phone +45 3528 2828 Fax +45 3528 2654

Memorandum of Understanding (MoU) between The Royal Veterinary and Agricultural University, Denmark and International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B)

A. Partners

1. International Centre for Diarrhoeal Diseases Research, Bangladesh (the Centre)
   GPO Box 128
   Dhaka 1000
   Bangladesh

2. The Royal Veterinary and Agricultural University of Denmark
   Rolighedsvej 30
   DK-1958 Frederiksberg C
   Copenhagen, Denmark

B. Background

The Royal Veterinary and Agricultural University, Denmark (KVL) and the International Centre for Diarrhoeal Disease Research, Bangladesh (the Centre) share common interests of expanding scientific research and promoting educational exchange through a collaboration in the areas of nutrition and food sciences. Researchers in the KVL Research Department of Human Nutrition have expressed an initial interest in working with Centre scientists on nutritional field studies to be conducted in Bangladesh. Other areas of collaboration are expected as an outgrowth of this initial research activity.

C. KVL

The Royal Veterinary and Agricultural University, Denmark (KVL) is a university that specialises in veterinary medicine, agricultural sciences, horticulture and food sciences. KVL through its Research Department of Human Nutrition awards advance degrees in Food Science and Nutrition and conducts nutrition research through its Department of Human Nutrition. As a means of expanding opportunities for field research in Bangladesh for its advanced degree candidates, KVL seeks to conduct nutrition research in collaboration with the Centre.

D. ICDDR, B—Centre for Health and Population Research

The Centre was established in 1978 as an international research institution and successor to the Cholera Research Laboratory created in 1960 to study the epidemiology, treatment and prevention of cholera. The mission of the Centre is to "develop and disseminate solutions to major reproductive and child health problems facing the developing world." Today the Centre conducts laboratory, clinical and public health research, trains health professionals and provides services in the following areas: child survival and child health, reproductive health, nutrition and emerging and re-emerging infectious diseases and vaccine evaluation.

E. Areas of Collaboration

It is agreed that both institutions will collaborate in studies in the fields of nutrition, and food sciences, which may be undertaken by the two institutions as and when funding becomes available. Other general areas of cooperation may include, staff exchanges, curriculum development and study abroad for students from KVL and staff from ICDDR,B. The roles and responsibilities of each party in carrying out research protocols and other collaborative arrangements may be decided on mutually agreeable terms. As a general principle, the cost of activities is to be reimbursed to the organisation where the activities are carried out.

It is also agreed that the research carried on under this joint collaboration will lead to the acquisition of knowledge that will be helpful in advancing research in the fields of nutrition and international health.

F. Publishable Results and Findings

Any research activity will receive requisite approval by Research Review Committee (RRC) and Ethical Review Committee (ERC) of ICDDR, B, and appropriate administrative channels of KVL. Any major changes in the implementation of research will be discussed between the parties in advance and, where necessary, will be submitted to the RRC and ERC for approval. At every stage of work (i.e. starting from protocol development, implementation of activities, up to publication of research papers, if any) both the parties will review, evaluate and decide on actions and out come of the work in joint meetings as mutually agreed.

Both the parties will be acknowledged for their contributions in the dissemination of research findings and outcomes.

3/4

G. Termination

Either party may request for termination of this MoU at any time. However, ongoing activities should be allowed to continue in accordance to the specifics outlined programwise.

I. Amendments

Amendments or changes to this MoU shall be made in writing and signed by the duly authorised representatives of both parties. The terms and conditions of any specific programme between the Centre and KVL will be discussed by both parties and set forth in a subsequent Agreement between the parties.

J. Effective Date

The Memorandum of Understanding shall become effective upon signature by the respective parties and shall remain in force until further notice.

Whereas, the undersigned do hereby agree to the terms set forth in this MoU.

Professor David A. Sack
Director
ICDDR,B

Bent Schmidt-Nielsen
Rector
The Royal Veterinary and
Agricultural University

Appendix 9.1

APPENDIX – 9.

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Voluntary Consent Form for Screening Children for Study

Before recruiting into the study, the study subjects 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 subjects 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 subjects must indicate his/her acceptance of participation by signing or thumb printing on this form

Title of the project:	The efficacy of vitamin A rich small fish in improving vitamin A status in children in Bangladesh
Protocol No.:	2001-025
Investigators:	M. A. Wahed[1], Katja Kongsbak[2], Sakuntala Thilsted[2]
Organisation:	[1] Head, Nutritional Biochemistry, Laboratory Sciences Division, ICDDR,B [2] Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (KVL)

Vitamin A deficiency is an important health problem in Bangladesh. It may lead to night blindness and other eye changes, and even to complete blindness if the deficiency is not identified and effectively treated. Moreover, vitamin A-deficient individuals cannot fight well against infectious diseases.

From earlier research studies we know that over half of the pre-school children in your area have marginal vitamin A deficiency, although they do not have any manifestation of deficiency. Your children, 3-7 years of age, might also have such deficiencies. We are from ICDDR,B conducting a research study in collaboration with The Royal Veterinary and Agricultural University, Copenhagen, Denmark. The main purpose of our study is to examine ways to improve vitamin A status of children who have marginal deficiency of vitamin A.

Since you have a child 3-7 years of age, we request for your permission to enroll her/him in our study, and if you agree the followings would be done:

1. We shall collect information on your socio economic status, the types of foods that your child usually takes including those containing vitamin A, and common illnesses that she/he had suffered from in the recent past. One of our study staff would visit your home to collect such information, which would take less than an hour. The study staff would also measure weight, height and mid upper arm circumference of your child to assess her/his nutritional status. A physician will examine your child to assess if she/he has any recent or chronic illness, and if an illness is identified she/he would either provide treatment at our research sub-centre located in your community or refer your child a health facility for necessary treatment.

2. Worm infestation is very common and majority children in your community have worms. We would collect a small sample of your child's stool for laboratory test and determine if she/he has worms and if they are few or too many. We would treat your child with an appropriate medicine for killing worms in her/his intestine, and repeat the dose 2 weeks later. We hope that this treatment would make your child free of worms.

3. To measure the amount of vitamin A (retinol), iron, and indicators of sub clinical inflammatory illness (acute phase proteins) we would perform certain laboratory tests. For this purpose, an experienced doctor will collect 4.0 ml (less than a teaspoonful) of blood from a vein in her/his arm. Other than momentary pain from the needle prick, small chance of discolouration of the skin surrounding the puncture site, and rare possibility of infection, collection of this amount of blood would cause no other harm to your child. We would use one-time-use, sterile syringes and needles, and also take other precautions to prevent these problems.

4. For the purpose of our study it is important that your child does not receive any type of vitamin A or multivitamin or mineral supplements during the study period without informing and discussing with our physician.

5. Some laboratory analyses take more time than expected; however, we would try to get the results as soon as possible. We would then get back to you with the results of serum retinol, and one of followings would be done: (i) if your child has severe vitamin A deficiency (i.e. serum retinol <0.35 μmol/L), we would treat your child with high potency vitamin A; (ii) if your child has marginal vitamin A deficiency (serum retinol <0.35-0.70 μmol/L), we would seek your permission to enroll your child in the next phase of our study; and (iii) if the retinol level is adequate, the child may not require vitamin A supplementation, and we would also not enroll her/him in the study.

6. All information collected from you/your child will be kept confidential, and none other than the investigators of this study would have an access to them. We would be happy to provide you information on medical problems, treatment, and results of the laboratory tests of your child.

7. There is no risk, except for the minor problems that might occur in relation to collection of blood. Possible benefits from participating in the study include identification of unrecognized vitamin A deficiency and other medical problems of your child and receive appropriate treatment for them, and also free treatment against intestinal worms.

8. Participation in this study is voluntary, and you are the only person to take decision either for or against participation of your child in the study. You would also be able to withdraw your child from the child at any time after enrollment. There is no penalty if you do not agree to our proposal of enrolling your child in the study, and also if you withdraw your consent after enrollment of your child in the study.

9. You would be able to ask questions to the doctor and the study staff about our study, health of your child, and the results of the laboratory tests performed on your child. You would also be able to communicate with the principal investigator, M. A. Wahed, at telephone number: 88 11 751

If you agree to our proposal for enrollment of our child in our study, please indicate that by putting your signature or left thumb impression at specified space below.

Thank you for your cooperation.

Parent/guardian's name	Parent/guardian's signature/LTI	Date
Investigator/representative's name	Investigator/representative's signature	Date:
Witness's name	Witness's signature	Date:

APPENDIX – 9

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Voluntary Consent Form for Screening Children for Study

Before recruiting into the study, the study subjects 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 subjects 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 subjects must indicate his/her acceptance of participation by signing or thumb printing on this form

Title of the project:	The efficacy of vitamin A rich small fish in improving vitamin A status in children in Bangladesh
Protocol No.:	2001-025
Investigators:	M. A. Wahed[1], Katja Kongsbak[2], Sakuntala Thilsted[2]
Organisation:	[1] Head, Nutritional Biochemistry, Laboratory Sciences Division, ICDDR,B [2] Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (KVL)

ঐচ্ছিক সম্মতি পত্র

ভিটামিন 'এ' এর অভাব বাংলাদেশে একটি মারাত্মক খাদ্য সমস্যা। যদি এই অভাব চিহ্নিত করা না হয় এবং তার ফলপ্রসূ চিকিৎসা করা না হয় তবে এর জন্য রাতকানা বা অন্যান্য চক্ষু রোগ এমনকি পূর্ন অন্ধত্বের কারন ও হতে পারে। তাছাড়া ভিটামিন 'এ' অভাবগ্রস্থ ব্যক্তি সংক্রামক রোগের বিরুদ্ধে সঠিক প্রতিরোধ গড়তে পারে না।

আমাদের পূর্ব গবেষনা থেকে আমরা জানি যে, আপনার এলাকার অধিকের ও বেশী স্কুলগামী ছেলে মেয়েরা নূন্যতম ভিটামিন 'এ' এর অভাবে ভুগছে। যদিও আপাত: দৃষ্টিতে এ ধরনের অভাব ধরা পড়ে না। আপনার ৩-৭ বছর বয়সী সন্তানের এ ধরনের অভাব থাকতেও পারে। কোপেনহেগেনস্থ রয়াল ভেটেরিনারী এন্ড এগরিকালচারাল বিশ্ববিদ্যালয় এর সাথে আমরা ICDDR,B থেকে যৌথভাবে একটি গবেষনা পরিচালনা করছি।

এই গবেষনার প্রধান উদ্দেশ্য হচ্ছে যে, সব ছেলেমেয়েদের নূন্যতম ভিটামিন 'এ'র অভাব রয়েছে তাদের শরীরের ভিটামিন 'এ'র পরিমান বাড়ানোর পদ্ধতি পরিক্ষা করা।

৪। আমাদের চিকিৎসকের সংগে সলা-পরামর্শ ব্যতিরেকে আপনার শিশুকে গবেষনাকালীন কোন প্রকার ভিটামিন 'এ' ও খনিজ পদার্থ জাতীয় খাবার খেতে দিবেন না।

৫। রক্ত পরীক্ষা সময় সাপেক্ষ ব্যাপার। রক্ত পরীক্ষার ফলাফল পাওয়াসাপেই আমরা আপনার নিকট আসবো :

ক) শিশুর রক্তে ভিটামিন 'এ'র পরিমান কম (০.৩৫ মাইক্রো গ্রাম) থাকলে তাকে উচ্চ মাত্রার ভিটামিন 'এ' দ্বারা চিকিৎসা করতে দেয়া হবে।

খ) পরিমান নূন্যতম (০.৩৫-০.৭০ মাইক্রো গ্রাম) এ শিশুকে পরবর্তী গবেষনায় অংশগ্রহনের জন্য আপনার অনুমতি চাওয়া হবে।

গ) পরিমান সন্তোষজনক থাকলে এ শিশুর বাড়তি ভিটামিনের প্রয়োজন নাই এবং আমাদের গবেষনায় তাকে অন্তর্ভুক্ত করা হবে না।

৬। আপনার নিকট থেকে সংগৃহীত যাবতীয় তথ্যাদি গোপন রাখা হবে। শুধু গবেষক বৃন্দ তথ্য সমূহ অবগত থাকবেন। আপনার শিশুর কোন স্বাস্থ্য সমস্যা, চিকিৎসা, রক্ত ও পায়খানা পরীক্ষার ফলাফল সমূহ আপনি জানতে পারবেন।

৭। এই গবেষনায় অংশগ্রহনের জন্য আপনার শিশুর কোন ঝুঁকি নাই তবে রক্ত নেওয়ার সময় সামান্য ব্যাথা হতে পারে। এই গবেষনায় অংশগ্রহনে কিছু লাভজনক হতে পারে; যেমন রক্তে ভিটামিন 'এ'র অভাব আছে কিনা তার সনাক্ত হওয়া, কৃমি মুক্ত হওয়ার জন্য চিকিৎসা, আনুসাংগিক কোন রোগ সমূহ নির্নয় বা চিকিৎসা এবং চিকিৎসকের উপদেশ পাওয়া।

৮। এই গবেষনায় অংশগ্রহন একান্তই আপনার ইচ্ছাধীন। আপনার শিশু অংশগ্রহন করবে কিনা তার পক্ষে-বিপক্ষে আপনিই একমাত্র সিদ্ধান্ত নেওয়ার মালিক। যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করে নিতে পারেন। আমাদের এই গবেষনায় আপনার শিশুর অংশগ্রহনে যদি

যেহেতু আপনার পরিবারে ৩-৭ বছর বয়সী শিশু রয়েছে, আমরা আমাদের গবেষনায় তার অর্ন্তভুক্তির জন্য আপনার সম্মতি কামনা করছি। যদি আপনি রাজী থাকেন, আমরা নিম্নলিখিত কার্যক্রম পরিচালিত করবো:

১। আমরা আপনার নিকট থেকে আপনার আর্থসামাজিক অবস্থা, আপনার শিশুর খাদ্য তালিকা, খাদ্যে ভিটামিন 'এ' জাতীয় খাবার কতটা থাকে এবং সম্প্রতি সে কোন রোগে ভুগে থাকলে সে সবের তথ্য সংগ্রহ করবো। আমাদের কর্মী এ ধরনের তথ্যাদি সংগ্রহের জন্য আপনার সঙ্গে আলাপ-আলোচনা করবে এবং তাতে এক ঘন্টারও কম সময় লাগবে।

গবেষনা কর্মী আপনার শিশুর ওজন, উচ্চতা এবং বাহুর মাপ নিবে। আমাদের চিকিৎসক আপনার শিশুর সাম্প্রতিক কোন রোগ-বালাই আছে কিনা তা পরীক্ষা করবেন। যদি কোন রোগ থাকে, তাকে আপনার এলাকায় অবস্থিত আমাদের উপকেন্দ্র থেকে চিকিৎসা দেয়া হবে। অথবা নিকটস্থ কোন স্বাস্থ্য কেন্দ্রে প্রয়োজনীয় চিকিৎসার জন্য উপদেশ দিবেন।

২। আপনার এলাকায় অধিকাংশ শিশুই কৃমি আক্রান্ত। আমরা আপনার শিশুর পায়খানার নমুনা সংগ্রহ করবো, তাতে কৃমি আছে কিনা বা থাকলে কি পরিমান আছে তা পরীক্ষা করবো। আমরা আপনার শিশুকে কৃমি নাশক প্রয়োজনীয় ঔষধ দিব। এবং এই ঔষধটি ২-সপ্তাহ পরে আবারো দেয়া হবে। আমরা আশা করি আপনার শিশু এ ধরনের চিকিৎসায় সম্পূর্নভাবে কৃমি মুক্ত হবে।

৩। ভিটামিন 'এ', লৌহ এবং প্রদাহ রোগের নির্দেশক আমিষ পরিমাপের জন্য আমরা কতিপয় ল্যাবরেটরী পরীক্ষা করবো। এ জন্য একজন অভিজ্ঞ চিকিৎসক শিশুর শিরা থেকে ৪.০ মি.লি. (এক চা চামচের ও কম) রক্ত সংগ্রহ করবে। রক্ত নেওয়ার সময় সূচের কারনে সামান্য ব্যাথা অনুভব হতে পারে, সূচ প্রবেশের স্থানে চামড়া সামান্য বিবর্ন হতে পারে, এ ছাড়া এই পরিমান রক্ত নেওয়ার জন্য আপনার শিশুর কোন ক্ষতির আশংকা নাই। এ জাতীয় সমস্যা প্রতিরোধের জন্য আমরা এককালীন ব্যবহার্য জীবানুমুক্ত সিরিঞ্জ ও সূচ ব্যবহার করবো।

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রাজী না থাকেন, সম্মতি দেওয়ার পরও তা প্রত্যাহার করে নেন তাতে আপনার কোন ক্ষতি বা জরিমানার সম্ভাবনা নাই।

৯। আমাদের উক্ত গবেষনা সম্পর্কে আপনার শিশুর স্বাস্থ্য সম্পর্কে, পরীক্ষাগারের ফলাফল সম্পর্কে আপনি আমাদের চিকিৎসক এবং গবেষনা কর্মীর নিকট যে কোন বিষয় জানতে চাইতে পারেন।

উপরন্তু উক্ত গবেষনার প্রধান গবেষক জনাব ডঃ ও. ওয়াহেদ (টেলিফোন ৮৮৯১৭৫৯) এর সাথে আপনি যোগাযোগ করতে পারেন।

আপনার শিশুকে যদি আমাদের উক্ত গবেষনা কার্যক্রমে অংশগ্রহনে রাজী থাকেন তাহলে নীচে স্বাক্ষর করুন অথবা বাম বুড়াঙ্গুলীর ছাপ দিন।

আপনার সহযোগিতার জন্য ধন্যবাদ।

পিতা মাতা/অভিভাবকের নাম

পিতা মাতা/অভিভাবকের সাই বা বাম বুড়াঙ্গুলীর ছাপ

তারিখ:

গবেষক/প্রতিনিধির নাম

গবেষক/প্রতিনিধির স্বাক্ষর

তারিখ:

স্বাক্ষীর নাম

স্বাক্ষীর স্বাক্ষর

তারিখ:

APPENDIX – 9

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Voluntary Consent Form for Feeding Trial

Before recruiting into the study, the study subjects 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 subjects 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 subjects must indicate his/her acceptance of participation by signing or thumb printing on this form

Title of the project:	The efficacy of vitamin A rich small fish in improving vitamin A status in children in Bangladesh
Protocol No.:	2001-025
Investigators:	M. A. Wahed[1], Katja Kongsbak[2], Sakuntala Thilsted[2]
Organisation:	[1] Head, Nutritional Biochemistry, Laboratory Sciences Division, ICDDR,B [2] Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (KVL)

You might remember our previous visit when we informed you on a research study that ICDDR,B is conducting in your community in collaboration with The Royal Veterinary and Agricultural University, Copenhagen, Denmark.
Just in case you have forgotten the details, we would like to remind you the purpose and procedures of the study. Vitamin A deficiency is an important health problem in Bangladesh. It may lead to night blindness and other eye changes, and even to complete blindness if the deficiency is not identified and effectively treated. Moreover, vitamin A-deficient individuals cannot fight well against infectious diseases.

The main purpose of our study is to examine ways to improve vitamin A status of children who have marginal deficiency of vitamin A. With your approval, we enrolled your child in the screening phase of the study, and found that she/he has marginal deficiency of vitamin A (serum retinol 0.35-0.70 μmol/L).

In this second "Feeding Phase" of the study, we want to examine if it would be possible to improve vitamin status of children with marginal vitamin A deficiency by providing them with a curry in their diet that contains a small, local fish, called "Mola", which contains good amounts of vitamin A. Since your child has marginal vitamin A deficiency, we would like to enroll her/him in this study. If you allow us to enroll your child, the followings would be done:

1. We would ask you to bring your child to the sub-centre for lunch, 6 days in a week, for 9 weeks, and also to bring her/him in the event she/he develops an illness. In the sub-centre we would provide lunch to all children with a blended curry containing one of the followings: (i) Mola fish, with high vitamin A content (ii) Ruhi fish with low or no vitamin A, and (iii) Ruhi fish with added vitamin A. We would divide all children into three groups and each group would receive one of the above curries during the entire duration of the study by pure chance, and thus your child would have equal chance to fall in any of these groups.

2. Before starting the feeding trial, we would select $1/4^{th}$ of the children in each of the 3 groups by a process similar to lottery. We would determine their Relative Dose Response (RDR), which is considered as a reliable indicator for individual's vitamin A reserve in the liver. This would require collection of 1.0 ml ($1/5^{th}$ of a teaspoonful) of fasting blood in the morning, and another 1.0 ml of blood 5 hours following an oral dose of retinyl palmitate (vitamin A). During this 5-hour period, the study children would stay at the research sub-centre where snacks and drinks, which do not contain vitamin A, would be provided. Your child would have a 25% chance to fall in this group of children. You would also be able to stay at the sub-centre with your child, and snacks and drinks would be provided to you.

3. During the 9-week study period, a study staff would visit your house every fortnight and collect information on foods that your child had taken during the previous 24 hours, and on illness that she/he might have had in the previous fortnight. If your child develops any feature of vitamin A deficiency such as night blindness or eye changes, or if she/he develops an illness that requires routine supplementation of vitamin A such as conditions with measles, pneumonia and diarrhoea, we would pull out your child from the study to safeguard her/his interest and provide her/him with high potency vitamin A as recommended by WHO. Study staff will also enquire and assess your perception about mola fish using open ended questionnaire as well as out of focus group discussion while you are assembled at the subcentre for child's feeding.

4. For the purpose of our study, your child will not be allowed to take any vitamin A or multivitamin or mineral supplements during the study without informing and discussing with our physician. At the end of 9-week trail, we would provide vitamin A to all study children.

5. At the end of the 9-week feeding trails, we would again measure her/his weight, height and mid upper arm circumference. An experienced doctor will collect 4.0 ml (less than a teaspoonful) of blood from a vein in her/his arm. Similar to before, we would select $1/4^{th}$ of the children from each of the 3 feeding groups for Relative Dose Response test. The procedures would be the same as described before, and it would require collection of an additional ml of blood, 5 hours after an oral dose of vitamin A. Your child would have a 25% chance to fall in this group of children.

6. Other than momentary pain from the needle prick, small chance of discolouration of the skin surrounding the puncture site, and rare possibility of infection, collection of a maximum of 6.0 + 5.0 = 11.0 ml of blood by 4/5 different pricks over a 9 week period would cause no other harm. We would use one-time-use, sterile syringes and needles, and also take other precautions to prevent such problems. Possible benefits from participating in the study include identification of vitamin A deficiency and other medical problems, and their appropriate treatment as well as possible improvement in their nutritional status.

7. All information obtained from you/your child will be kept confidential, and none other than the investigators of this study would have an access to those information. We would be happy to provide you information on medical problems, treatment, and results of the laboratory tests of your child. Some laboratory analyses take more time than expected; however, we would try to get the results as soon as possible.

10. Participation in this study is voluntary, and you are the only person to take decision either for or against participation of your child in the study. You would also be able to withdraw your consent at any time during the study. There would be no penalty if you do not agree to our proposal of enrolling your child in the study, and also if you withdraw your consent at a later time.

11. You would be able to ask questions to the doctor and the study staff about our study, health of your child, and the results of the laboratory tests performed on your child. You would also be able to communicate with the principal investigator, M. A. Wahed, at telephone number: 88 11 751

Thank you for your cooperation.

Parent/guardian's name	Parent/guardian's signature/LTI	Date
Investigator/representative's name	Investigator/representative's signature	Date:
Witness's name	Witness's signature	Date:

APPENDIX – 9

International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B)
Voluntary Consent Form for Feeding Trial

Before recruiting into the study, the study subjects 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 subjects 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 subjects must indicate his/her acceptance of participation by signing or thumb printing on this form

Title of the project:	The efficacy of vitamin A rich small fish in improving vitamin A status in children in Bangladesh
Protocol No.:	2001-025
Investigators:	M. A. Wahed[1], Katja Kongsbak[2], Sakuntala Thilsted[2]
Organisation:	[1] Head, Nutritional Biochemistry, Laboratory Sciences Division, ICDDR,B [2] Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Copenhagen, Denmark (KVL)

ঐচ্ছিক অঙ্গীকার পত্র

আপনার হয়তো মনে থাকতে পারে যে ইতিপূর্বে ..... আন্তর্জাতিক উদরাময় অবেষনা কেন্দ্র ও কোপেনহেগেনস্থ রয়েল ভেটেনারী ও কৃষি বিদ্যালয়ের একটি যৌথ অবেষনার ব্যাপারে অবগত করা হয়েছিল। আপনি হয়তো ভুলে যেতে পারেন। তাই, আমরা এ অবেষনার উদ্দেশ্য ও পদ্ধতিসমূহ পুনরায় আপনাকে জানাতে চাই। ভিটামিন এ'র অভাব বাংলাদেশের জন্যে একটি গুরুত্বপুর্ন স্বাস্থ্য সমস্যা। এই ধরনের অভাব কতকগুলি চোখের বিভিন্ন অসুখ এমন কি পুর্ন অন্ধত্ব ঘটাতে পারে যদি না তাকে সময়ে চিহ্নিত করা না হয় ও ফলপ্রসু চিকিৎসা না দেওয়া হয়। ..... এ ধরনের অভাবের কারনে মানুষের সংক্রামক ব্যাধির প্রতিরোধ ক্ষমতা কমে যায়।

আমাদের এই অবেষনার প্রধান উদ্দেশ্য হচ্ছে যে সমস্ত শিশুর শরীরে ভিটামিন এ'র ন্যুনতম অল্পতা আছে তাদের ভিটামিন এ'র মান উন্নত করার বিভিন্ন পন্থা নিরূপন করা। ইতিপূর্বে আপনার শিশুর রক্ত পরীক্ষা করা হয়েছে এবং ফলাফল অনুসারে আপনার শিশুর ভিটামিন এ'র ন্যুনতম অল্পতা আছে।

আমাদের হয় পর্যায়ের অবেষনায় আমরা দেখতে চাই যে, একধরনের রান্না মাছ খেলে মাছ যাতে প্রচুর পরিমান ভিটামিন এ' আছে, এ ধরনের মাছ দিয়ে রান্না করা খাবার খাইয়ে যেসমস্ত শিশু ন্যুনতম ভিটামিন এ'র অল্পতায় ভুগছে তাদের রক্তে ভিটামিন এ'র মান উন্নয়ন করা যায় কিনা। যেহেতু আপনার শিশুর ভিটামিন এ'র ন্যুনতম অল্পতা আছে এজন্য আমরা চাই আপনার শিশু অবেষনায় অংশগ্রহণ করুক। আপনি রাজী থাকলে নিম্নলিখিত পদ্ধতি পরিচালনা করতে আপনার সহযোগীতা চাই:

১। আপনি আপনার শিশুকে ১ম সপ্তাহ বিরে সপ্তাহে ৬ দিন দুপুরের খাবারের জন্য আমাদের উপকেন্দ্রে নিয়ে আসবেন। কোনকারণ অসুস্থ বিসুস্থ হলেও তাকে উপকেন্দ্রে নিয়ে আসবেন। এই উপকেন্দ্রে আপনার শিশুকে নিম্নবর্ণিত ৩ টির যে কোন এক ধরনের রান্না করা মাছ সহ দুপুরের খাবার দেওয়া হবে

ক। গলা মাছ যাতে প্রচুর ভিটামিন 'এ' আছে
খ। কুই মাছ যাতে খুব কম অথবা ভিটামিন 'এ' নেই বললেই চলে।
গ। কই মাছ ও বাড়তি অ;যোজিত ভিটামিন 'এ'

আমরা অসুস্থ শিশুকে লটারীর মাধ্যমে ৩ টি দলে ভাগ করবো এবং প্রতি দলের শিশুরা উপরে বর্ণিত যে কোন একটি খাবার অবেষনাকালীন সময় খাবে। আপনার শিশু যে কোন দলে অন্তর্ভুক্ত হতে পারে।

২। এই খাবার কার্যক্রম শুরুর আগে প্রতি দল থেকে ৫ জনের ১ ভাগ শিশুকে লটারীর মাধ্যমে নির্বাচিত করা হবে। এই নির্বাচিত শিশুদের যকৃতে ভিটামিন 'এ' র পরিমানের নির্দেশক হিসাবে রিবোটেস পরীক্ষা (রিলেটিভ ডোজ রেসপন্স) করা হবে। এই পরীক্ষার জন্য সকালে খালি পেট অবস্থায় বাচ্চার শিরা থেকে ১ মি.লি (১/৫ চা চামচ) রক্ত নেওয়া হবে। ১ ডোজ ভিটামিন 'এ' খাওয়ার ৫ ঘন্টা পর আবারো ১ মি.লি. রক্ত নেওয়া হবে। এই ৫ ঘন্টা সময়ে আপনি ও আপনার শিশু আমাদের উপকেন্দ্রে অবস্থান করবেন। আপনার শিশুকে ভিটামিন 'এ' বিহীন খাবার দেওয়া হবে। আপনাকেও জলযোগ করানো হবে। এই পরীক্ষায় আপনার শিশুর অন্তর্ভুক্ত হওয়ার সম্ভাবনা ২৬%।

৩। এই ৯ সপ্তাহ ধরে আমাদের অবেষনা-কর্মী আপনার সাথে সার্বক্ষনিক যোগাযোগ রাখবেন। আমে ২ বার আপনার শিশুর পূর্ববর্তী ২৪ ঘন্টার খাদ্য তালিকা, পূর্ববর্তী সময়ে অসুখ বিসুখের তথ্যাদি সংগ্রহ করবে। এই সময় আপনার শিশুর এমন কোন অসুখ যথা বাতজ্বর, হাম, নিউমোনিয়া, ডায়রিয়া যা ভিটামিন 'এ' ঘাটতিজনিত প্রতিক্রমন হিসেবে উক্ত শিশুকে অবেষনা থেকে প্রত্যাহার করা হতে পারে। বিশেষজ্ঞ ডাক্তার সুপারিশ মোতাবেক তাদের উচ্চমাত্রার ভিটামিন 'এ' খাওয়ানো হবে। গলা মাছের ব্যাপারে আপনার অভিমত পরিমাপ ও যাচাই করার জন্য অবেষনাকর্মী আপনার সাক্ষাৎকার গ্রহন করবে এবং আপনি উপকেন্দ্রে থাকার সময় দলবদ্ধভাবে আলোচনা করবে।

৪। অবেষনাকালীন সময়ে আমাদের চিকিৎসকের পরামর্শ ব্যাতিরেকে আপনার শিশুকে কোনকারণ বাড়তি ভিটামিন 'এ', মাল্টি ভিটামিন-সিরাপ অথবা খনিজ সিরাপ খাওয়াবেন না। ৯ সপ্তাহের অবেষনা শেষে অবশিশুকে আমরা ভিটামিন 'এ' খাওয়াবো।

৫। ৯ সপ্তাহ অবেষনা শেষে আমরা পুনরায় শিশুর ওজন, উচ্চতা

বাক্সের ন্যায় পরিমাপ করবো। রক্ত হিমোগ্লবিন এ' লৌহ ও প্রদাহ রোগের নির্দেশক আমিষ পরিমাপের জন্যে একজন অভিজ্ঞ ডাক্তার আপনার শিশুর শিরা থেকে ৫.০ মি.লি (১ চা চামুচের কম) রক্ত সংগ্রহ করবে। পূর্বের ন্যায় আবারো প্রতি দল থেকে ১/৪ শিশুর রিলেটিভ ডোজ রেসপন্স টেস্ট করা হবে। পরিমিত ১ ডোজ ভিটামিন 'এ' খাবার ও ঘন্টা পর অতিরিক্ত ৫.০ মি.লি. রক্ত নেওয়া হবে। আপনার শিশুর এই দলে অর্ন্তভুক্ত হওয়ার সম্ভাবনা ২৫%।

৬। রক্ত নেওয়ার সময় সূচের কারণে সামান্য ব্যথা হতে পারে, সূচ প্রবেশের স্থানে চামড়া সামান্য বিবর্ণ হতে পারে এবং রোগ সংক্রমনের ক্ষীণ সম্ভাবনা থাকতে পারে। তাছাড়া ৯ সপ্তাহের মধ্যে ৪-৫ বার সর্বমোট ২১ মি.লি. রক্ত নেওয়াতে কোন ক্ষতির সম্ভাবনা নেই। আমরা জীবাণুমুক্ত একবালীন ব্যবহারযোগ্য সিরিঞ্জ ও সূচ ব্যবহার করবো যাতে এ ধরনের সমস্যা না হয়। এই গবেষণায় অংশ গ্রহণে আপনার শিশুর ভিটামিন 'এ'র অভাব চিকিৎসা, অন্যান্য রোগ-বালাই নির্ধারণ ও চিকিৎসা এবং সম্ভাব্য পুষ্টি উন্নয়ন হতে পারে।

৭। আপনার নিকট থেকে সংগৃহীত যাবতীয় তথ্যাদি গোপন রাখা হবে, শুধু গবেষক বৃন্দ তথ্যগুলোই অবগত থাকবেন। আপনার শিশুর কোন স্বাস্থ্য সমস্যা, চিকিৎসা, রক্ত ও পায়খানা পরীক্ষার ফলাফল সমূহ আপনি জানতে পারবেন। রক্ত পরীক্ষা সময় সাপেক্ষ ব্যাপার তবুও আমরা তাড়াতাড়ি ফলাফল পাওয়ার চেষ্টা করবো।

৮। এই গবেষণায় অংশ গ্রহণ একান্তই আপনার ইচ্ছাধীন। আপনার শিশু অংশগ্রহণ করবে কিনা তার পক্ষে/বিপক্ষে আপনিই একমাত্র সিদ্ধান্ত নেওয়ার মালিক। যে কোন সময় আপনি আপনার সম্মতি প্রত্যাহার করে নিতে পারেন। আমাদের এই গবেষণায় আপনার শিশুর অংশগ্রহণে যদি রাজী না থাকেন, সম্মতি দেওয়ার পরও তা প্রত্যাহার করে নেন তাতে আপনার কোন ক্ষতি বা জরিমানার সম্ভাবনা নাই।

৯। আমাদের এই গবেষণা সম্পর্কে, আপনার শিশুর স্বাস্থ্য সম্পর্কে, পরীক্ষাগারের ফলাফল সম্পর্কে, আপনি আমাদের চিকিৎসক এবং গবেষণা-কর্মীর নিকট যে কোন বিষয় জানতে চাইতে পারেন।

এই গবেষণার প্রধান গবেষক জনাব এম.এ ওয়াহেদ (টেলিফোন ৮৮-১১৭৫১) এর সংগেও আপনি যোগাযোগ করতে পারেন।

আপনার শিশুকে যদি আমাদের এই গবেষণা কার্যক্রমে অংশগ্রহণে রাজী থাকেন তাহলে নিচে স্বাক্ষর করুন অথবা বাম বৃদ্ধাঙ্গুলীর ছাপ দিন।

আপনার সহযোগিতার জন্য ধন্যবাদ।

পিতা-মাতা / অভিভাবকের নাম

পিতা-মাতা / অভিভাবকের সই
বা বাম বৃদ্ধাঙ্গুলীর ছাপ

তারিখ:

গবেষক / প্রতিনিধির নাম

গবেষক / প্রতিনিধির স্বাক্ষর

তারিখ:

স্বাক্ষীর নাম

স্বাক্ষীর স্বাক্ষর

তারিখ:

Appendix 10.1

1 reviewer (1st round)

Review of Prospectus "The effectiveness of vitamin A-rich small fish in improving vitamin A status in children (7-11 yrs) in Bangladesh" (MA Wahed)

1. **Impact on child health and well-being:** The issue of sustainable (i.e., food based) solutions to marginal vitamin A deficiency requires attention since coverage with twice-yearly supplements is never universal, not sustainable in many areas, and almost never reaches children over the age of five years. Consumption of vitamin A-rich small fish, as proposed here, is a likely approach in environments in which these types of fish are or can be grown and are accepted as food for children. Impact could be significant.

2. **Practical applications:** The approach has practical applicability in many parts of Asia at least, and perhaps in other areas where aquaculture is or can be practiced. Within the local area, the applicant does not discuss the extent to which cultivation and consumption of mola is already part of the household economy for households similar to those of the children to be studied in the present project, nor whether if mola were cultivated there are significant barriers or facilitators (culturally and economically) to these fish being utilized as food for children. Because this is a potentially empowering approach, evaluation of the efficacy of the intervention is justified only if there is reasonable expectation of its being adopted and utilized on a fairly wide scale.

3. **Purposes and Objectives**: The proposal is well written and the objectives clearly stated. Interactions with iron status, infection, and potential confounding variables are well formulated. I would be pleased to see further explication of how the results will be utilized to lead into practical application.

4. **Project Design**: In general, the design (randomized intervention with a positive and a negative control) is appropriate for the questions being addressed. The attention to detail of data collection and of intervention (i.e., preparation and delivery of the test meals) is also a strength. However, there are several aspects of design that can be clarified or improved.

a) Although I am generally in agreement that 7-11 year old, non-school attending children are an appropriate study population for this project, the selection of this age group is not well justified by the applicants. Their data on prevalence of subclinical vitamin A deficiency is in younger children. The calculation of sample size and power is based in dePee's work on 7-11 year old Indonesian children; this is quite appropriate but the applicants should discuss whether they believe their Bangladeshi sample will exhibit similar or different prevalences and distribution of low serum retinol. The justification for selecting non-school attending children is the assumed higher risk of vitamin A deficiency in this group than in school-attenders, but there are no estimates or data provided as to what proportion of children are non-school attenders. Since this variable is likely to be very non-random across age (with fewer younger children being non-attenders than older children) and probably non-random across gender, the implications need to be thought out and specified. Also, among non-school attending children, what are the logistics of a centralized meal daily? This obviously depends on the childrens' activities during the day and their ability to get to the feeding site reliably.

b) Whether low serum retinol levels or other indicators of mild vitamin A deficiency are seasonal in this population is not discussed. If seasonality is characteristic, the design should take this into account and will be considerably complicated.

c) Clearly the assignment to intervention group will not be (and probably cannot be) blinded. However, it is essential that data analysis be blind to group assignment (see #5 below) and this is not mentioned.

d) Assessment of "habitual food intake" with a single 24-hour recall is proposed both at baseline and followup, and is not adequate to estimate habitual intake. At least it should be supplemented with some kind of qualitative targeted food frequency instrument that addresses significant sources of vitamin A in the diet. Additionally, a more comprehensive assessment of diet and household resources will be essential to moving from this study toward practical interventions. ICDDRB has quite a good track record in the area of dietary assessment, and there should be plenty of resources in the immediate institutional enviornment to strengthen this aspect of the study.

e) I would feel more comfortable about the ultimate utility of the work if there were a concrete plan for working on integrating the intervention into the local community on a longer-term basis.

5. **Evaluation:** The importance of assuring a single-blind (i.e., data analysis done without knowledge of intervention group assignment; see #4c above) cannot be over-emphasized. No mention is made of how the qualitative data (mother's perceptions, etc) will be addressed. Additionally, iron status is treated in the paragraph on statistical analysis as a potential confounding variable; in fact, a separate analysis of the effects of the intervention on iron status (with iron status as the outcome variable) needs to be elaborated and the implications for sample size explored.

6. **Project Site:** The project site is appropriate and accessible; the investigator and the institution have a record of work in the area.

7. **Replicability:** If the outcome is positive, there will be little need for replication per se. The logical next questions have to do with application and generalizability of the interevention. Particularly, since the additional food will be completely subsidized during the research, what are the implications for sustainability?

8. **Funding Considerations**: The budget is quite reasonable for the work proposed, and considerable co-funding is demonstrated.

2. reviewer (1st round)

Appendix 10.2

TRF Prospectus Review

Project Name: The effectiveness of vitamin A-rich small fish in improving vitam A status in children (7-11 yrs) in Bangladesh. Submitter: M.A. Wahed

Comments :

1. Impact on child health and well-being.

The project addresses a child health problem in developing countries, in my opinion however, pre-school children should be the target of this study because they have higher risk for the consequences of vitamin A deficiency. With the current level of malnutrition among pre-school children in Bangladesh, it will be more beneficial to child health and well-being to focus first on this target group. Furthermore, recommendations from this research can also be used in the Bangladesh National Nutrition Program, which will also focus on improving nutrition of the under-five. If it is planned in this way, Thrasher involvement would likely be a significant factor in making progress toward solving malnutrition among millions of children in the country where the prevalence of this problem is currently one of the highest in the world.

2 . Practical application

Food-based strategies are long term, sustainable and culturally accepted ways of controlling and preventing vitamin A deficiency in developing countries. Studies in Bangladesh had indicated both direct and indirect benefits of increasing the production and consumption of green leafy vegetables and fruits rich in pro vitamin A carotenoids, especially B-carotene in rural populations through home gardening programs. However, plant sources for vitamin A are known to have lower bioavailability.

Since a commonly consumed small fish such as mola fish has been found to have high contents of preformed vitamin A (2400-3000 RE/100g raw edible fish), a recommendation to add the fish to commonly consumed diets of young children will enhance their vitamin A status.

In my opinion, the project potentially more or less will result in immediate benefit to Bangladeshi children. However, more benefit will be obtained if the study design would also include an additional intervention group of dietary high vitamin A intake from a minimum mola fish in combination with suitable amount of

locally available vitamin A–rich vegetable(s) and/or fruit(s). Since it is known that vitamin A rich plant sources are more accessible for the poor, this study will then offer more knowledge as to how much mola fish should poor families add into their child's mixed diets in order to improve vitamin A status.

3. Purpose and objectives

As already indicated, I would prefer a study that would address food-based strategies in a more comprehensive manner in developing countries. Though it might be useful to prove whether an intake of 45 g vitamin A rich small mola fish will or will not improve vitamin A status in Bangladeshi children with marginal vitamin A deficiency. I think it would be more helpful for the poor to know a minimum amount of mola fish that should be added to a menu that combines with locally available vitamin A rich vegetable(s) and/or fruit(s). In this way, we will come up with a recommendation that will tell the poor about the feasible combination of vitamin A rich foods. Also, the smaller the amount of the fish, the more feasibility for poor families. Thus, it might be easier for the poor to link knowledge into practice in the long run.

4. Project design

Suggestion of an additional intervention group of minimum Mola fish plus locally available vitamin A-rich vegetable(s) and/or fruit(s).

5. Evaluation

The evaluation component should also include the acceptance of the proposed menu among target children and mothers or food providers if they have to cook the suggested foods.

6. Project site

In my opinion, food-based strategies should be integrated with food and agriculture development in the country. In this way, the project site for this study should be in a rural community rather than a slum community.

7. Replicability

If the design can be modified as indicated above, the project results will be very useful for sustainable vitamin A improvement in

Bangladesh. As indicated earlier, if the research is planned along the same line with the National Nutrition Program, replicability can be quite high.

8. Funding considerations
A rather expense research with scientific, top-down approach.

Appendix 10.5

3. Reviewer (1st round)

Review of: Wahed, M.A. et al.
The Effectiveness of Vitamin A Rich Small Fish in Improving Vitamin A Status in Children in Bangladesh

1. Impact on child health and well-being
The project does address one of the main nutrient deficiency in the developing world, in a country where childhood malnutrition rates are excessively high.

There are various approaches to alleviating the problem of vitamin A deficiency – supplementation, food fortification, plant breeding, dietary modifications (also referred to as food-based approaches). Of all these approaches, dietary modifications are the least advanced in terms of our knowledge of their efficacy and effectiveness. The present study addresses the important question of the efficacy of a dietary approach targeted to children and can therefore be an important contribution to our current understanding of strategies to alleviate vitamin A deficiency.

Thrasher's involvement in the evaluation of all types of interventions targeting vitamin A deficiency is important and food-based approaches are the interventions that have received the least attention to date. There is an urgent need for funding high-quality, well-designed food-based interventions to explore their real potential in solving the problem of vitamin A deficiency.

2. Practical applications
If school-age children are vitamin A deficient in Bangladesh, the project definitely has the potential to be beneficial to children. The authors document the fact that the solution is likely to be culturally acceptable and financially feasible. However, it is not clear to me that the intensity of the intervention (feeding fish to children 6 days a week) could be replicable at the level of households for both cultural and financial reasons. The authors are proposing to test the maximum potential impact that this intervention can have (if children consume the targeted amount of fish, 6 days a week), in an efficacy trial. However, they do not discuss what could feasibly be achievable in a real life situation if parents had to purchase, prepare and feed this amount of fish every day to their child (as in an effectiveness trial). This needs to be discussed in the proposal.

3. Purpose and objectives
The study has clear objectives and the proposed intervention is well selected from a cultural point of view. The authors, however, are confusing the terminology for efficacy and effectiveness of interventions. The title of the proposed study, as well as the definition of the research questions refer to an effectiveness study, when in fact, the proposed study is clearly an efficacy trial. In other words, the study is proposing to test the *efficacy* of giving 45 g of vitamin A rich fish to school age children and the delivery of the intervention as well as the compliance will be highly controlled. The study is basically testing the biological impact of feeding this amount of fish to children 6 days a week, when the intervention is carried out under ideal, highly controlled conditions. This is the definition of an *efficacy* trial. An effectiveness trial would replicate 'real life

situation', meaning that households would be encouraged to give the fish to the children, for instance, but the possibility of non-compliance would be factored in the sample size calculations and in the overall design of the study. Effectiveness trials are recommended when the efficacy of the intervention has been demonstrated first. The proposed efficacy trial is therefore an important first step in the process.

4. Proposed design

The proposed design is appropriate for an efficacy trial (see point 3 above), but not for an effectiveness trial (as the authors refer to).

There are 2 ethical issues that are not addressed properly in the proposal:

1) Having a 'control' group of children who will receive no vitamin A when they are marginally deficient (the big fish group).
2) Withholding anti-helminthic treatment when helminths are known to reduce vitamin A absorption.

The other problem with the plan to not deworm children is that it also causes a problem for the *efficacy* evaluation. If the experiment is really a true efficacy trial, an effort should be made to maximize the chances of success of the intervention by providing optimal conditions. The project does intend to provide optimal conditions related to compliance with the intervention (having a center where children's intake is monitored closely). The project should provide deworming to all children before the intervention because worms are known to interfere with vitamin A absorption. In order to maximize the potential impact of the intervention, all children should be dewormed prior to trial.

Another problem related to the design of the intervention is the selection of the age group –. school age children. Although the authors argue that vitamin A deficiency is a problem among school children in Bangladesh, this age group is certainly not the most vulnerable and the most at risk of the potentially devastating consequences of vitamin A deficiency – blindness and death. Solutions for the problem of vitamin A deficiency are more urgently needed for pregnant and lactating women and for children 6-36 months of age (the age group most likely to suffer the long-term dramatic consequences of vitamin A deficiency) than among school age children. The authors need a more convincing justification of their choice of age group for the proposed intervention.

5. Evaluation

The proposed evaluation component is adequate.

6. Project site

The proposed project site has been well thought through and seems entirely feasible.

7. Replicability

The specific intervention (small vs large fish) is highly context-specific and is unlikely to be replicable in other regions of the world and even in other countries of the region. It entirely depends on the availability of local sources of vitamin A in the food system. The

design of the proposed efficacy trial, however, can be replicated in other contexts, using available vitamin A rich products.

8. Funding considerations

The budget seems appropriate and reasonable.

Appendix 10.4

4. reviewer (1st round)

Thrasher Research Fund—Prospectus Review

P.I: Abdul Wahed Title: Effectiveness of vitamin A-rich small fish in improving vitamin A status in children (7-11 yrs) in Bangladesh

1. Impact on Child Health and Well-Being

VAD is a major problem in Bangaladesh with major health effects among < 6year olds. Much less is known as to the magnitude of the problem in older children who will be studied in this project. Presumably it is a problem but unlikely to have the severe adverse health consequences seen in younger ages. VAD is preventable through diet and this approach through promotion of horticultural interventions has had an impact on family vitamin A intakes, as well as has supplement distribution in the younger age groups. I am unaware of previous studies in Bangladesh where the addition of a serving size of vitamin A-rich fish has been evaluated.

2. Practical Application

The inclusion daily of an RDA level serving size of fish in children of the specified age is practical in this fish-eating culture provided that these fish could be harvested or made available in sufficient quantity at the community level. It is not clear from the text as to how practical this would be on a national or regional basis.

3. Purpose and Objectives

The hypothesis's focus and the overall aim is on improving vitamin A status while the specific objectives are more extensive including effects on iron status and to included some measures of parasitic infections. Since the criteria for selection of study subjects specifies that children will have hemoglobin levels below the cut-off for anemia, measurement in change of hemoglobin would likely be sufficient to test the hypothesis—the other parameters of iron status would be nice to have as they are more specific to iron status, but increase the laboratory costs considerably. By randomly subsampling, the number of analyses required might be reduced considerably. The new aspect of the proposal is the quantitative evaluation of a small fish in improving vitamin A serum levels.

4. Project Design

Design does not adequately evaluate change in vitamin A status because serum levels in this age group are likely to be less responsive, particularly if the children are not dewormed prior to screening and selection of subjects for the intervention. Chronic parasitic infection is likely to limit the plasma response and may mask the true vitamin A status on screening. To overcome this there would be need for

an indirect measure of vitamin A stores using the RDR or of total body stores using isotope dilution at least on a subset of subjects.

5. Evaluation

As noted in #4, the proposal could be strengthen by more in-depth evaluation of vitamin A status at least on a subsample of subjects in each experimental group.

6. Project Site

Site is appropriate

7. Replicability

Limited to areas where this specific species of retinol-rich small fish (or other species if identified to be similarly rich sources) are available or could be promoted.

8. Funding considerations

Over budgeted for the laboratory analyses. Not all of these analyses are critical to achieving the project aims.

A full proposal should be solicited provided the investigators can make a more convincing argument for the feasibility of production of this species of fish in adequate quantities to serve a significant population of at-risk children.

Prospectus Review 5 reviewer (1st round) Thrasher Research Fund

Appendix 10.5

Principal Investigator: Wahed	Project Name: Mola fish
Institution/Organization: ICDDR,B	
Reviewers	Date Please return by

Review Criteria	Comments (Make additional comments on the back of this page.)
1. Impact on Child Health Consider the following: Does the project address a major child health problem? How many children are affected? Where are they found? If applicable, is the problem preventable/treatable by already available methods, procedures, agents, etc.? Is Thrasher involvement likely to be a significant factor in making progress toward solving the problem?	This significant problem, affecting over 125 million children worldwide, and leading to premature death from infectious diseases, can be prevented. The most sustainable dietary diversification has not had the scientific attention paid to it that it should, and hence it is often seen as a lower priority. This is a critical part of the evidence needed to promote this intervention approach.
2. Practical Application Will the project potentially result in immediate benefit to children? Is the solution likely to be culturally acceptable, financially feasible, and technologically practical?	Results of this research could be readily applied and if they were, would result in immediate benefit. It is very much the most culturally acceptable of interventions for this problem, as well as being financially and technologically feasible.
3. Purpose and Objectives Evaluate the strength of the project's aims and objectives, particularly new and unique features.	The overall aim and specific objectives are appropriate, although 3.2. 3rd bullet point needs more specificity in a subsequent proposal. It is a fundamental scientific question, albeit very applied that is being asked. Not knowing if there is a biological impact is seriously inhibiting support for food-based programs.
4. Project Design Evaluate the adequacy of the project design and methodology.	The design should get the answer being looked for. Technically sound background of the two organizations involved.
5. Evaluation Does the proposed project have an adequate evaluation component?	Generally adequate. For 4.8 (compliance), it would be helpful to state how this will be measured and ensured, besides just "carefully informing."
6. Project Site Is it feasible to carry out the project as proposed in the specified location(s)?	Yes, definitely.
7. Replicability If applicable, is the project potentially transferable and adaptable to other settings and/or geographic areas?	Yes
8. Funding Considerations Is the budget request reasonable? Are there other ready resources for funding this type of project?	Yes to both questions. There are also lots of funds in operational research going to other related areas which will help eventual implementation, but this piece needs funding (and is sufficiently food-based, that this may not come from elsewhere.)

1/1

Appendix II.1

1. reviewer (2nd round)

Impact on Child Health

The project proposal addresses a major child health problem of global proportions and importance.Vitamin A deficiency (VAD) is seen in al most all developing countries of the world: in Southeast Asia,Africa Central and South America, India and in the Middle East.The statistics quoted in the proposal are the best estimates available and impart the magnitude of the problem.The problem of VAD is wholly preventable but food-based approaches which are appropriate and sustainable in developing countries has seriously lagged behind periodic vitamin A capsule distribution and administration in selected populations.Fortification, a potentially promising method is limited as it does not reach more remote rural populations as many people are barely in the cash economy and cannot purchase the fortified foodstuffs. The proposed research would contribute to food-based solutions for a serious problem which causes high morbidity and mortality from respiratory, diarrheal and other infections and is the leading cause of blindness in developing countries.The Thrasher Fund would contribute considerably to combatting VAD by funding this well-designed research study by what appears to be a very competent and experienced team.Also the collaborating two institutions are well known and highly respected.

Practical Application

The project has the high likelihood of bringing immediate improvement in the nutritional status of the study children.All groups would benefit from the extra food received all with an excellent source of fish protein and iron, calcium, riboflavin and zinc.The Mola fish group and the low vitamin fish plus the added retinal would both improve the retinol status of the recipients as well.

if the logistics of the local raising of the mola fish and the daily feeding is shown to improve the VAD, iron and general nutrition status, an is acceptable to the recipients then this would be a great advance in the prevention of VAD and ameliorate iron deficiency anemia.The intervention of the raising and eating of mola fish is very likely to be culturally acceptable, financially feasible and technically practical.In preparation of this proposal a an adequate and successful pilot study was carried out with promising results.Also other groups such as the World Bank are demonstrating the large scale -raising of the mola fish which appears to be accepted by the population.Other parts of the world ,where fresh water fish is eaten ,would likely be open to the introduction of the mola or similar small vitamin A- rich fish.Although not mentioned, the mola or other small fish would improve calcium status as well.

Funding Considerations

The budget appears reasonable although reduction in the frequency of certain measurements would not compromise the study design and ability to come up with scientific results There is almost overkill in the case of having weekly 24 hour food intakes and vitamin food frequency intakes.The burden of data entry and analyses for nutrients will be very large and will not add that much information.Also it appears excessive to me to be measuring the nutrient composition of the intervention feedings for macro and micro-nutrients.

Purpose and Objectives

The first two and last specific objectives of the project are clear,well articulated and precise as to what ,when , by whom and how the interventions and measurements will be carried out.The approach of using a small retinal-rich fish which contains the approximate daily recommended intake of retinol, is generally accepted by the population and is easily raised at an affordable cost, is an innovative approach to a very serious health problem.

The third objective is unwieldy and includes far too many co-variates than can be simultaneously analyzed in multivariate fashion given the sample size.There are about 5 or 6 research questions posed in a single specific objective.It overlaps to an extent with the second objective of "investigating the impact of vitamin A -rich fish on iron status etc vs the other intervention groups.
It would be better to simplify this into several objectives or omit this.The parasites, infection,morbidity food intake ,anthropometry can be used as co- variates wherever appropriate in the other objectives.

Project Design

The project design overall seems sound.However i have some concerns.With such strict and restrictive enrollment criteria I question if there will be an adequate number of children available who fit the criteria.Also, given the nature of the population who live in slum surroundings and children who are not enrolled in school I wonder what the daily attendance will be like at the intervention feeding ,with meals being skipped and perhaps a high overall attrition rate excluding children with clinical infection does not make sense as they are bound to have infections from time to time while in the study.Even without obvious infection they could have infection detectable only with markers of infection such as C-reactive protein(CRP) and/or alpha 1 ACT). Tuberculosis may not be clinically apparent.I wonder why children with mild xerosis or night blindness (an early sign) but no other clinical signs of VAD are being excluded?I would suggest that at least 80 or more children , not 60 be enrolled and the frequency of the food intake measures and food nutrient analyses be reduced in frequency a mentioned under "funding considerations"Also where there are so many co-variates included for data collection, for multivariate analyses ,of any sort, the sample sizes may be too small.

I question if three months of feeding during only one season is too short a period.Given the sizable investment in such a study would not 5 or 6 months of feeding which takes place in more than one season,with less frequent measures as suggested above ,not make for a stronger study?This might increase the cost , but not by much since the infra structure would already be present.sdemonstrating the large scale -rais

I have some questions about some of the measurements.Taking one 24 hr. recall of food intake at baseline does not measure usual food intake as stated.Using the screening food intake data as the baseline value .It would be good to repeat the 24 hr recall at the baseline for a better estimate of the usual intake.

As for the test meals ,I question the ad lib intake of rice.Would it not be better to weigh out the rice based on the child's requirements based on size and age etc.If a child is sick he/she may have decreased intake at a time when they need more energy intake? Also since anthropometry is being measured energy intake would be important to measure as best as one can.or would the exact rice intake be measured after the intervention meals?where is the precribed amount of calories stated?Also in regard to food intake, why should there not be a food frequency for iron containing foods plus the enhancers of iron absorption such as citrus as well as phytate and fiber, tea, milk etc. which reduce bioavailablility of the iron.

As for biochemical studies I have a few questions.Transferrin receptor studies are very expensive ($15 per test).Ferritin becomes elevated with infection and inflammation.The ratio of ferritin to transferrin receptor values would be distorted.At least the infection markers will be used. to help interpret elevated ferritins.Although serum iron is reduced in the face of infection this may still be a useful measure.

It would be very important to determine vitamin A stores.Instead of the RDR test requiring 2 venipunctures at a set interval the modified MDR using Vitamin A-2, an anolog of retinol can be done with one one blood sample obtained 4-5 hours after the vitamin A-2 dose.The field worker could administer this dose at a home visit or a the feeding site.The A-2 we obtained from the University of Wisconsin from the Dept of Nutrition (will send the name if interested).For this study it would cost about $2000 for the vitamin A-2.

I am surprised to hear that deworming for helminths is considered ineffective in reducing the worm burden and improving retinol ststus.Ascaris can adversely affect retinol absorption and for this reason deworming would be important.

As for a marker for protein -malnutrition ,pre- albumen may be superior to albumen and has a similar methodology.

As for the data analyses this section is weak.A statistician with expertise in longitudinal data analyses is required.as change over time for a number of indicators are required.There is little mention of the appropriate statistical methods to accomplish this.A more detailed statistical analytic plan is needed.Lastly, the third objective ,as written ,would be an analytic challenge!!!and should be simplified and rewritten.

Evaluation

DNA

Project Site

The project site is most suitable for this study. The research facility is close to the subject population and is in a concentrated area.Also the children will benefit from being in the study irregardless of the type of intervention plus the exams and testing and will receive care if seriously ill.(It is sad to think that so many children in this slum do not attend school.)

Replicability

The intervention has a reasonable chance of being sustainable and replicable ,if the findings are positive and give results comparable to those receiving vitamin A supplements.The proposed study has the potential for successfully preventing or ameliorating VAD .Provided that people have the sites for small fish raising and would eat fresh water fish this intervention should it prove to have positive findings would ,I believe, be highly transferable to many other areas, given that the mola or similar fish can thrive in a variety of environments.

Summary

Overall, the project is in the low superior rank in my judgement.It is a practical ,potentially very valuable and important intervention feeding study with promise of yielding very practical and results applicable to many other areas of the world.The problem of VAD is a major cause of death and disability and morbidity and affordable, feasible and sustainable solutions are badly needed to prevent and ameliorate the situation.The study has been well researched with up to date literature ,has overall a sound design and methodology and will be carried out by experienced and highly qualified and proven researchers from two well-respected institutions with excellent research track records.

Appendix II.2

2. reviewer (2nd round)

Project Review: The Efficacy of Vitamin A Rich Small Fish in Improving Vitamin A Status of Children in Bangladesh

PI.: M.A. Wahed, ICDDR,B

Impact on child health:

Vitamin A deficiency is a major problem in Bangladesh. Although progress has been made in alleviating the problem by periodic distribution of medicinal vitamin A supplements, particularly when integrated into campaigns for immunization, there is question whether this progress is sustainable. Campaign days for immunization are scheduled to be phased out as polio immunizations have achieved the broad coverage needed for eradication. Therefore, the project proposed addresses a more sustainable way of preventing and controlling vitamin A deficiency based on a local, culturally acceptable food-based resource. Most deficient children in Bangladesh are found in rural areas where potentially available sources of vitamin A are underutilized. The most affordable sources in these areas are green leafy vegetables (GLV) which have the problem of reduced bioavailability compared to animal sources. This means consumption of larger quantities of GLV are required than necessary if preformed sources of vitamin A were consumed (i.e., animal sources such as fish). Vitamin A deficiency is preventable by vitamin A supplements but the question is whether a medicinal source is sustainable over the long term. Most experts agree that sustainability is linked to better utilization of local food-based sources of vitamin A, and preferably preformed vitamin A to overcome problems of bioavailability.

Practical Application:

The project proposed should result in immediate benefit to child health of those involved and it is very likely to be culturally acceptable in this community where fish is a traditional part of the diet. The approach using a small fish that is available in Bangladesh is financially and technologically feasible if its production is fostered on a local level through development programs.

Funding considerations:

The budget request is excessive in the amount designated for support of the PI, Wahed. He is already lists commitment of 135% of his time to other projects. Presumably these project carry compensation for salary. Therefore, it is not acceptable to provide additional salary for commitment to this project where he says that he will spend another 25% of his time (160% time commitment!). Otherwise, the budgetary commitments seem justified. A bilateral agency, i.e., DANIDA already is substantially supporting part of this initiative in the form of salaries for the Danish collaborators who will be the real on-site implementers. Other groups also funded the pilot study completed earlier.

Purposes and objectives:

The research questions addressed by the project are appropriate, as are the overall and specific aims. The problem of transferability of the findings revolve around whether it is reasonable to expect that a daily consumption of small fish will occur in economically strained populations where fish is likely to be relatively expensive. The research protocol calls for daily consumption for 9 weeks. It is questionable whether such frequency of consumption would be sustainable over longer periods when the community would have to commit resources to daily purchase of the fish. More likely, consumption would occur less frequently than on a daily basis. Therefore, the research design would evaluate effectiveness on a daily basis but not on a less frequent basis, which is more likely. The investigators do plan to question mothers on their perception about the use of mola and constraints that they might encounter in continuing its use following end of the research.

Project design: The project design overall is appropriate. They will select participants on the basis of low serum retinol levels from a large screening of those potentially eligible. This should, on a population basis, identify those with serum levels expected to respond to an intervention. The investigators will only be able to evaluate intervention impact on a population basis, not on an individual basis. Preferably, they would have done a dose response test on a subsample of the participants before and after the intervention since serum retinol levels can be artificially lowered by concurrent infection. Unfortunately there is no information in the study area on the distribution of serum levels in the age group selected for this study (7-11 years), although they report a high percentage among the preschool age with low serum levels. They will be able to correct in part for infection based on the CRP and ACT analysis. I strongly recommend a revision of the protocol to include a dose response on a subsample because it would confirm low vitamin A status and improvement by the intervention in terms of body stores. I am not sure why both serum retinol and RBP are being analyzed as well as serum albumin. RBP and serum retinol should measure virtually the same thing and the albumin will give a better reflection of long term protein status. I would put the money allocated for RBP determination into doing a dose response test.

Preliminary work on recipe testing and acceptability indicates that the three different meals will be acceptable to the children-presumably the daily consumption will be tolerable for 9 weeks.

In the positive control group, it is not clear whether the retinyl palmitate will be added to the meal during preparation of the curries or if it will be added to individual meals of each child when served.

This is a very ambitious study that will require close supervision to prevent mix up among children in different intervention groups. To feed 180 children daily in one building at the same time will not be easy. This age group may be more difficult to control than a younger group, but this is only speculation and hopefully the investigators have confidence that sustained cooperation for 6 days for 9 weeks is feasible.

Evaluation:

The evaluation component is adequate.

Project Site: The project site is appropriate. A facility on-site for meal preparation and serving makes it feasible for management of the intervention. It will be taxed, however, by serving 180 children daily at about the same time. Previous projects conducted successfully in the area suggest community acceptance of the research team.

Replicability: The project is potentially replicable to other areas with access or potential for production of the particular fish (mola) that has an unusually high content of vitamin A. If the project demonstrates impact on vitamin A status, it would need support and promotion on a national scale.

Summary:

The proposed project design is appropriate to Bangladesh where vitamin A deficiency is a major public health problem. It addresses a more sustainable intervention that that of periodic vitamin A supplementation which is the current preventive control measure. The design of the project should provide answers to the question of efficacy but may not fully answer the question of efficiency, i.e., will the intervention have an impact in a non-research context controlled at the community/household level where it is unlikely that a daily intake of the noted high retinol fish would occur and where the supply of fish may be variable.

Appendix II.3

3. reviewer (2nd round)

Review of: Wahed, M.A. et al.
The Efficacy of Vitamin A Rich Small Fish in Improving Vitamin A Status in Children in Bangladesh

Comments for authors

1. Impact on child health and well-being

The project does address one of the main nutrient deficiencies in the developing world, in a country where childhood malnutrition rates are excessively high.

There are various approaches to alleviating the problem of vitamin A deficiency – supplementation, food fortification, plant breeding, dietary modification (also referred to as food-based approaches). Of all these approaches, dietary modifications are the least advanced in terms of our current knowledge of their efficacy and effectiveness. The present study addresses the important question of the efficacy of a dietary approach targeted to children and can therefore be an important contribution to our current understanding of strategies to alleviate vitamin A deficiency.

Thrasher's involvement in the evaluation of all types of interventions targeting vitamin A deficiency is important and food-based approaches are the interventions that have received the least attention to date. There is an urgent need for funding high-quality, well-designed food-based interventions to explore their real potential in solving the problem of vitamin A deficiency.

My main concern, however, is the age group targeted by the project. While school age children are at risk of vitamin A deficiency, they are beyond the period of potentially irreversible eye damages caused by vitamin A deficiency. It is not clear to me why the project is designed to target school-age children rather than weaning-age children who are at much higher risk of severe vitamin A deficiency and of its dramatic health consequences such as increased morbidity from infectious diseases, irreversible eye damage and mortality.

2. Practical applications

The authors argue that school-age children in Bangladesh are vitamin A deficient, and thus, they have the potential to benefit from the intervention.

The authors document the fact that the solution is likely to be culturally acceptable and financially feasible. However, it is not clear to me that the intensity of the intervention (feeding fish to children 6 days a week) could be replicable at the level of households for both cultural and financial reasons. The authors are proposing to test the maximum potential impact that this intervention can have (if children consume the targeted amount of fish, 6 days a week), in an efficacy trial. The authors also argue that increased

production of mola is feasible and that efforts are currently underway to promote greater production and consumption of mola. However, the authors do not specifically discuss the issue of cost and whether or not it is possible for households to afford consuming sufficient amounts of mola 6 days a week on a regular basis. Even if increased production of mola results in increased household income from the sale of the product, it does not necessarily mean that intakes of mola will increase among the most vulnerable household members or that increases in income will translate into improved dietary quality overall. Households have a variety of pressing needs in addition to food and may chose to invest increased income in other basic necessities.

The issues of cost and cultural acceptability need to be discussed in the proposal.

3. Purpose and objectives

The study has clear objectives and the proposed intervention is well selected from a cultural point of view.

4. Proposed design

The proposed design is appropriate for an efficacy trial.

There are 2 ethical issues, however, that are not addressed properly in the proposal:

1) Having a 'control' group of children who will receive no vitamin A when they are marginally vitamin A deficient (the big fish group): I do not find the justification provided in the "Ethical Issues" section satisfying. The authors indicate that the ethical issue is not a problem since only one meal of the usual diet will be replaced. Children, even marginally deficient, should be supplemented.

2) Withholding anti-helminthic treatment among infested children when helminths are known to reduce vitamin A absorption. Even if one study conducted in the targeted population showed no impact of anti-helminthic treatment on change in vitamin A status, there is sufficient evidence from the published literature that helminths interfere with vitamin A absorption. Providing anti-helminthic treatment would both be beneficial for children's health and would help to maximize the efficacy of the intervention. In efficacy trials, efforts should be made maximize the chances of success of the intervention by providing optimal conditions. The project does intend to provide optimal conditions related to compliance with the intervention (having a center where children's intake is monitored closely).The project should also provide deworming for all infested children prior to the trial.

As noted above, another problem related to the design of the intervention is the selection of the age group –. school age children. Solutions for the problem of vitamin A deficiency are more urgently needed for pregnant and lactating women and for children 6-36 months of age (the age group most likely to suffer the long-term dramatic

consequences of vitamin A deficiency) than among school age children. The authors need a more convincing justification of why they did not select pre-school children, or pregnant or lactating women for the proposed intervention.

5. Evaluation

The proposed evaluation component is adequate.
The research includes collection of information on many potential confounding factors. If properly analyzed, this information will be invaluable to understand the mechanisms by which the intervention affected the outcomes, and also to document whether some subgroups benefited more than others from the intervention. For this latter purpose, however, the authors will need to test the statistical significance of two-way interactions between treatment groups and the characteristics of interest. These types of analyses should be added to the analytical plan.

6. Project site

The proposed project site has been well thought through and seems entirely feasible.

7. Replicability

The specific intervention (small vs. large fish) is highly context-specific and is unlikely to be replicable in other regions of the world and even in other countries of the region. It entirely depends on the availability of local sources of vitamin A in the food system. The design of the proposed efficacy trial, however, can be replicated in other contexts, using available vitamin A rich products.

8. Funding considerations

The budget seems appropriate and reasonable.

Appendix 12

1. Responses to first reviewer's comments

1.1 Practical Application

1.1.1 Reviewer's comment:
Although not mentioned, the mola or other small fish would improve calcium status as well.
Response:
In C. Project Background, it is stated that "... small fish also contribute greatly to intakes of other nutrients such as calcium iron and zinc (Thilsted and Roos, 1999). Even though there are no studies that show the effect of small fish on calcium status in humans, a human study conducted by the co-investigators showed that the bioavailability of calcium from small fish is as high as that from milk (Hansen *et al*, 1998).

1.2 Funding Considerations

1.2.1 Reviewer's comment:
There is almost overkill in the case of having weekly 24 hour food intakes and vitamin food frequency intakes. The burden of data entry and analyses for nutrients will be very large and will not add that much information. Also it appears excessive to me to be measuring the nutrient composition of the intervention feedings for macro and micro-nutrients.
Response:
a) Habitual food and nutrient intake will be measured by one 24 h recall at screening (n=700) as stated in Experimental Design and Methodology in the application form. These results are adequate for assessing food and nutrient intake at group level.
b) Habitual intake of vitamin A rich foods and vitamin A intake will be measured using a food frequency questionnaire on vitamin A rich foods at screening (n=700) as stated in Experimental Design and Methodology in the application form. The results will be used to assess habitual intake of vitamin A rich foods and vitamin A intake at individual level.
c) We agree that weekly 24 h recall of food intake (except test meals) during the feeding trial is perhaps too frequent. We have reduced the number of 24 h recall from weekly to biweekly. The results will be used to assess the total daily intakes of nutrients in each treatment group (mean of five recalls). This will also be used to compare with habitual food and nutrient intakes.
d) In Experimental Design and Methodology in the application form, it is stated that "duplicate sample of the dishes will be taken each day and pooled weekly samples of each dish will be used for chemical analyses of dry matter, vitamin A, energy, protein, fat, iron, calcium and zinc".
We agree with the reviewer's comment that the burden of the nutrient analyses of dishes is great and therefore this has been revised. One sample each of cooked rice (n=5) and vegetable curry

(n=5) will be taken biweekly for nutrient analyses. For the three fish dishes, one sample each will be taken every other day in each week and pooled weekly (n=9) for nutrient analyses.

1.3 Purpose and Objectives

1.3.1 Reviewer's comment:
The third objective is unwieldy and includes far too many co-variates than can be simultaneously analyzed in multivariate fashion given the sample size. There are about 5 or 6 research questions posed in a single specific objective. It overlaps to an extent with the second objective of "investigating the impact of vitamin A -rich fish on iron status etc vs the other intervention groups. It would be better to simplify this into several objectives or omit this. The parasites, infection, morbidity, food intake, anthropometry can be used as co- variates wherever appropriate in the other objectives.
Response:
The third objective does not overlap with the second objective. The first and second objectives refer to the results from the feeding trial (n=180, one test meal for 9 weeks). The third objective refers to the results for health, morbidity and nutritional status from the screening (pre-feeding trial) of about 700 children. In order to study the relationship between the indicators for vitamin A and iron status, and parasites, infection, morbidity, food intake and anthropometry, (multi)-analysis of variance will be used.
We agree that the results for parasites, infection, morbidity, food intake and anthropometry can be used as covariates in objectives 1 and 2 for the three groups of children (n=180 in total).
For indicators for changes in vitamin A and iron status, analysis of covariance will be used, testing group effect as a factor with parasites, infection, morbidity, food intake and anthropometry as covariates. Thus, comparisons of changes in the indicators between the three groups will be analysed. In addition, the effect of parasites, infection, morbidity, food intake and anthropometry on changes in the indicators for vitamin A and iron status will be explored. If an effect is found, sub group analysis will be performed.

1.4 Project Design

1.4.1 Reviewer's comment:
With such strict and restrictive enrollment criteria I question if there will be an adequate number of children available who fit the criteria. Also, given the nature of the population who live in slum surroundings and children who are not enrolled in school I wonder what the daily attendance will be like at the intervention feeding, with meals being skipped and perhaps a high overall attrition rate excluding children with clinical infection does not make sense as they are bound to have infections from time to time while in the study.
Response:
We believe from our knowledge of the area and population that the required number of children will be met in spite of the selection criteria. The daily attendance will be high (>90%), based on

past experience with research studies carried out in the area as well as the good rapport and constant contact with the families. The efforts of the local field staff will ensure a high participation. Children with clinical infection from time to time while in the study will not be excluded.

1.4.2 Reviewer's comment:
I wonder why children with mild xerosis or night blindness (an early sign) but no other clinical signs of VAD are being excluded?
Response:
All children who show any signs of vitamin A deficiency (mild or severe) will be excluded from the study.

1.4.3 Reviewer's comment:
I would suggest that at least 80 or more children, not 60 be enrolled and the frequency of the food intake measures and food nutrient analyses be reduced in frequency a mentioned under "funding considerations"Also where there are so many co-variates included for data collection, for multivariate analyses, of any sort, the sample sizes may be too small.
Response:
We believe that the numbers of children (60) in each group (total 180) are adequate for analysis of covariance.
Regarding "food intake measures and food nutrient analyses", please refer to response under "Funding Considerations", point 1.2.1.

1.4.4 Reviewer's comment:
I question if three months of feeding during only one season is too short a period.Given the sizable investment in such a study would not 5 or 6 months of feeding which takes place in more than one season,with less frequent measures as suggested above ,not make for a stronger study?This might increase the cost , but not by much since the infra structure would already be present.sdemonstrating the large scale -rais.
Response:
This study is designed as an efficacy trial and based on the results of de Pee et al (1998a), the time frame of 9 weeks is deemed to be adequate.

1.4.5 Reviewer's comment:
I have some questions about some of the measurements. Taking one 24 hr. recall of food intake at baseline does not measure usual food intake as stated.Using the screening food intake data as the baseline value .It would be good to repeat the 24 hr recall at the baseline for a better estimate of the usual intake.
Response:
Measuring one 24 h recall at screening is adequate to measure habitual food intake at group level.

1.4.6 Reviewer's comment:

As for the test meals, I question the ad lib intake of rice.Would it not be better to weigh out the rice based on the child's requirements based on size and age etc. If a child is sick he/she may have decreased intake at a time when they need more energy intake? Also since anthropometry is being measured energy intake would be important to measure as best as one can.or would the exact rice intake be measured after the intervention meals?where is the precribed amount of calories stated?

Response:

The test meal is just one meal per day. The child eats two meals per day at home. Thus it is not necessary to calculate the exact amount of rice for each child from the test meal in relation to energy requirement. All children are given the same fixed amount of rice based on the amount of rice eaten in a meal in the pilot project. The few children who ask for more will be given more. The food intake of each child from the dishes in the test meal will be recorded daily. The energy intake of each child will be calculated from the test meals as well as the 24 h recalls of other foods eaten.

1.4.7 Reviewer's comment:

Also in regard to food intake, why should there not be a food frequency for iron containing foods plus the enhancers of iron absorption such as citrus as well as phytate and fiber, tea, milk etc. which reduce bioavailablility of the iron.

Response:

This is a very good suggestion, but it is not feasible to carry out the work involved, including the required nutrient analyses. The food composition tables are incomplete with respect to data on inhibitors such as phytic acid and fiber.

1.4.8 Reviewer's comment:

It would be very important to determine vitamin A stores.Instead of the RDR test requiring 2 venipunctures at a set interval the modified MDR using Vitamin A-2, an anolog of retinol can be done with one one blood sample obtained 4-5 hours after the vitamin A-2 dose.The field worker could administer this dose at a home visit or a the feeding site.The A-2 we obtained from the University of Wisconsin from the Dept of Nutrition (will send the name if interested).For this study it would cost about $2000 for the vitamin A-2.

Response:

MRDR cannot be used as an indicator in this study as vitamin A in fish is present both as retinol and dehydroretinol (vitamin A-2). In mola, 80% of the total vitamin content is dehydroretinol (Roos. 2001). However at screening, MRDR tests will be taken on a sub sample (10% of the children, n=70) to compare with values for serum retinol.

RDR cannot be used as retinyl palmitate is used in the positive control group apart from the fact of 2 blood in 5 hours interval..

1.4.9 Reviewer's comments:

I am surprised to hear that deworming for helminths is considered ineffective in reducing the worm burden and improving retinol ststus.Ascaris can adversely affect retinol absorption and for this reason deworming would be important.

Response:

We did not state that deworming is ineffective to reduce the worm burden. However, as stated in Experimental Design and Methodology in the application form, we have shown that deworming is not essential to improve vitamin A status when β-carotene supplementation is given to preschool children in Mirpur (Wahed et al, 2001).

The study design will be changed to include deworming. At screening, all children will be given deworming medication as it is common that children in Bangladesh have worms and in the study area, it has been found that >95% children are infected. After 2 weeks, prior to the feeding trial, a stool test will be done and children who are still infected will again be given deworming medication. At the end of the feeding trial, a stool test will be done to determine the degree of reinfection if there is any.

1.4.10 Rewiewer's comment:
As for a marker for protein -malnutrition ,pre- albumen may be superior to albumen and has a similar methodology.
Response:
We agree completely with the above comment and pre-albumin will be measured instead of albumin.

1.4.11 Reviewer's comment:
As for the data analyses this section is weak.A statistician with expertise in longitudinal data analyses is required.as change over time for a number of indicators are required.There is little mention of the appropriate statistical methods to accomplish this.A more detailed statistical analytic plan is needed.
Response:
We agree that the data from the feeding trial is longitudinal as in each individual, each indicator is measured more than once over time.
We acknowledge that we need expert advice from a qualified statistician. We will be assisted by a statistician from The Royal Veterinary and Agricultural University, Denmark, during the statistical analysis of data. He has been involved in the planning of the study design, and the details of the analysis are being developed as the study plan and implementation progress.

1.4.12 Reviewer's comment:
Lastly, the third objective ,as written ,would be an analytic challenge!!!and should be simplified and rewritten.
Response:
As stated in the response to "Purpose and Objectives", point 1.3.1, the first and second objectives refer to the results from the feeding trial (n=180, one test meal for 9 weeks). The third objective refers to the results for health, morbidity and nutritional status from the screening (pre-feeding trial) of about 700 children. In order to study the relationship between the indicators for vitamin A and iron status, and parasites, infection, morbidity, food intake and anthropometry, (multi)-analysis of variance will be used.

2. Responses to second reviewer's comments

2.1 Funding considerations

2.1.1 Reviewer's comments:
The budget request is excessive in the amount designated for support of the PI, Wahed. He is already lists commitment of 135% of his time to other projects. Presumably these project carry compensation for salary. Therefore, it is not acceptable to provide additional salary for

commitment to this project where he says that he will spend another 25% of his time (160% time commitment!).

Response:

The above calculations are incorrect. When this project will be initiated, the Principal Investigator, Mr. M. A. Wahed will have completed the project "Nutrition Center of Excellence (NCOE)", which presently accounts for 75% of the annual salary. The remaining projects account for 60% of the annual workload, while this present project accounts for 25% of the annual workload.

2.2 Purposes and objectives

2.2.1 Reviewer's comments:

The problem of transferability of the findings revolve around whether it is reasonable to expect that a daily consumption of small fish will occur in economically strained populations where fish is likely to be relatively expensive. The research protocol calls for daily consumption for 9 weeks. It is questionable whether such frequency of consumption would be sustainable over longer periods when the community would have to commit resources to daily purchase of the fish. More likely, consumption would occur less frequently than on a daily basis. Therefore, the research design would evaluate effectiveness on a daily basis but not on a less frequent basis, which is more likely.

Response:

This proposed study is an efficacy trial. Small fish is a commonly consumed food in Bangladesh, after rice and vegetables. Small fish is bought in small quantities for a small price by the poor and in the rural areas, small fish is caught in the wild. If this study proves successful, there are many agencies in Bangladesh, including governmental institutions which will initiate increased production, accessibility and consumption of vitamin A rich small fish. The present study is not an effectiveness trial and does not address the "real life situation", which are steps which will follow the successful completion of this study.

2.3 Project design

2.3.1 Reviewer's comment:

Preferably, they would have done a dose response test on a subsample of the participants before and after the intervention since serum retinol levels can be artificially lowered by concurrent infection. Unfortunately there is no information in the study area on the distribution of serum levels in the age group selected for this study (7-11 years), although they report a high percentage among the preschool age with low serum levels. They will be able to correct in part for infection based on CRP and ACT analysis. I strongly recommend a revision of the protocol to include a dose response on a subsample because it would confirm low vitamin A status and improvement by the intervention in terms of body stores.

Response:
MRDR cannot be used as an indicator as vitamin A in fish is present both as retinol and dehydroretinol (vitamin A-2). In mola, 80% of the total vitamin content is dehydroretinol (Roos. 2001). However at screening, MRDR tests will be taken on a sub sample (10% of the children) to compare with values for serum retinol.
RDR cannot be used as retinyl palmitate is used in the positive control group. The study design will be changed to include deworming of children prior to the feeding trial.

2.3.2 Reviewer's comments:
I am not sure why both serum retinol and RBP are being analyzed as well as serum albumin. RBP and serum retinol should measure virtually the same thing and the albumin will give a better reflection of long term protein status. I would put the money allocated for RBP determination into doing a dose response test.
Response:
Serum albumin as an indicator was dropped, instead prealbumin will be measured as an indicator for protein malnutrition.

2.3.3 Reviewer's comment:
In the positive control group, it is not clear whether the retinyl palmitate will be added to the meal during preparation of the curries or if it will be added to individual meals of each child when served.
Response:
The retinyl palmitate will be added to the individual meal of each child at serving.

3. Responses to <u>third</u> reviewer's comments

3.1 Impact on child health and well-being

3.1.1 Reviewer's comment:
My main concern, however, is the age group targeted by the project. While school age children are at risk of vitamin A deficiency, they are beyond the period of potentially irreversible eye damages caused by vitamin A deficiency. It is not clear to me why the project is designed to target school-age children rather than weaning-age children who are at much higher risk of severe vitamin A deficiency and of its dramatic health consequences such as increased morbidity from infectious diseases, irreversible eye damage and mortality.
Response:
At the initial phase of designing this study, it was very obvious that the target group should be selected from the at risk groups – infants, pregnant and lactating women. In further developing the study, deciding on an intervention study, determining the length of the intervention and the

distribution and consumption of the test meals, it became quite clear that this study cannot be carried out in the above at risk groups. For example, to ensure that there is compliance, the test meals must be distributed and consumed in a central location. This is not possible in a field setting in Bangladesh as women cannot take time off from their work to come to the central feeding location every day, or bring their infants. Therefore, the age group selected is the most appropriate group for carrying out this intervention, taking into consideration the practical and logistical conditions.

This trial is an efficacy trial using school age children. However, the results of the study, will certainly be applicable to other age groups, including weaning-age children.

The Bangladesh Integrated Nutrition Project (BINP), funded by The World Bank is currently planning a pilot project to implement a fisheries component pilot study in which mola together with carps will be produced in small seasonal ponds belonging to 100 poor rural households. If this intervention trial is successful, BINP will increase the number of households and also include a nutrition education component focusing on the value of mola to pre school children and women.

3.2 Practical applications

3.2.1 Reviewer's comment:

However, it is not clear to me that the intensity of the intervention (feeding fish to children 6 days a week) could be replicable at the level of households for both cultural and financial reasons. However, the authors do not specifically discuss the issue of cost and whether or not it is possible for households to afford consuming sufficient amounts of mola 6 days a week on a regular basis.

Response:

As stated above, this study is an efficacy trial testing the maximun potential impact. Fish, mainly small fish is an everyday food for poor Bangladeshis. Mola is a common, popular small fish, making up a considerable amount of the small fish intake. A Ph.D. study, recently conducted in Bangladesh by a Danish scholar has shown that there is great potential for increasing mola production in the current fish production strategies being practised in Bangladesh (Roos, 2001). The poor in Bangladesh obtain small fish, including mola from capture fisheries and the local markets. Increased production of mola will lead to greater availability and also perhaps reduced price. The poor in Bangladesh have limited resources to buy food. However, small fish is an integral part of the diet and it has been shown that when fish availability is decreased, the poor continue to purchase fish, but in smaller quantities. Even though the poor cannot afford much mola every day, the high vitamin A density makes even small amounts an extremely valuable vitamin A source.

3.2.2 Reviewer's comment:

Even if increased production of mola results in increased household income from the sale of the product, it does not necessarily mean that intakes of mola will increase among the most vulnerable household members or that increases in income will translate into improved dietary quality overall.

Households have a variety of pressing needs in addition to food and may chose to invest increased income in other basic necessities.

The issues of cost and cultural acceptability need to be discussed in the proposal.

Response:

Studies in rural Bangladesh have shown that with increased production of fish, large fish (carps) and small fish, the households sell most of the large fish and some of the small fish while consuming a large proportion of the small fish (Roos, 2001). Small fish breed in the ponds and other water bodies and must be harvested continually. This harvesting pattern leads to an increased consumption. Large fish are stocked and are harvested all at once, leading to sale of these fish. In addition, small fish are tasty, well liked and are an integral part of the every day diet.

3.3 Proposed design

3.3.1 Reviewer's comment:

There are 2 ethical issues, however, that are not addressed properly in the proposal:

1) Having a 'control' group of children who will receive no vitamin A when they are marginally vitamin A deficient (the big fish group): I do not find the justification provided in the "Ethical Issues" section satisfying. The authors indicate that the ethical issue is not a problem since only one meal of the usual diet will be replaced. Children, even marginally deficient, should be supplemented.

Response:

We agree that all children, even marginally deficient under ideal conditions should be supplemented. In order to prove the efficacy of mola in improving vitamin A status, it is essential to include a negative control group (and therefore cannot receive supplementation during the course of the trial) as changes in serum retinol over time may be related to factors other than mola. After the 9 weeks of trial, all children in the control group will be given a recommended oral vitamin dose as medically advisable. This procedure has been carried out in previous studies conducted by ICDDR,B, after approval by the ethical committee.

3.3.2 Reviewer's comment:

2) Withholding anti-helminthic treatment among infested children when helminths are known to reduce vitamin A absorption. Even if one study conducted in the targeted population showed no impact of anti-helminthic treatment on change in vitamin A status, there is sufficient evidence from the published literature that helminths interfere with vitamin A absorption. Providing anti-helminthic treatment would both be beneficial for children's health and would help to maximize the efficacy of the intervention. In efficacy trials, efforts should be made maximize the chances of success of the intervention by providing optimal conditions. The project does intend to provide optimal conditions related to compliance with the intervention (having a center where children's intake is monitored closely).The project should also provide deworming for all infested children prior to the trial.

Response:

The study design will be changed to include deworming. At screening, all children will be given deworming medication as it is common that children in Bangladesh have worms and in the study area, it has been found that >95% children are infected. After 2 weeks, prior to the feeding trial, a stool test will be done and children who are still infected will again be given deworming medication. At the end of the feeding trial, a stool test will be done to determine the degree of reinfection.

3.3.3 Reviewer's comment:

The authors need a more convincing justification of why they did not select pre-school children, or pregnant or lactating women for the proposed intervention.

Response:

As stated in "Impact on child health and well being", point 3.1.1.:

At the initial phase of designing this study, it was very obvious that the target group should be selected from the at risk groups – infants, pregnant and lactating women. In further developing the study, deciding on an intervention study, determining the length of the intervention and the distribution and consumption of the test meals, it became quite clear that this study cannot be carried out in the above at risk groups. For example, to ensure that there is compliance, the test meals must be distributed and consumed in a central location. This is not possible in a field setting in Bangladesh as women cannot take time off from their work to come to the central feeding location every day, or bring their young children. Therefore, the age group selected is the most appropriate group for carrying out this intervention, taking into consideration the practical and logistical conditions.

This trial is an efficacy trial using school age children. However, the results of the study, will certainly be applicable to other age groups, including weaning-age children.

The Bangladesh Integrated Nutrition Project (BINP), funded by The World Bank is currently planning a pilot project to implement a fisheries component pilot study in which mola together with carps will be produced in small seasonal ponds belonging to 100 poor rural households. If this intervention trial is successful, BINP will increase the number of households and also include a nutrition education component focusing on the value of mola to pre school children and women.

3.4 Evaluation

3.4.1 Reviewer's comment:

The research includes collection of information on many potential confounding factors. If properly analyzed, this information will be invaluable to understand the mechanisms by which the intervention affected the outcomes, and also to document whether some subgroups benefited more than others from the intervention. For this latter purpose, however, the authors will need to test the statistical significance of two-way interactions between treatment groups and the characteristics of interest. These types of analyses should be added to the analytical plan.

Response:

The results for parasites, infection, morbidity, food intake and anthropometry can be used as covariates in objectives 1 and 2 for the three groups of children (n=180 in total).

For indicators for changes in vitamin A and iron status, analysis of covariance will be used, testing group effect as a factor with parasites, infection, morbidity, food intake and anthropometry as covariates. Thus, comparisons of changes in the indicators between the three groups will be analysed. In addition, the effect of parasites, infection, morbidity, food intake and anthropometry on changes in the indicators for vitamin A and iron status will be explored. If an effect is found, sub group analysis will be performed.

We acknowledge that we need expert advice from a qualified statistician. We will be assisted by a statistician from The Royal Veterinary and Agricultural University, Denmark, during the statistical analysis of data. He has been involved in the planning of the study design, and the details of the analysis are being developed as the study plan and implementation progress.

3.5 Replicability

3.5.1 Reviewer's comment:

The specific intervention (small vs. large fish) is highly context-specific and is unlikely to be replicable in other regions of the world and even in other countries of the region.

Response:

In many developing countries, for example in South East Asia with water resources, fish is a commonly consumed food. Many countries, international organisations (for example International Center for Living Aquatic Resources Management (ICLARM)), bilateral organisations, (for example Danish International Development Assistance (Danida)) and non governmental organisations (for example CARE), in their efforts to increase fish production, availability and consumption have focused on large fast growing fish, such as carps. No attention has been given to the nutrient dense small indigenous fish, commonly consumed by the poor. Thus, the specific intervention (small vs. large fish) is replicable in many regions.

Appendix 13

Thrasher Research Fund
15 E. South Temple Street, 3rd Floor
Salt Lake City, UT 84150-6910 USA
Phone: (801) 240-4753
www.thrasherresearch.org

7 August 2001

M.A. Wahed
Assoc. Scientist & Head
Nutritional Biochemistry Division
ICDDR,B: Ctr. for Health and Population Research
Mohakhali
Dhaka-1212
Bangladesh

Dear Mr. Wahed:

This Award Letter is to advise you that the Thrasher Research Fund ("Fund") has approved your application for a grant (the "Grant") described below, to be made upon the following terms and conditions:

1. The Principal Investigator is M.A. Wahed. The project name is "The effectiveness of vitamin A-rich small fish in improving vitamin A status in children (3-7 yrs.) in Bangladesh." The total project grant award for the period of 1 October 2001 to 30 September 2004 is $203,287. The Supervising Institution is ICDDR,B: Centre for Health and Population Research.

2. This Grant is awarded on two conditions. The first condition is that the changes to the research protocol to which you have previously agreed be incorporated. These changes include those listed in your response to reviewers' comments (received 18 June 2001, copy attached), the use of children 3-7 years of age rather than 7-11 years of age, and performance of relative dose response tests on a subsample of each study group, both pre-and post-intervention. The second condition is that the attached revised budget dated 27 July 2001 supersedes any previous requests.

3. By acceptance of the Grant, the Principal Investigator and the Supervising Institution expressly acknowledge and accept all of the conditions in this Award Letter as well as the conditions stated in the document entitled Conditions of Grant, a copy of which is attached and made part of this contract by this reference. Please pay particular attention to the details stated in this Award Letter as well as in the Conditions of Grant, and retain them for future reference. Unauthorized reallocation of funds or failure to submit semiannual reports as required will give the Fund the right, but not the obligation, to suspend the Grant. If suspended, the Grant can only be reinstated upon written authorization from the Fund. Unless otherwise specifically stated in this Award Letter, this Grant shall be paid to the Supervising Institution under whose supervision the Principal Investigator shall be responsible for the research and other activities required to complete the project.

4. The Principal Investigator and Supervising Institution agree to adhere to this Award Letter and the Conditions of Grant, the violation of any provision of which shall be reason for suspension of the Grant for which reinstatement shall require written appeal from the Principal Investigator and Supervising Institution.

5. This paragraph is included in accordance with the Royalties, Patents, Publications paragraph of the Conditions of Grant. The Principal Investigator hereby agrees to fully disclose and assign and transfer to the Supervising Institution, its successors and assigns, the entire right, title and interest in and to any ideas, techniques, inventions, or designs, patentable or unpatentable, conceived, made, developed, acquired, or supported, in whole or in part, during performance of the project funded by the Grant, or which, in the opinion of the Supervising Institution or the Fund, relate in any way to activities supported, in whole or in part, by funds, supplies, equipment or facilities furnished or administered by the Fund (all hereinafter collectively referred to as "Inventions"). Such assignment shall include the right to obtain letters patent or design patent in the name of the Supervising Institution or otherwise of any Inventions in the United States or in any applicable foreign countries covering such, all costs for such patenting to be paid by the Supervising Institution. Both the Supervising Institution and Principal Investigator agree to grant and by these presents do hereby grant to the Fund a perpetual, transferable, paid-up, non-exclusive license to make, have made, use, import, sell, and offer for sale any and all Inventions and to exercise any rights under any patents or patent applications which may exist covering any Inventions.

6. To accomplish the purposes set forth above, the Principal Investigator will (a) disclose promptly and report fully all Inventions to the Supervising Institution and to the Fund; (b) not disclose such Inventions to any third parties without first notifying both the Supervising Institution and the Fund of such proposed disclosure; and (c) deliver to the Supervising Institution and to the Fund all relevant notes, drawings, blueprints and papers upon request and to give other reasonable assistance to the Supervising Institution and the Fund or their assignees, in the preparation or defense of patents or patent applications. The Principal Investigator agrees to sign all papers and make all disclosures necessary for the filing of any application for such letters patent or design patent.

7. The Supervising Institution and the Principal Investigator hereby agree that all amounts of gross revenue received, whether as up-front fees, royalties, or otherwise, from any Inventions shall be divided as follows:

a. Until the Fund shall receive an amount equal to all amounts supplied by the Fund for the Grant, the gross revenue shall be divided 50% to the Fund, and 50% to the Supervising Institution and the Principal Investigator to be divided as they shall agree.

b. Thereafter the gross revenue shall be divided 20% to the Fund, and 80% to the Supervising Institution and the Principal Investigator to be divided as they shall agree.

8. In determining gross revenue, no deduction shall be allowed for the costs of applying for or prosecuting U.S. and foreign patents, or development and marketing costs before the percentages of paragraph 7 become applicable.

9. In the event that by one year from 30 September 2004, the Supervising Institution has not formally pursued any patent or other intellectual property protection for any Inventions, the Supervising Institution and the Principal Investigator hereby transfer and assign to the Fund all right, title and interest in and to any and all Inventions with full discretion as to whether to pursue any patents or other intellectual property protection therefor at the expense of the Fund. In such event, the gross revenue from any Inventions shall be divided solely in accordance with paragraph 7a, and paragraph 7b shall not apply. The Supervising Institution and Principal Investigator agree to sign or execute any assignments or other documents reasonably necessary to complete such assignment or transfer to the Fund. If the Fund, in its sole discretion, extends the above-indicated end date in writing, this paragraph 9 shall apply one year from such extended end date.

10. This Agreement supersedes your Grant proposal application and all other prior dealings between you and the Fund regarding your proposal.

11. In the event the Principal Investigator transfers from the within-named Supervising Institution to another institution and this Grant or the project funded thereunder is continued with such other institution, such other institution will become a Successor Supervising Institution, and such Successor Supervising Institution will be bound by the terms and conditions of this Award Letter and Conditions of Grant. Except as provided herein, the parties hereto may not assign, encumber or otherwise transfer this agreement, and any such attempt at assignment, encumbrance, or transfer will be void.

12. This Agreement shall be construed in accordance with the laws of the State of Utah and the United States of America. Any disputes concerning this Award Letter Agreement or the Conditions of Grant shall be resolved through binding arbitration in Salt Lake City, Utah, pursuant to the rules of the American Arbitration Association.

When both the Supervising Institution and Principal Investigator have executed this Agreement, please return one fully executed copy of this Agreement to each of the parties.

We wish you success in your research.

Very truly yours,

THRASHER RESEARCH FUND

By _____
A. Dean Byrd, Ph.D., MBA
President

ACCEPTED:

SUPERVISING INSTITUTION

ICDDR,B: Centre for Health and Population Research

By _____
David A. Sack, M.D.
Title _____
Director
Dated this 16 day of August, 2001

PRINCIPAL INVESTIGATOR

M.A. Wahed

By _____

Dated this 16 day of August, 2001

Appendix 14

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Vegetable curry
(with lau, shosha, potato and mug dal)

(200 children)

12,000 g	Potato
12,000 g	Lau (bottle gourd)
12,000 g	Shosha (cucumber)
2,000 g	Onions
3,000 g	Mug dal (washed and) soaked in 375 g water
600 g	Salt, iodised
280 g	Ginger paste
280 g	Garlic paste
240 g	Turmeric (Tiger) paste (turmeric:water 1:2)
200 g	Chilli seeds (dried and roasted)
480 g	Soybean oil
19,200 g	Water (May be adjusted according to the size of portion)

Fried onions:
2,000 g Onions
1,000 g Soybean oil

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1. Soak the **mug dal** for approximate 20 min. (max 30 min)
2. Put the **water, salt, ginger paste, garlic paste, turmeric paste, chilli seeds, soybean oil and potatos** into the pot and bring it to boil **without** the lid. Cook it for 5 min (without the lid).
3. Add the **soaked mug dal and onions** and boil the curry **with the lid** for additional 10 min.
4. Add **Lau** and boil for another 5 min **(with the lid)**.
5. Add **shosha** and boil for 20 min more **(with the lid)**.
6. When the curry is cooked take it of the burner (total cooking time is app. 40 min).
7. ***Frying of the onions:*** Heat the oil in a separate pot. Add the onions and fry. Add the fried onions and oil into the vegetable curry. Put the lid on pot until serving.
   The whole curry is weighted before serving. Note the duration from the curry is cooked until portions are weight.

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Mola curry

(approximate 65 children)

3,000 g Mola mach (weight before freezing of the cleaned mola)
1,000 g Onions

20 g Ginger paste
80 g Garlic paste
50 g Turmeric (Tiger) paste (turmeric:water 1:2)
27 g Chilli seeds roasted
70 g Salt, iodised

370 g Soybean oil

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1. Put Mola fish and all the water from the freezing bag into the pot.

2. Add onions, species and oil and mix thoroughly with hand.

3. Put the pot over the fire. Bring the ingredients to boiling temperature **without** the lid and boil the fish curry for 4-5 min (without the lid). Turn the fish upside down, lower the gas (**weak boiling** !) and put on the lid. Boil for additional 10 min. (with lid).

4. Store the fish curry with the lid on the pot. Blend into a homogenised dish.

5. The whole fish curry is weighed before weighing into portions. Note the duration from the curry is cooked until portions are weight.

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Rui curry

(approximate 130 children)

A. Boiling of the whole rui fish:

0.26 g water per kg whole rui fish. Note the amount of water added

1. Note the weight of the fish on the plastic bag.
2. Put the whole fish as well as all the water from the freezing bag into the pot
3. The whole fish is cooked/steamed in excess water with the lid until it is finish (10-15 min).
4. Separate the meat from the bones by hands. Weight meat (an bones). Throw the bones away.
5. Use the meat and skin for cooking the rui fish curry (see below).
6. Pass the leftover water through a chani and use it for cooking the rui curry (se below).

B. Cooking of the rui curry

6,000 g as RAW Rui (weight before freezing) (calculate what the 6,000 g of *raw* rui meat correspond to in *cooked* rui meat)

2,000 g Onions

40 g Ginger paste
160 g Garlic paste
100 g Turmeric (Tiger) paste (turmeric:water 1:2)
53 g Chilli seeds roasted
140 g Salt, iodised

740 g Soybean oil
1340 g Water

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1. Mix thoroughly with hands: cooked fish, onions, species, oil and water.

2. Put the pot over the fire. Bring the ingredients to boiling temperature **without** the lid and boil the fish curry for 4-5 min (**without** the lid). Turn the fish upside down, lower the gas **(weak boiling !)** and put **on the lid**. Boil for additional 10 min. (with lid).

3. Store the fish curry with the lid on the pot. Blend into a homogenised dish.

4. The whole fish curry is weighed before weighing into portions. Note the duration from the curry is cooked until portions are weight.

International Centre for Diarrhoeal Disease Research, Bangladesh
Fish intervention on vitamin A status in children – a study in Mirpur

Rice

(200 children)

36,000 g Rice
82.500 g Water

WASHING:
Wash by hands the rice in excess water. Through the water away. Repeat 3 –4 times.

The washing water is not used in the cooking.

COOKING IN A FIX AMOUNT OF WATER:
Put the rice and the water into a pot. Bring it to boil and cook in fix amount of water for about 45-50 minutes.