Effects of Vitamin A and Iron Fortified Supplementary Food on Vitamin A and Iron Status of Rural Preschool Children in Vietnam*

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RINGKASAN

Seperti banyak negara yang sedang berkembang, Vietnam menghadapi berbagai masalah kesehatan yang berkaitan dengan gizi pada berbagai kelompok umur dan penduduk. Diantara masalah-masalah tersebut, kekurangan vitamin A, anemia defisiensi besi dan gangguan pertumbuhan pada anak-anak prasekolah pada waktu ini mendapat perhatian khusus. Prevalensi kekurangan vitamin A pada tahun 1985-1988 sebesar 0,07%, 7 kali lebih besar dari "cut off point" 0,01% yang ditetapkan WHO (1976). Survai mengenai kurang vitamin A secara nasional pada tahun 1994 menunjukkan prevalensi (0.005%) yang lebih rendah dari pada kriteria (0.01%) yang ditetapkan WHO (1976) untuk anak prasekolah dan lebih rendah secara bermakna dari prevalensi 6 tahun yang lalu (0.07%). Anemia defisiensi besi didapati pada 60,5% anak 6-24 bulan dan 29,8% pada kelompok 24-60 bulan, sedangkan 44,9% "stunted". Program Nasional Pemberian Suplementasi kapsul Vitamin A setiap 6 bulan yang diselenggarakan sejak tahun 1988 berhasil meningkatkan status vitamin A anak-anak prasekolah. Namun demikian, beberapa penelitian di luar Vietnam menunjukkan bahwa cara suplementasi semacam ini tidak dapat mempertahankan kadar retinol serum sampai pemberian suplementasi berikutnya. Di Vietnam tidak ada program intervensi zat besi dan suplementasi dengan bahan makanan yang difortifikasi untuk anak -anak prasekolah. Suatu strategi yang mungkin dapat dilaksanakan untuk mencegah penurunan kadar retinol serum, mengurangi prevalensi anemia defisiensi besi yang tinggi dan gangguan pertumbuhan anak prasekolah Vietnam adalah intervensi dengan memberikan bahan makanan yang difortifikasi vitamin A dan zat besi. Berbagai bahan makanan yang disukai anak-anak seperti susu skim, susu sapi segar, tepung terigu, biskuit dan gula telah diteliti untuk dipakai pada fortifikasi bahan makanan dengan vitamin A dan zat besi. Susu kedelai biasa dikonsumsi di Vietnam, disukai anak- anak prasekolah, harganya murah dan mengandung protein bermutu tinggi. Apakah susu kedelai dapat dipakai untuk fortifikasi? Tujuan penelitian ini mempelajari pengaruh konsumsi susu kedelai yang difortifikasi dengan vitamin A dan zat besi secara teratur, untuk mencegah penurunan kadar retinol dan meningkatkan status zat besi anak prasekolah Vietnam setelah mendapat satu kali suplemen vitamin A dosis tinggi dan tunggal (200.000 UI). Penelitian ini adalah studi masyarakat yang terdiri dari lima bagian: tes "relative bioavailability" susu kedelai yang difortifikasi, tes "acceptability" susu yang difortifikasi dengan vitamin A (300 µgRE) dan zat besi (5 µg pyrophosphate) pada anak- anak prasekolah, studi pada anak-anak prasekolah daerah pinggir kota, studi pada anak prasekolah daerah pedesaan, dan studi intervensi. Anak-anak prasekolah yang diteliti adalah pengunjung "day care centers"/tempat penitipan anak dan berasal dari keluarga berpenghasilan rendah. Tes "bioavailability" susu kedelai yang difortifikasi dilakukan pada sukarelawan dewasa di laboratorium SEAMEO-TROPMED-RCCN, Jakarta dan hasilnya 45%/ Tes "acceptability" susu kedelai yang difortifikasi dilakukan pada anak-anak prasekolah dari daerah pedesaan di tempat penitipan anak di Vietnam. Ternyata susu kedelai yang difortifikasi disukai oleh semua anak. Studi di daerah pinggir-kota dilakukan dalam bulan Januari 1995 pada 438 anak prasekolah dari 2 kelompok masyarakat pinggir kota Hanoi. Studi di daerah pedesaan diselenggarakan dalam bulan Juli 1995, pada 417 anak prasekolah dari sekelompok masyarakat pedesaan propinsi Hatay. Kedua studi ini diadakan satu bulan setelah anak-anak mendapat kapsul vitamin A dosis tinggi tunggal, dari Program Pembagian Kapsul Vitamin A Nasional di tempat penitipan anak, pada anak laki-laki dan perempuan berumur 8-62 bulan. Hasil studi menunjukkan konsumsi makanan sehari-hari anak- anak tidak adekuat, terutama besi-heme dan retinol rendah. Angka infeksi saluran pernafasan akut lebih tinggi secara bermakna (30- 60%) di daerah pinggir kota dibanidngkan dengan di daerah pedesaan (7,9%). Persentase diare tidak berbeda bermakna antara daerah pinggir kota (3,2%) dan daerah pedesaan (2,6%). Infeksi Ascaris tinggi, di daerah pinggir kota 64,6% dan di daerah pedesaan 64,5%. Infestasi Trichuris lebih tinggi secara bermakna di daerah pinggir kota (16,2%) dibandingkan dengan di daerah pedesaan (10,7%). Tidak terdapat infestasi cacing tambang. Kadar retinol serum anak-anak prasekolah daerah pedesaan lebih rendah secara bermakna dari pada daerah pinggir kota (1,0±0,21 versus 1,34±0,24 µmol/L). Persentase Kurang Vitamin A (retinol serum <1,05 µmol/L) lebih tinggi secara bermakna di daerah pedesaan (57,5%) daripada di daerah pinggir kota (6,9%). Persentase anemia (Hb<110 g/L) 20,6% di daerah pinggir kota dan 20% di daerah pedesaan. Angka rata-rata ferritin serum anak-anak daerah pedesaan 29,3±13,7 µg/L dan 26,5% anak-anak kadar ferritin serumnya kurang dari 20 µg/L. Hasil penelitian juga menunjukkan bahwa angka Hb anak prasekolah di kedua daerah penelitian sejajar dengan angka-angka retinol serum. Keadaan pertumbuhan fisik anak- anak di kedua daerah buruk tetapi persentase "stunting" lebih tinggi secara bermakna pada anak prasekolah di daerah pedesaan (55,6%) dari pada di daerah pinggir kota (37,4%). Persentase "underweight" juga lebih tinggi secara bermakna di daerah pedesaan (53%) daripada di daerah pinggir kota (44,1%). Kedua studi menunjukkan bahwa status gizi anak-anak prasekolah daerah pinggir kota dan pedesaan buruk, tetapi lebih buruk di daerah pedesaan. Berdasarkan hasil-hasil tersebut diatas studi intervensi dilakukan di daerah pedesaan yang sama dan dengan jumlah serta subjek yang sama. Studi intervensi dilakukan pada 417 anak-anak prasekolah berumur 6-62 bulan, dari kelompok masyarakat yang sama dan anak-anak tidak menderita penyakit berat dan alergi makanan. Tinja subjek diperiksa sebelum mereka dibagi dalam 3 kelompok secara acak; subjek dengan tinja yang mengandung telur cacing kemudian mendapat obat cacing Mebendazole. Setiap anak dalam kelompok, mendapat, per hari, 30 gram susu kedelai yang difortifikasi (FSBM), 30 gram susu kedelai yang tidak difortifikasi (UFSBM) dan 12 *gram cassava cookie (CC). Masing- masing suplemen FSBM, UFSBM dan CC mengandung berturut-turut 150, 150, 45 kcal, 300, 50,0 µgRE dan 6.8, 1.8, 0.7, mg zat besi. Suplementasi diberikan setiap hari, 6 hari seminggu kecuali Minggu, selama 5 bulan, di tempat penitipan anak. Makanan sehari- hari subjek memberikan 88,8% energi dan 151,2% protein dari RDA menurut WHO/FAO/UNU (1985) dan 10,4% zat besi serta 78,5% vitamin A dari RDA menurut FAO/WHO (1988). Hasil intervensi selama 5 bulan menunjukkan bahwa anak-anak prasekolah menyukai dan menghabiskan makanan "suplemen" yang diberikan. Makanan sehari-hari para subjek tidak berubah selama intervensi. Makanan "suplemen" yang dikonsumsi setiap hari, selama intervensi memberikan tambahan energi dan zat-zat gizi sebagai berikut : FSBM, 12% energi, 70% vitamin A, 30% zat besi; UFSBM, 12% energi, 12% vitamin a, 8% zat besi; CC, kira-kira 3% energi, 0% vitamin A dan zat besi, dari RDA. Penilaian keadaan kesehatan para subjek menunjukkan persentase anak-anak yang menderita diare sebelum dan setelah intervensi tidak berbeda bermakna pada semua kelompok penelitian (rentang 0- 2,6%) tetapi lebih banyak anak yang menderita radang saluran pernafasan setelah intervensi (saluran pernafasan atas 21,2- 2,31%, saluran pernafasan bawah 0,9-2,5%). Infestasi Ascaris dan Trichuris terdapat di ketiga tiga kelompok intervensi, yaitu masing-masing 27,4-33% dan 5,8-9,4%. Hasil intervensi selama 5 bulan juga menunjukkan bahwa retinol serum, Hb, dan feritin serum meningkat di ketiga kelompok; kenaikan pada kelompok FSBM dan UFSBM hampir sama, UFSBM lebih besar dan kenaikan terkecil pada kelompok CC. Perubahan kadar retinol serum tidak berbeda bermakna diantara kelompok FSBM, UFSBM dan CC, dengan rentang 0,24±0,22, 0,20±0,21 dan 0,16±0,25 µmol/L. Perubahan dalam konsentrasi Hb berbeda bermakna diantara ketiga kelompok dan rentang 6,5±9,3, 5,7±10,5 dan 3,3±10,7 g/L. Kenaikan kadar ferritin serum berbeda bermakna hanya pada kelompok FSBM (3,1±12 µg/L). Pertumbuhan fisik memakai indikator berat badan, tinggi badan MUAC, HAZ-score, WAZ-score dan triceps- skinfold meningkat secara bermakna pada semua kelompok dan makin muda usia, makin baik peningkatannya. Studi intervensi ini menunjukkan bahwa suplementasi setiap hari, memakai susu kedelai yang difortifikasi dengan vitamin A (300 µgRE) dan zat besi (5 mg iron pyrophosphate) yang dilakukan setelah Program Suplementasi kapsul Vitamin A Nasional, dapat mempertahankan kadar retinol serum cukup tinggi selama 5 bulan dan tidak ditemukan tanda keracunan. Berbagai faktor berperan pada peningkatan serum retinol, Hb dan pertumbuhan fisik para subjek setelah intervensi. Peningkatan Hb dan serum retinol pada ketiga kelompok dapat dikaitkan dengan kenaikan umur mengingat intervensi dilakukan selama 5 bulan. Kecukupan makanan sehari-hari meningkat dengan pemberian makanan suplemen. Beberapa studi menunjukkan bahwa vitamin A mempengaruhi pelepasan zat besi dari hepar untuk pembentukan Hb dan pada penelitian ini kadar serum retinol sejajar dengan kadar Hb sebelum intervensi. Interaksi antara vitamin A dan zat besi dapat menerangkan peningkatan Hb anak-anak di daerah pedesaan. Faktor lain adalah kondisi kesehatan subjek yang tercermin pada persentase anak-anak yang menderita diare dan radang saluran nafas bawah, yang rendah, dan tidak berubah sebelum dan sesudah intervensi. Kenaikan persentase radang saluran nafas atas dapat dikaitkan dengan waktu intervensi dilakukan yaitu perubahan dari musim panas (Juli) ke musim dingin (Desember). Pengaruh pemberian obat cacing tidak dapat diabaikan; persentase anak-anak yang Ascaris-positip dan Trichuris-positip menurun. Jika anak-anak bebas cacing, nafsu makan, absorbsi dan utilisasi zat-zat gizi akan meningkat. Pada penelitian ini peningkatan nafsu makan tidak tercermin pada peningkatan konsumsi makanan sehari-hari, mungkin karena subjek berasal dari keluarga kurang mampu dimana persediaan makanan terbatas. Peningkatan serum retinol, Hb dan pertumbuhan fisik pada kelompok CC mungkin sekali juga dipengaruhi "efek intervensi" (Hawthorn effect). Walaupun studi ini telah menunjukan peningkatan status vitamin A, Hb dan pertumbuhan fisik, tetapi kadar feritin serum tidak meningkat secara bermakna dan "bioavailability" zat besi yang dipakai pada penelitian rendah. Untuk mendapatkan efek intervensi yang lebih baik perlu dilakukan penelitian lebih lanjut untuk menemukan senyawa zat besi yang cocok untuk fortifikasi dan mempunyai "bioavaibility" yang lebih tinggi, selain itu perlu diteliti efek penggunaan kadar zat besi yang lebih besar daripada yang dipakai pada studi intervensi ini (3 mg elemen zat besi/30 gram bubuk susu kedelai) dikombinasi dengan vitamin C. Mengingat susu kedelai dapat dipakai untuk fortifikasi maka fortifikasi susu kedelai dengan vitamin A dan zat besi mungkin dapat dipergunakan sebagai makanan suplemen untuk meningkatkan status gizi anak-anak prasekolah. Program suplementasi makanan jangka panjang memakai susu kedelai, vitamin A dan zat besi sebaiknya disertai paket pendidikan gizi. Paket sebaiknya menggaris bawahi aspek produksi dan konsumsi makanan kaya vitamin A, Beta karoten dan makanan dengan "bioaviability" zat besi yang tinggi. Pemberian obat cacing seharusnya menjadi bagian dari program suplementasi makanan dan atau vitamin A serta zat besi untuk meningkatkan efektivitas program.*

BACKGROUND INFORMATION AND PROBLEM

Like many developing countries several nutrition related health problems exist in Vietnam like vitamin A, iron, iodine deficiency and growth retardation. Among those problems, vitamin A, iron deficiency anemia and growth retardation are at present under focus of attention.

The prevalence of severe xerophthalmia as a result of vitamin A deficiency (VAD) in Vietnam between 1985-1988, was (0.07%) seven times higher than the cut-off point (0.01%) established by the World Health Organization.[1] From 1988, with the support of UNICEF, a universal distribution campaign started and has expanded over the last four years. Annual evalua-

* *Summary of the dissertation. Leading to the Doctorate Degree in Nutrition at the University of Indonesia under the auspices of the Rector Professor Dr. M.K. Tadjudin, for the public defense at the Senate Committee University of Indonesia, on Tuesday, 7 January 1997*

tions by the National Institute of Nutrition (NIN) in some areas covered by the program, showed a definite decline in the rates of xerophthalmia and increase in the serum vitamin A level in the population surveyed since the start of the program. The national vitamin A deficiency survey conducted in 1994 showed the prevalence of severe xerophthalmia in the total sample (0.005%) was lower than the criteria (0.01%) of the WHO[1] of a significant public health problem in preschool children and significantly lower than (0.07%) those identified six years ago.[2] Iron deficiency anemia (IDA) in preschool age children was 60.5% in the age group 6-24 months and 29.8% in the age group 24-60 months.[3] Furthermore, results of the National Surveys in 1994 showed a high prevalence of growth retardation in preschool children. The prevalence of underweight was 44.9% and stunting 46.9%.[2] There are many factors which influence the nutritional status of preschool age children. The most important are inadequate dietary intake as well as infectious diseases, and parasite infestation. Diets low in energy intake are usually also low in protein, and other important nutrients like iron, vitamin A, zinc, copper, calcium.[4]

There is a clear association between infection, especially diarrhea and growth retardation.[5] Infestation with parasites such as *Giardia,* Hookworm, *Trichuris* and *Ascaris* is common in infants and young children in developing countries. It may cause malabsorption of nutrients such as protein, vitamin A, iron and loss of iron.[6,7] Hence parasites infestation may influence growth, vitamin A and iron status. Some studies have shown that there is an interaction between vitamin A and iron. Vitamin A deficiency in rats causes anemia associated with increased iron concentrations in liver and spleen[8,9,10] suggesting interference of vitamin A with the mobilization of iron from tissue stores and improving iron status. There would be several possibilities to solve the VAD, IDA problem and growth retardation of Vietnamese preschool children. Taking into consideration the operational efficiency the most proper one would be possibly through food fortification with vitamin A and iron. Many supplementary feeding programs outside Vietnam had shown its benefits such as for the treatment and prevention of growth retardation, protection against the negative effects of diarrheal diseases on child- growth,[11,12,13] promotion of the normal development including psychological development.[14] Vitamin A and iron fortification of supplementary food resulted in increase of vitamin A and iron status as well as physical growth.[15,16,17,18]

In Vietnam, iron interventions and fortified food supplementation programs have not been implemented for preschool children. Single high dose of vitamin A capsules supplementation every six months for preschool children since 1988 improved the vitamin A status of the Vietnamese preschool children. However this strategy does not maintain the serum retinol until the next distribution of vitamin A capsules.[19,20]

Various foods fortified as vehicles of vitamin A and iron were investigated in many countries. Dried skim milk, fresh cow's milk, monosodium glutamate were vehicles of vitamin A fortification.[15,21,22] Milk powder, wheat flour, dried cereals, drinking water were vehicles for iron fortification.[23,24,25] Biscuits and sugar were vehicles for a combination of vitamin A and iron fortification.[18,26]

In Vietnam soybean milk is commonly consumed and acceptable for preschool children, is sold at low price, has high quality protein and high fat content. Thus soybean milk is expected to be a good vehicle for vitamin A and iron fortification.

The question arises whether the vitamin A deficiency, iron deficiency anemia and growth retardation of Vietnamese preschool children could be reduced by a daily feeding of vitamin A and iron fortified soybean milk through a school-feeding program.

BENEFITS OF THE STUDY

Findings of this study will give inputs :

A/ On the effectiveness of the fortified soybean milk with vitamin A and iron to improve the nutritional status of preschool children in terms of :

- The daily supplementation of soybean milk fortified with a combination of vitamin A (300 µgRE) and iron (5 mg iron pyrophosphate) on the retention of vitamin A status of preschool children after a single high dose (200,000 UI) of vitamin A capsule supplementation.
- Improvement of iron status.
- Daily supplementation of soybean milk, an acceptable food stuff would improve of the physical growth status of preschool children in terms of reduced the prevalence of stunting and underweight.

B/ For consideration in the planning of programs to improve the nutritional status of preschool children.

CAUSAL MODEL OF THE STUDY

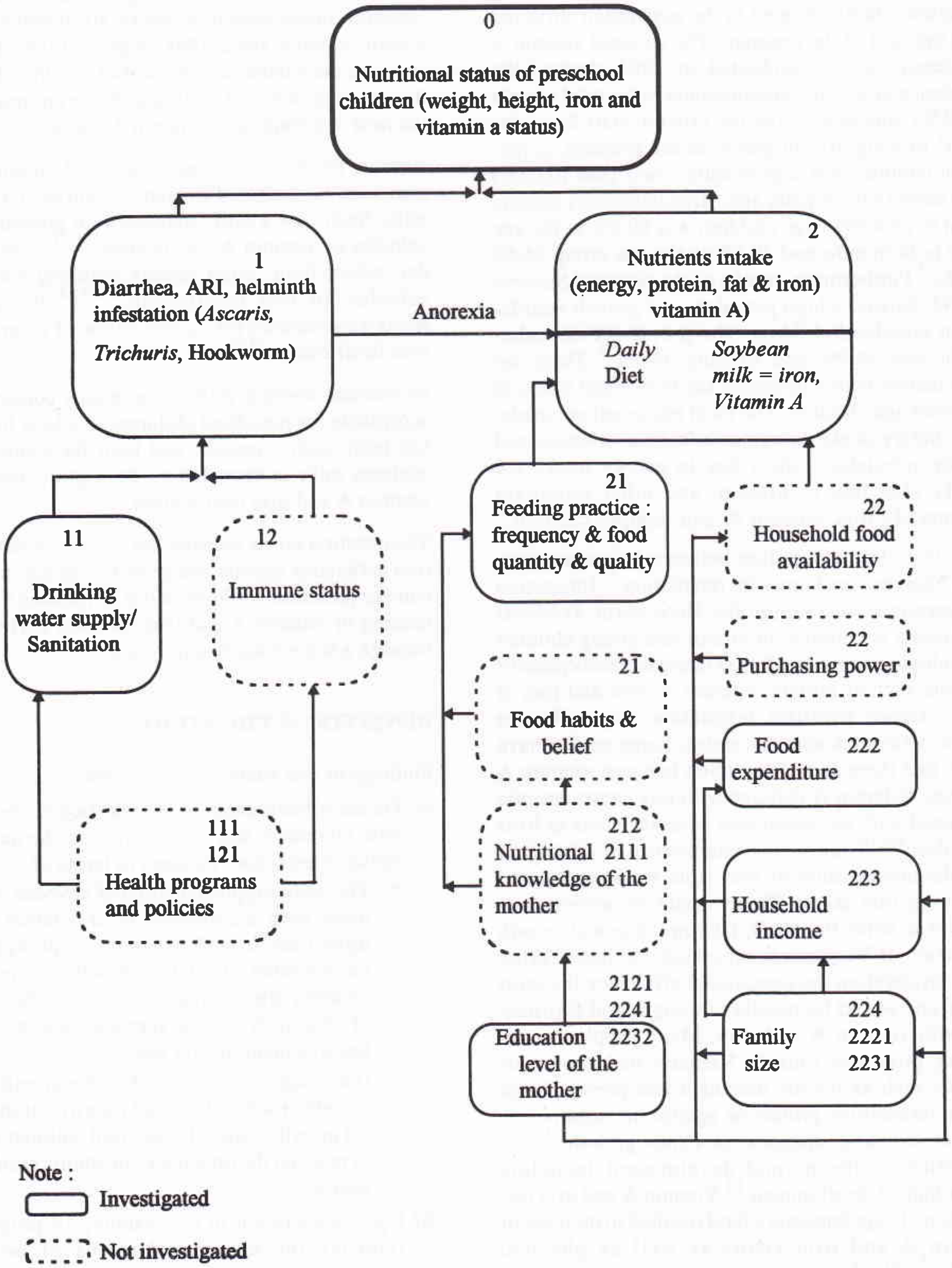

Figure 1. Causal model of the study

RESEARCH HYPOTHESIS

General hypothesis

A supplement of soybean milk fortified with a combination of vitamin A and iron will improve the nutritional status of Vietnamese preschool children.

Specific hypothesis

1. Serum retinol concentration of preschool children will remain at adequate level if daily vitamin A and iron fortified soybean milk (SBM) were given to preschool children who have received a single high dose of vitamin A capsules supplementation whereas retinol concentration falls below adequate level in preschool children that did not receive daily vitamin A and iron fortified SBM.
2. Soybean milk fortified with vitamin A and iron have better effects on iron status in preschool children than soybean milk alone.
3. Supplementary feeding leads to an improvement of anthropometric indices in preschool children.

OBJECTIVES OF THE STUDY

General Objective

To investigate the effects of regular consumption of iron and vitamin A fortified soybean milk on the retention of vitamin A and iron status of Vietnamese preschool children after a single high dose (200,000 IU) of vitamin A supplementation.

Specific objectives

1. To test the acceptability of soybean milk fortified with vitamin A (300 µgRE) and iron (5 mg of iron pyrophosphate) among Vietnamese preschool children.
2. To test the bioavailability of iron in fortified soybean milk
3. To investigate:
   a) the iron, vitamin A status and physical growth of a selected group of peri-urban and rural Vietnamese preschool children attending day-care centers.
   b) the health status, food consumption and socio-economic conditions of a selected group of peri-urban and rural Vietnamese preschool children.
4. To compare the nutritional status of the peri-urban and rural preschool children.
5. To compare the effects of regular consumption of fortified soybean milk, unfortified soybean milk and cassava cookies on the iron, vitamin status and anthropometric indices of Vietnamese preschool children.

MATERIAL AND METHODS

The study is a community trial and consists of 5 parts
* The relative bioavailability test of iron in fortified soybean milk,
* The acceptability test of fortified milk for preschool children,
* The study in the peri-urban area
* The study in the rural area
* Intervention study in the rural area.

The Relative Bioavailability Test of Iron in Fortified Soybean Milk

Objective, study site and subjects

The objective of this test was to assess the relative bioavailability of iron sulphate added to fortified soybean milk powder. The test was done on 7 female and 3 male healthy adult volunteers aged 43.8 ± 4.6 years. Nine of them were Master of Science students and one staff member of the SEAMEO-TROPMED Center, Jakarta, Indonesia. As the supplementary feeding would be implemented for preschool children, the test should be done on preschool children, however, because for this test 7 times venous blood (3.0 ml/time) must be drawn on the same day, based on ethical consideration, it was impossible to carry out this test on preschool children. Due consideration will be given in applying the findings on preschool children.

Materials

The materials used for the experiment consisted of :

a/ Standardised meal : consisting of 40 g of white bread, 10 g of butter, 10-15 g of pineapple jam, and water.

b/ Thirty grams of fortified soybean milk (FSBM) powder composed of 150 kcal, 7.6 grams protein, 8.4 grams lipid, 1.8 mg of iron, 1.6 mg vitamin C, and fortified with 300 µgRE, 5 mg iron pyrophosphate. It was mixed with 95 mg elemental iron equivalent to 475 mg of Fe(II)-sulphate 7 H_2O and mixed with hot water to make a 200 ml mixture.

c/ A reference solution was made of 100 mg iron equivalent to 500 mg of Fe(II)-sulphate 7 H_2O mixed with water to make a 200 ml mixture.

d/ Commercial kits for plasma iron determination (Merckotest, Merck, Darmstadt, Germany).

Methods

The Ferrozin method was used to compare the change in plasma iron during a period of 10 hours after the consumption of test substances. The procedure consisted of preparation of the subjects, tests on the first day and one week later.[27]

* ***Preparation of the subjects***: The subjects can not consume alcohol, coffee, teas or solid food during the 12 hours prior to the test. On the day of the experiment, the subjects received a standardised meal. The subjects were measured for weight, height and Hb before consumption of the iron reference solution on the first day.
* ***The first day of the test***: The subjects were given 200 ml of reference solution. at 8.00 am.
* ***One week later:*** The subjects received 30 g of FSBM product (containing 5 mg of iron) and 95 mg of iron solution mixed with hot water to make a 200 ml mixture at 8:00 am.
* ***Blood samples collection***: on the first day of the test and one week later. On both days, the schedule of drawing venous blood, test substances consumption and taking meal was as follows.

Activities / Time	7.55	8.00	9.00	10.00	12.00	14.00	16.00	18.00
Consumption of test substances		√						
Taking standardised mean					√			
Venous blood drawn	√		√	√	√	√	√	√

Analysis of results

The relative bioavailability value (RBV) of iron mixed with soybean milk was calculated based on the following formula (Pietrzik et al 1990):

$$\% \text{ Bioavailability} = (AUC^*_{\text{test substances per os}} \times 100) : AUC^*_{\text{reference per os}}$$

AUC* (area under the curves) is calculated using the plasma iron concentration values.

The Acceptibility Test of Fortified Soybean Milk and Fortified Soybean milk

Objective, subjects, study site and sample size

This was one kind of sensory test following the guideline of Tech[28] to determine the acceptance of the soybean milk powder fortified with 300 µgRE and 5 mg of iron pyrophosphate (FSBM) per 30 g product and unfortified soybean milk (UFSBM) by preschool children. The acceptability tests was carried out on 150 preschool children, aged 8-62 months, both boys and girls, at the rural day-care center, in Vietnam.

Materials

The FSBM & UFSBM were produced by the company "Nestle's Indonesia" and was packed in 30 grams per one foil sachets.

* ***The UFSBM*** consisted of 30 grams soybean milk powder which contained 150 kcal, 7.6 grams protein, 8.4 grams lipid, 1.8 mg of iron, 1.6 mg of vitamin C and the FSBM was soybean milk powder fortified with 300 µgRE and 5 mg of iron pyrophosphate per 30 grams of the product.

Methods

The subjects were randomly divided into 2 groups (Group 1 & Group 2) and each group consisted of 30 children age 8-<36 months and 35 children of age 36-62 months. Each group was given FSBM and UFSBM on 3 consecutive days. Daily records were made on the number of children who consumed/accepted the FSBM and the UFSBM as well as the amount of supplement consumed in percentage, the acceptability of the supplements was based on the following criteria :

1. Consumption of more than two-third of the FSBM or UFSBM given.
2. Number of subjects who accepted each kind of supplement.

Analysis of results

Chi-square (X^2) test was used for testing the acceptability of the FSBM and UFSBM

The Studies in Peri-Urban and Rural Area

Design, objective and subjects

The studies in the peri-urban and rural area were cross-sectional studies with the following objectives:

1. To assess the nutritional status of preschool children in the peri-urban and rural area
2. To determine the influencing factors
3. To compare the condition of the peri-urban and rural area.

Subjects were boys and girls who visited day-care centers, aged 15-72 months with the mean age of 48.4 ± 13.4 months in the peri- urban and aged 9-66 months with the mean age of 40.5 ± 12.5 months in the rural day-care center. Only those children who obtained the consent from their parents were selected for the study.

Sample size

Using the formula for cross-sectional study with a 95% confidence interval, prevalence of anemia for each area plus 10% n for drop outs, the sample sizes were

* Peri-urban area study, 380 with anemia prevalence of 33% in the urban area.
* Rural study 422 preschool children with the anemia prevalence of 49.3% in the rural area.[29]

Measurements and methods of assessment

The measurements and methods used were the same for the peri- urban and rural study

1. ***Socio-economic condition***: by interview
2. ***Dietary assessment***: by 24 hours food recall and observation on two consecutive days
3. ***Clinical assessment:*** Physical examination combined with interviewing the mother to assess acute respiratory tract infection (ARI) and diarrheal disease during one previous week.
4. ***Anthropometric measurements***: *Weight, the recumbent length* of children less than 24 months and *height* of children above 24 months, the *mid-upper-arm-circumference (MUAC), Skinfold thickness* were measured following the guideline of WHO (1983).
5. ***Stool examination***: to determine the type, and intensity of worm infestation (*Ascaris, Trichuris,* and Hookworm) by Kato-Katz method.[30]
6. ***Blood examination***
   - *Serum retinol determination* : by High Pressure Liquid Chromatography method (HPLC)[31] using the Sigma standard (Sigma, Saint Lois, MO, USA).
   - *Hemoglobin determination*: by cyanmethemoglobin method (INACG 1985).
   - *Serum ferritin determination*: by Microparticle Enzyme Immunoassay (MEIA)[32] using computerized IMx System (Abbott Laboratories Diagnostics Division, Abbott Part, Illinois).

Statistical analysis

1. Data management

All data collected were coded, entered and analysed in the Statistical Package for the Social Sciences (SPSS) for windows (version 6.0, SPSS Inc, Chicago) and Epi-Info 6 (USD Inc, West Park, G.A). The nutrient intake was analyzed in the nutrient analysis program Demeter 1.5.

2. Data analysis

All continuous variables were first checked for normal distribution. Descriptive characteristics were expressed as Mean, Median, Standard Deviation, Standard Error (for *Continuous variables*), Frequency and Percentage (for *categorical variable*). The statistical analysis tests used were: t-test, ANOVA test, COANOVA test, Mann-Whitney test, Chi-square test, Bivariates correlation and Multiple linear regression analysis (MLRA).

The Intervention Study

After the assessment of the condition in the peri-urban and rural area, the rural area was selected for the intervention study. The same area, and the same number and subjects from the rural study were enrolled the intervention study.

Design, objectives, study site and subjects

The design of this study was a community nutrition trial. All subjects were allocated into 3 groups by simple random sampling method into FSBM, UFSBM and cassava cookies (CC) group. The objective was to assess the effects of vitamin A (300 µgRE) and iron (5 mg of iron pyrophosphate) fortified soybean milk on vitamin A, iron status and physical growth of rural Vietnamese preschool children. The subjects were healthy boys and girls who visited daycare centers, aged 8-62 months with the mean age of 40.5 ± 12.5 months. The subjects were free from severe diseases and food allergy. Only those children who obtained the consent of their parents were selected for the study.

Sample size

The sample size was calculated using the formula for community trial with 95% confident interval and 80% chance to detect an expected change in SR (5.1 ± 10.2 µg/dL) Hb (8±16 g/L), for eventually prevent drop out (10%) and the presumption that the response to fortified supplement may occur only in the anemic subjects (50%). A total of 420 rural preschool children were allocated into 3 groups by simple random sampling method into FSBM, UFSBM and CC groups. The samples size was 140 children for each group.

Materials

Deworming

Dewormed drug used was Mebendazole 120 mg x 3 consecutive days.

Food supplements

The food supplements consist of FSBM, UFSBM and Cassava cookies (CC). each child will be given of 30 g of FSBM or UFSBM or 12g of CC. The FSBM is soybean milk fortified with 300 μgRE and 5 mg of iron pyrophosphate. The amount of vitamin A and iron for fortification were determined taking into consideration the ***requirement, actual dietary intake and toxicity of vitamin A and iron***. The composition of the supplements is presented in Table 1.

Table 1. The composition and the price of FSBM, UFSBM and cassava cookies.

Compositions	Fortified SBM (30 g)	Unfortified SBM (30 g)	Cassava cookies (12 g)
Energy (kcal)	150	150	45
Protein (g)	7.6	7.6	0.5
Fat (g)	8.4	8.4	0
Iron from food (mg)	1.8	1.8	0.7
Vitamin C (mg)	1.6	1.6	0
Vitamin A (μgRE)[2]	300†	50✤	0
Iron pyrophosphate fortification (mg)[1]	5	0	0
The price (US$/kg product)	1.9	1.7	0.9

[1] Iron Pyrophosphate, *(equivalent to 1.25 mg elemental iron)*, [2]Retinyl acetate
† Information from Nestle Indonesia, ✤Determined at the NIN, Hanoi, Vietnam

Conduct of the intervention study

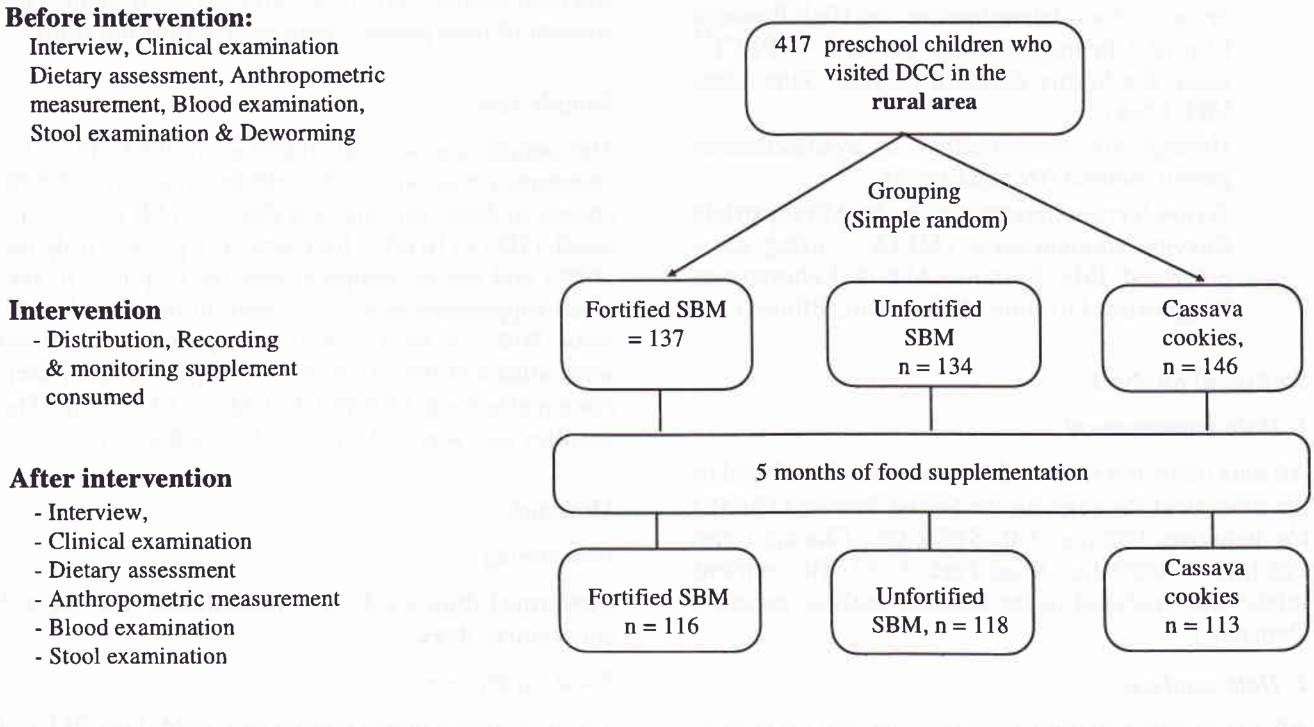

Figure 2. Conduct of the intervention study

Methods

1. Interview to get information on: Diseases and ARI
2. Clinical assessment: physical examination
3. Dietary assessment on 2 consecutive days by 24 hours food recall and observation.
4. Anthropometric measurement: Weight, height, MUAC, Skinfold thickness,
5. Blood examination: SR, Hb and Serum ferritin determination
6. Stool examination: worm infestation
7. Deworming: Before intervention, all subjects with egg-positive stools for parasites infestation were given Mebendazole 120 mg during 3 consecutive days
8. Distribution, recording and monitoring of supplementary foods.

The intervention was conducted by giving supplements to the subjects daily, on 6 days per week, except on Sunday, at the day- care centers and around 2.00 pm, (3 hours after lunch). The trained-child attendants prepared and regularly distributed the supplement was prepared individually for each child.

Monitoring of supplementary food was done by the researcher on uninformed visits to the day care centers twice or three times a week.

Statistical analysis

1. Data management

The same data management programs for the peri-urban and rural study were used.

2. Data analysis

All continuos variables were first checked for normal distribution.

Descriptive analysis: Mean, Standard deviation, Median, Standard error (for *continuous variables*), Frequency and Percentage (for *categorical variables*). The statistical analyses tests used were: t-test and Repeated- measure-MANOVA test, Kruskal-Wallis, Chi-square test, Wilcoxon- test, Bivariate correlation, and Spearman's correlation coefficient and Multiple linear regression analysis (MLRA).

RESULTS AND DISCUSSIONS

This study consisted of five parts: The first part and second part of the study were to test the relative bioavailability of iron in fortified soybean milk and the acceptability of fortified SBM with vitamin A and iron by preschool children. The third part was the study in the peri-urban Hanoi and the fourth part was the study in the rural Hatay province in North of Vietnam to get the information about and compare the nutritional status of preschool children in these two areas. These cross sectional studies were carried out one month after the children got a single high dose of vitamin A capsule from the National Vitamin A Capsules Distribution Program.

The relative bioavailability of iron in soybean milk was 45% compared with iron alone and the fortified SBM was very well accepted by the preschool children. The results of the cross- sectional studies showed that the nutritional status of the children were poor in both the peri-urban and rural areas. As the rural children had poorer nutritional status than the peri-urban children, the fifth part, the intervention study, was conducted on the same rural preschool children.

The characteristics of Fortified Soybean Milk Supplementation

The relative bioavailability of iron in the fortified soybean milk

The relative bioavailability of iron was assessed on 7 female and 3 male adult volunteers and the result was 45.8 ± 13.7%. The finding of this study is in agreement with the findings by Cook et al.[33] Hallberg & Rossander[34] indicating an inhibitory effect by a wide range of soybean produts. The factors that caused the inhibition of iron absorption in the soybean milk product could be the high phytate content of soybean milk components, which has a range from 0.18-0.43 mg%. Other factors influencing absorption are phytate, protein and calcium interaction.[35,36] Several studies showed that the bioavailability of non-heme iron can be improved by vitamin C.[34,36]

The composition and acceptability of fortified soybean milk

The results showed that both UFSBM and FSBM with 5 mg of iron pyrophosphate (equivalence to 1.25 mg of elemental iron) and 300 μgRE were excellently accepted by the children (100%). These supplements would give an additional 12% of energy, 33% of protein and ± 70% of vitamin A of RDA for the preschool age children. With regards to iron, taking under consideration the Relative bioavailable of iron in the FSBM was 45%, a presumed absorption rate of 20% in iron deficient subjects and about 1.25 mg of iron from FSBM, the additional iron provided by FSBM was presumed to be 29% of the RDA.[37]

The Studies in the Peri-Urban and Rural Area

The peri-urban study was conducted in the winter, January, 1995 and the rural study in the summer, in July, 1995. The study subjects were preschool children who went to the day-care centers, 438 children from two communities in the peri-urban Hanoi and 417 children from one rural community from Hatay province. Based on the information of the mothers there was no significant difference in the yearly, monthly household and monthly per capita income of the peri-urban and rural households. However, the peri-urban expenditure on food and education was significantly higher than the rural households.

The dietary intake of preschool children

Food recall results on energy and nutrients intake showed that the subjects in both peri-urban and rural areas, on average, had low energy, heme iron, and vitamin A intake compared with the RDA established by WHO/FAO/UNU[38] and FAO/WHO 1988[37] (Table 2). Rural children had a fairly good intake of energy which was reflected by an 88.8% of RDA intake and that level was significantly higher than the peri-urban children (78.9%). The rural children had a higher intake of vitamin A than the peri-urban chilren, however the main

Table 2. Energy and nutrients intake of the children from peri-urban and rural households

	Peri-urban (n=207)				Rural (n=417)				
	Mean ± SD	Median	RDA *(Mean)*	Adequency *(%)*	Mean ± SD	Median	RDA *Mean*	Adequency *(%)*	p-value
Energy (kcal/day)	963.5± 185.2	926.7			1003.4 ± 167.4	1029.4			* < 0.1
Energy (kcal/kgBW)	75.7±17.4	73.2	95.8	78.9	86.8±14.0	85.7	97.8	88.8	*<0.001
Protein (g/kgBW)¶	1.51±0.37	1.46	1.11	139	1.67 ± 0.29	1.65	1.08	151.2	* <0.001
Animal protein (%)	29.8±13.1	28.6			25.0±9.9	24.2			*<0.001
Calcium (mg)	169±54	171	439	38.6	194±191	169	433	44.7	m<0.05
Phosphorus (mg)	396±84	386	333	119.8	384±67	384	321	120.4	*>0.05
Thiamin (mg)	0.45±0.18	0.44	0.7	69.8	0.40±0.11	0.4	0.6	67.5	*<0.05
Vitamin C (mg)	27±18	23	20	137.7	46±35	36	20	228.2	*<0.001[a]
Total iron (mg)	5.26±1.19	5.1	5.4	97.5	5.6±1.09	5.53	5.3	104.9	*<0.001
Heme iron (mg)	0.79±0.58	0.59		0.71±0.47	0.56				m<0.05
Heme iron (%)	14.7±9.5	12.1		12.9±8.6	10.9				*<0.01[a]
Vitamin A (mgRE)	230.2±156.9	194	400	57.5	314.3±202.5	269.7	399	75.8	m<0.001
b-Carotene	1014±634	905	1651±1125	1390					m<0.001
Retinol (μg)	60.5±115.1	3.9			37.5±78.2	0.4			m<0.001
Retinol (%)	17.7±24.3	3.6			10.4±17.1	0.2			m<0.001

*t-test, m Mann-Whitney test, [a]Based on the Log 10-transformation values
¶Adjusting for digestibility 85% and acid amino score 75%
Adequacies were calculated based on RDA from FAO/WHO, 1988 and WHO/FAO/UNU 1985

source of vitamin a was β-carotene. The finding on β-carotene and vitamin C in the diet may be related to the time the study was conducted, in the rural area in summer and the peri- urban are in the winter. In the summer more sources of β-carotene and vitamin C like dark green vegetables and yellow fruits are available. The intake of retinol of rural children was significaty lower than the peri-urban children. The percentage of heme iron and animal protein intake of rural chilren were significantly lower than the peri-urban children. Protein intake of both peri-urban and rural children fulfilled their RDA, however, the main part came from plant protein, especially for the rural children.

The findings of this study are little different from studies in other countries. Jasen et al showed that the dietary intake was worse in the urban than in the rural area of Brazil. Other findings indicated that general urban diets provide a more adequate energy and nutrient supply than rural diets.[39,40] Energy intake in the rural area of this study was less adequate than in rural Kenya[41] and poor rural areas of China.[42]

The health status of the preschool children

Independent of their living location, nearly two-third of the children were infected with *Ascaris* in both areas and 16.2% of the peri-urban and 10.7% of the rural children were infected with *Trichuris*. The percentage of children infected with both *Ascaris* and *Trichuris* was 12.9% in the peri-urban area and 8.9% in the rural area. However, none of the children showed any presence of Hookworm infestation. Peri-urban children suffered significantly more from acute respiratory tract infections (30.6%) than the rural group (7.9%). The percentage of diarrhea in the peri-urban and rural children were 3.2% and 2.6%, respectively.

Vitamin A and iron status of preschool children in the peri-urban and rural area

Table 3 shows that the retinol level of the peri-urban preschool children was significantly higher than those in rural area. Using the cut-off point for serum retinol concentration 1.05 μmol/L after vitamin A capsule supplementation,[43] the study showed that 57.5% of rural children and 6.9% of the peri-urban children had low retinol concentration (p<0.001). This condition can be explained by the difference in the retinol and animal protein intake, which are lower in the rural area, although β-carotene intake was high. With regards to diarrhea and *Ascaris* infestation, the same condition was found in the two areas.

Table 3 also shows the mean Hb of the children which was 119.2 g/L in the peri-urban and 119.3 g/L in the rural preschool children. After corrected for age it was 118.3 g/L in the peri- urban area and significantly (p=0.02) lower than that of rural children (120.2 g/L). The percentage of anemia of the peri-urban and rural preschool children (20.6 and 20%, respectively) did not differ significantly. The mean of serum ferritin (SF) concentration in the rural children was 29.3±13.7 µg/L and 26.5% of them had a SF concentration below 20 µg/L. The percentage of anemia in this study was lower than the result of the National Anemia Survey conducted in 1995. Why was it lower? May be because this study was conducted one month after the children got a single high dose of vitamin A capsules (200,000 UI) from the National Vitamin A Capsules Distribution Program. This study supports the finding of a study by Bloem et al[44] which showed an improvement of iron status after only 2 weeks supplementation of a single high dose of vitamin A.

The relationship between serum retinol and hemoglobin level of preschool chilren

Recently studies have shown that vitamin A status influences the iron status through the release of storage iron by vitamin A. An experiment on rats showed that marginal vitamin A deficiency produced low blood Hb concentration[8,10] and Staab et al's study[9] on rats support the hypothesis of vitamin A as regulating agent in iron release from the liver. In both these study areas the SR levels parallel the Hb levels (Table 4). The observation of the relationship between Hb and SR found in the present study has also been reported in other countries.[20,45]

Table 3. Vitamin A status and iron status of the preschool children from peri-urban and rural area

	Peri-urban	Rural	p-value
Serum retinol (µmol/L)	1.34±0.24 (391)	1.0±0.21 (398)	b§<0.001
Hemoglobin (g/L)	119.2±11.0 (398)	119.3±11.3 (410)	§ < 0.05
Serum ferritin (µg/L)	-	29.3±13.7 (347)	
Serum retinol <0.70 µmol/L	0	30 (7.5%)¶	
Serum retinol <1.05 µmol/L	27 (6.9%)	229 (57.5%)	†<0.001
Hemoglobin <110 g/L	82 (20.6%)	82 (20%)	†<0.001
Serum ferritin < 20 µg/L	-	92(26.5%)	†ns

§ Corrected for age (ANOVA model test), † Chi-square test, * Mean±SD (n)
¶ n(%) [b]p-value based on the transformed serum retinol (Square root of serum retinol)

Table 4. Means of hemoglobin and serum ferritin level* by serum retinol concentration categories

	Serum retinol concentration		
	< 0.70 µmol/L	0.70-<1.05 µmol/L	≥ 1.05 µmol/L
Peri-urban			
Hemoglobin (g/L)		113.6±10.4(23)	119.5±10.9[1](343)
Rural			
Hemoglobin (g/L)	113.6±10.4(30)	118.0±11.3(198)	121.4±10.7[2](164)
Serum ferritin (µg/L)	29.1±18.2 (16)	26.5±12.5 (93)	36.6±20.6 (78)

* Mean±SD(n) [1]p=0.05., [2]p<0.01 significant difference between serum retinol classes, (ANOVA Model test, control for age)

Physical growth of the peri-urban and rural pre-school children

The studies in the peri-urban and rural area showed that the physical growth of rural preschool children was worse (Table 5). The rural children had a significantly lower mean body weight, height and MUAC compared to their peri-urban counterparts. More rural children were stunted (55.6%), underweight (53%) and had low MUAC (60%). Compared with the National Survey in Vietnam (1994) which showed 46.9% prevalence of stunting and 44.9% underweight,[2] the condition of the preschool children of peri- urban was better, but the rural children was worse. Data on physical growth from developing countries in Asia, Africa and America vary. According to Braun et al[46], Graham et al[47], Valverde et al[48] prevalence of growth retardation of rural preschool children is greater than of the urban areas. On the other hand, other studies in Costa Rica, Guatemala, the condition was worse in poor areas of cities than rural areas.

The Five Months Intervention on the Rural Pre-school Children

Considering the condition of the rural area in terms of vitamin a status, physical growth, it was decided to do intervention study in the rural area. The intervention study was done with the same number of subjects, of the same area of the rural study. Four hundred and seventeen children enrolled with the mean age of 40.5±12.5 months, consisting of 215 boys and 202 girls. The subjects with egg-positive stool were given deworming before randomly allocated into 3 groups: FSBM, UFSBM, and CC group. The FSBM, UFSBM, CC provided respectively FSBM 12% of energy, 70% of vitamin A, 30% of iron; UFSBM 12% of energy, 12% of vitamin A, 8% of iron; CC about 3% of energy, 0% of vitamin A and iron. The daily food of the subjects provided 88.8% energy, 104% iron, 78.5% vitamin A of RDA. During the study 70 subjects (16.5%) dropped out because some became sick, some hadn't got the consent of their parents for blood taking and some blood samples were hemolysed. Two hundred and seven subjects were assessed for serum ferritin.

The socio-economic condition

Based on the information of the mothers the results showed that the yearly of the rural household was very low (less than 600 US$) and monthly per capita income was about 11 US$. The food expenditure was 57.2% of the total household income. Only 48.4% of the rural household have television. This situation indicated that the children in this study came from poor household with low income.

Table 5. Age and selected anthropometric indices of the preschool children from peri-urban and rural area

	Peri-urban (n=438) Mean±SD	Rural (n=417) Mean±SD	p-value
Age (months)	48.4± 13.4	40.5±12.5	<0.001¶
Weight (kg)	13.0±2.1	11.7±2.0	§<0.001¶
Height (cm)	95.0±7.8	88.8±7.8	§<0.001¶
HAZ-Score	-1.62±1.17	-2.14±0.90	§<0.001¶
WAZ-Score	-1.80±0.86	-2.06±0.72	§<0.001¶
WHZ-Score	-1.06±0.89	-1.04±0.66	§ns¶
MUAC (cm)	14.9±1.0	14.3±1.0	§<0.001¶
Biceps skinfolds (mm)	5.2±1.0	5.4±1.0	§>0.05¶
Stunting (%)[1]	37.4	55.6	<0.001†
Underweight (%)[2]	44.1	53.0	<0.01†
Wasting (%)[3]	12.6	7.2	<0.01†
Low MUAC (%)[4]	29.5	60.0	<0.001†

¶t-test, †Chi-square test, §Corrected for age (ANOVA test), ns no-significant difference,
[1]HAZ-Score<-2SD, [2]WAZ-Score<-2SD, [3]WHZ-Score<-2SD),
[4]MUAC<14.5 cm

The dietary intake of subjects

Regular dietary intake

Before intervention there was no significant difference in food consumption patterns among the three groups. Energy intake of rural preschool children met 88.8% of RDA and protein intake was 151.2% of RDA,[38] however the animal protein intake was low (25%). The iron intake of the study subjects met the RDA established by FAO/WHO 1988,[37] however the heme iron intake was also low (12- 15%). The mean vitamin a intake met 80% of RDA[37], but the main part came from β-carotene. Retinol intake was only about 10% of the total vitamin A intake.

After intervention, there was no significant difference in the regular diet between the three groups, except for vitamin A intake; it was significantly lower in the USBM group compared to the FSBM group ($p<0.05$, Kruskal-Wallis test). Comparing of the regular diet consumption before and after intervention showed that in general there was no significant difference on the consumption of energy, protein, heme iron and retinol intake. However the intake of vitamin A, β-carotene and vitamin C were significantly lower after intervention. The condition was the same in the three groups. It means that the animal food consumption remained as before intervention but the vegetables consumption was reduced after intervention. The reasons could be that the last month of intervention, was winter in Vietnam so the dietary intake of dark green vegetables and yellow fruits available in the summer such as *Water spinach, Vinespinach, Jute potherb, Mustard green and Mango* were lower. A study in Northeast Thailand on children 1-8 years old on the effects of a single high dose of vitamin A capsule supplementation showed that after two months, the levels of retinol and RBP in serum increases significantly both in the supplemented (73%) and in the control group (47%). The authors suggested that seasonal effects of dietary intakes may have impact of the intervention with vitamin A status.[20]

Food supplement consumed

The results of monitoring of the foods supplement consumption showed that the children liked and consumed all fortified SBM, unfortified SBM or cassava cookies that were given. There was no left over. So the supplementary food consumed daily during the intervention provided from FSBM, UFSBM and CC respectively: *150, 150, 45 kcal; 300, 50, 0 mgRE and 6.8, 1.8, 0.7 μg of iron*. In percentage of their RDA, the food supplements gave the addition as the following: FSBM 12% of energy, 70% of vitamin A, 30% of iron; UFSBM 12% of energy, 12% of vitamin A, 8% of iron; CC about 3% of energy, 0% of vitamin A and iron.

The health status of preschool children

Selected infections

Most of the studies of supplementary feeding have not evaluated it's impact on morbidity. Beaton and Ghassemi[49] pointed out that of the 200 programs that they reviewed only eight included information on morbidity outcomes. However, it couldnot be concluded that the lower morbidity rate was the result of the improvement of nutritional status.

The results shows that before and after intervention, there was no significant difference between-group in the percentage of infectious disease, however within-group there was a significant increase in the percentage of upper-respiratory tract infection after intervention. Before intervention (5.9-7.1%) percent of children who suffered from upper-respiratory tract infection which was significantly higher after intervention in all three groups (21.2-31%). The percentage of lower-respiratory tract infection was low and did not significantly differ before (0- 1.7%) and after intervention (0.9-2.5%) in all three groups. Before intervention the percentage of diarrhea of three groups varied between 1.8-3.4%. After intervention it was reduced to 0.9-2.6%, however there was no significant difference before and after intervention in all three groups.

The condition of diarrhea and lower-ARI could be related to the improvement of vitamin a status. Evidence from intervention study in Chinese and Vietnamese preschool children indicated supplementation with large doses of vitamin A decreased the incidence and severity of diarrhea and respiratory disease in supplemented children, possibly through enhanced activity of the immune system.[50] With regard to the effect of the improved iron status in this study, it supports most of the studies evaluating the effect of oral iron therapy in iron deficiency subjects which showed a reduction in morbidity.[5] With regard to the upper-ARI in this study which was increased after intervention, this could be explained that when the intervention study was ending, it was winter in Vietnam and children easily suffer from upper-ARI. Environmental factors which were not examined in this study, can not be neglected because it influence infectious diseases like diarrhea and ARI.

Helminth infestation

Stool examination before deworming and intervention showed that there was no case of hookworm infestation; However *Ascaris* and *Trichuris* were found in children of all groups. 59.4%, 65.2% and 67.9% of children suffered from *Ascaris* infestation respectively in the FSBM, UFSBM and CC group and 13.5%, 8%, 11.3% suffered from *Trichuris* infestation; respectively in the FSBM, UFSBM and CC group. The percentage of *Ascaris* and *Trichuris* infestation was not significantly different between the three groups (p>0.01). Just before starting the intervention the children suffering from *Ascaris* and *Trichuris* were given Mebendazole for deworming.

After intervention the deworming subjects showed the following picture, about 30% remained infected by *Ascaris* and varied 0.21.4% by *Trichuris.*

The subjects who were not infected and did not receive deworming before intervention who became infected (by *Ascaris* varied between 18.4-31.6%, and by *Trichuris* varied 3.3-8.7%) after intervention.

After intervention the percentage of *Ascaris* infestation in the total studied subjects was significantly reduced in all groups (p<0.001). It was 27.9%, 33%, and 27.4% respectively in the FSBM, UFSBM and CC group.

The decrease of worm infestation could have led to the improvement vitamin A status. In this study showed that SR of the *Ascaris*-free subjects and *Ascaris* infected one increased and the increase in the *Ascaris*-free subjects was significantly higher in the *Ascaris* infected subjects in the FSBM group. Studies in Brazilian and Indonesian preschool children have shown that deworming led to significantly improvement vitamin A status.[51,52]

The improvement of iron status increased only in the FSBM which was infected with *Ascaris* and *Trichuris.* In other study where only iron fortified was used and combined with deworming on the subjects who were also infested by *Ascaris* and *Trichuris* showed the significantly improvement of iron status, however the percentage of *Trichuris* infestation was higher.[17] The study of Stephenson et al[6] included by subjects infested by hookworm infestation showed that the iron status of the dewormed subjects was maintained whereas it was reduced in the control subjects. It could be related to the result of deworming before intervention.

This study showed that physical growth measured by weight, height, MUAC, Triceps skinfolds, WAZ-score, HAZ-score and WHZ- score increased significantly in the CC group (after controlling confounding factors of age). The same finding was found in the study by Stephenson et al.[6]

Vitamin A and iron status of preschool children

Table 6 shows that there were no significant differences among the three groups in the mean values of SR, Hb, and SF level before intervention. After intervention, both FSBM, UFSBM and CC groups showed a significant increase in SR concentration (p<0.001 for all) within the respective groups. The amount of SR increase after intervention was marginally significant different between the three groups (p=0.07). There were also significant improvements in the mean values of Hb concentration within a group of all three groups (p<0.001 for all). There was between groups differences in supplement effect for Hb (p=0.02). SF concentration increased significantly within the fortified SBM group only (p<0.01). There was no significant difference for the mean values of SF between the three groups after intervention. To assess the effect of the supplement, potential confounding factors such as age, initial values of SR, Hb status of subjects, *Trichuris* and *Ascaris* infectation which were controlled by Repeated-measure MANOVA test (Table 6).

Table 6. Changes in serum retinol and iron status after intervention

	n	Before Mean±SD	After Mean±SD	Changes Mean±SD
Serum retinol (μmol/L)				
Fortified SBM	102	0.98±0.21	1.22±0.21	0.24±0.22§[1]
Unfortified SBM	109	0.99±0.22	1.19±0.19	0.20±0.21§
Cassava cookies	100	1.01±0.20	1.18±0.22	0.16±0.25§
Hemoglobin (g/L)				
Fortified SBM	112	118.7±10.4	125.2±9.4	6.5±9.3§[2]
Unfortified SBM	113	119.2±10.6	124.9±9.9	5.7±10.5§
Cassava cookies	108	119.3±11.9	122.6±9.8	3.3±10.7¶
Serum ferritin (μg/L)				
Fortified SBM	77	27.5±13.9	30.6±13.6	3.1±12.0¶
Unfortified SBM	82	27.7±13.4	29.3±12.6	1.6±10.4
Cassava cookies	88	29.1±13.8	31.0±13.6	1.9±10.8

¶p<0.01, §p<0.001 significant changes within group (Paired t- test)
Between groups difference in changes of SR: [1]p=0.076, of Hb: [2]p=0.02 (controlled for age, initial Hb, SR, *Trichuris and Ascaris* infestation by Repeated-measure MANOVA test)

In Table 7 shows the changes of percentage of VAD, anemia and iron deficiency after intervention. Based on the SR values <0.70 μmol/L, the change was 100% in the FSBM and UFSBM groups. Whereas the reduction in the CC group was less than the two SBM groups,

Table 7. Changes of percentage of vitamin A deficiency, anemia and iron deficiency before and after intervention

	n	Before n(%)	After n(%)	Effect of intervention n(%) become normal
Serum retinol < 0.70 μmol/L				
Fortified SBM	102	8(7.8%)	0(0%)^3	8(100%)
Unfortified SBM	109	10(9.2%)	0(0%)^3	10(100%)
Cassava cookies	100	7(7%)	1(1%)^3	6(85.7%)
Serum retinol < 1.05 μmol/L				
Fortified SBM	102	70(68.6%)¶	21(20.6%)^3	49(70%)
Unfortified SBM	109	58(53.2%)	23(21.1%)^3	35(60.3%)
Cassava cookies	100	59(59%)	24(24%)	35(59.3%)
Hemoglobin < 110 g/L				
Fortified SBM	112	21(18.8%)	6(5.4%)^2	15(71.3%)
Unfortified SBM	113	22(19.5%)	6(5.3%)^2	16(72.8%)
Cassava cookies	108	27(25.0%)	10(9.3%)^2	17(62.8%)
Serum Ferritin < 20 μg/L				
Fortified SBM	78	25(32.1%)	18(23.1%)	7(28%)
Unfortified SBM	83	25(30.1%)	21(25.3%)	4(15.9%)
Cassava cookies	87	25(28.7%)	23(26.4%)	2(8%)

^{2} p<0.01, ^{3}p<0.001 significant decreases within group (Wilcoxon- test)

85.7% (p<0.001 for all group). If the cut-off point of SR <1.05 μmol/L was used, then the reduction in prevalence is less for all the 3 groups. With regard to iron status, the Hb used the cut-off point <110 g/L, the effect was larger in the FSBM and UFSBM groups than in the CC group. Using the cut-off point of serum ferritin < 20 μg/L, there was no significantly change was observed, however it was still higher in the FSBM group.

The result showed that intervention there was a clear positive improvement of vitamin a status of all three groups. The question arises why the SR of all 3 groups went up after intervention? The factors like deworming, diet and infectious disease may have controbuted the condition.

The increase of SR in the FSBM group was **0.08 μmol/L** higher than in the CC group which could be the effect of *300 μgRE fortified* SBM. The benefits of vitamin A fortified food was studied in several countries, in Guatemala[16], Indonesia[53], Brazil.[54] The present study found an increase of SR **0.08** ***μmol/L*** which is in the range of previously reported findings. It also could be the reason that beside the increase of SR, the storage of vitamin A also increased in the liver. When liver vitamin a concentrations are between the limits (20-300 μg/g liver), SR concentrations are controlled, levels remain relatively constant and do not reflect total body reserves of vitamin A.[55]

The *dietary intake* doesn't seen have to contribute to improvement of vitamin A status, because the dietary of β-carotene was reduced and retinol did not change after intervention. The increases of SR in the FSBM and UFSBM groups could be contributed on the one part by the increases of *fat* (8.4 g) and *protein* (7 g) intakes from the supplement. The UFSBM also contains 50 μgRE which could lead to increase of SR. Both absorption and utilization of preformed vitamin A and provitamin A are improved with current dietary fat and protein intake.[56,57,58] When adequate dietary proteins were given to the patients with kwashiorkor, the serum vitamin A levels rose and provided the patients had sufficient liver reserves of vitamin A.[59] Fat is an important factor which need for vitamin A absorption. *Ascaris* infestation impairs fat absorption. After treatment for *Ascaris* infestation, faecal fat decreased from 9.5 to 2.3%.[60]

Deworming treatment of the children before intervention is one of the factors which could explain the significant improvement in the SR concentration of all three groups. Before intervention nearly two-third of children suffered from *Ascaris* infestation and it reduced to about 30% after deworming following the intervention. the present intervention study showed that the subjects without *Ascaris* infestation had a 2.5 times higher in increase of SR than those who remained *Ascaris* infestation in the FSBM group (p=0.005). The increase of SR in the control group was **0.16 µmol/L** in the present study which was fully in agreement with the study in Indonesian and Brazilian children by Schultink et al[52], Marinho et al.[51] Vitamin a supplementation for children suffering from intestinal parasites was ineffective/or less effective.[51,61]

The *infectious diseases* also did not change significantly after intervention Finally, the SR of the CC group increase could be the *"intervention"* (Hausthorn) effect.

Five months intervention resulted in significant increases of Hb concentration among the three groups. However, the largest increase was in the FSBM group which was significantly higher than that in the CC group (3.2 g/L). The reasons for the Hb increase in all three groups, in theory could be several, *Firstly,* the *increase in age* of children after 5 months of intervention could be the reason of the Hb increase. It was reported that there is a gradual rise in values of Hb throughout childhood.[62] The present study also showed that there was a positive correlation between age and Hb concentration before intervention study. *Secondly*, the increases of Hb concentration could be related to effect of the *increase of SR* during the intervention period. Results of this study and many studies have shown that vitamin A status is related to the iron status.[16,63,64] Vitamin A deficiency in rats causes anemia.[8,9,10] The hypothesis is that vitamin A plays a role in the release of iron from the liver for Hb production.[65]

Beside the above reasons, the increase of Hb in the FSBM group partially reflects also the effect of *5 mg of iron purophosphate (≈ 22% of RDA) fortified* SBM. The benefits of iron fortification food have been shown by many studies.[17,18,28,66]

After 5 months of intervention, SF increased significantly within the FSBM group only. No significant difference between the three groups existed. The result of the present study is lower than the result of a study by Dutra-de-Oliveira,[25] Rivera et al.[66]

In this study the difference between the FSBM and the C group in the increase of SF was 1.2 µg/L. In infants and children, 1 µg of SF is equivalent to about 120 µg iron storage/kg body weight.[67,68] Based on this suggestion we can estimate the amount of iron storage increase as 4.8 mg in FSBM group, 2.1 mg in the UFSBM and 2.9 mg in the CC group. The present study showed that the increases of SF was small in the FSBM group. The reason could be that the amount of iron supplemented was not enough to build sufficient iron storage and iron absorption inhibited in SBM.

The reason for the increase of SF in the three group could be related to the increase in age of the children after 5 months of intervention. Changes in the concentration of SF is known to parallel changes in age and iron stores.[62] The present results showed a positive correlation between the age and the SF before intervention (n = 247, r=42, $p < 0.001$). Serum ferritin did not improve significantly which in theory serum retinol improve doesn't lead to improve serum ferritin.[65] Ferritin was a main part (70%) of stored iron in the body, when SR increases iron will be mobilized to the bone marrow for Hb production, thus the SF will not increase. A study by Mejia & Arroyave[16], Mejia & Chew[69] supports the hypothesis that vitamin A status increase did not lead to increase of SF.[65]

Physical growth of preschool children

The change in physical growth or the recovery rate from growth retardation was defined as the proportion of children who were initially stunted, underweight, wasted, or low MUAC for age and whose HAZ-Score, WAZ-Score, WHZ-Score or MUAC was ≥ 2 SD after intervention. After intervention, every age group of children showed significant increases in anthropometric indices, however the changes were significantly different between age groups. The younger age group showed more increase in anthropometric indices than the older age group (Repeated-measure MANOVA test, control for *Ascaris* and *Trichuris* infestation), that the younger children got more benefit from food supplements than the older ones.

Table 8. Changes in anthropometric indices after intervention

	Fortified SBM (n=116)	Unfortified SBM (n=118)	Cassava cookies (n=113)
Weight (kg)	1.13±0.41[3†]	1.05±0.36[3✤]	0.94±0.34[3]
Height (cm)	3.8±0.9[3¶]	3.7±1.1[3¶]	3.4±1.1[3]
MUAC (cm)	0.3±0.6[3]	0.3±0.6[3]	0.2±0.7[2]
HAZ-Score	0.23±0.27[3¶]	0.22±0.32[3✤]	0.15±0.33[3]
WAZ-Score	0.29±0.25[3✤]	0.25±0.22[3]	0.23±0.24[3]
WHZ-score	0.25±0.35[3]	0.21±0.35[3]	0.20±0.33[3]
Triceps skin-fold (mm)	0.5 ±1.5[3]	0.4±1.4[3]	0.4±1.4[2]

[2]p<0.01, [3]p<0.001 significant difference within group changes (Pair t-test).
[✤]p<0.05, [¶]p<0.01, [†]p<0.001 significant different from CC group (Repeated-measure MANOVA test controlled for age & Ascaris, *Trichuris* infestation).

Table 8 shows that 5 months of intervention resulted in significant increases in weight, height, MUAC, TSF as well as HAZ-score and WAZ within each intervention group. The mean values of the FSBM group for weight ($p<0.001$), height ($p<0.01$), HAZ- Score ($p<0.05$) and WAZ-Score were higher than the values for the CC group. There was also a significant difference for the mean values of weight, height, and the Z-score of height for age between the UFSBM and the CC group. There was no significant difference in increase of TSF between the three groups.

Table 9. The impact of supplements to recovery from growth retardation

	Proportion of subjects who recovered from growth retardation (%)		
	HAZ ≥-2SD n(%)	WAZ≥-2SD n(%)	MUAC≥-2SD n(%)
Fortified SBM	23(33.8%)	17(32.6%)	8(28.6%)
Unfortified SBM	12(19.9%)	14(21.6%)	11(27.4%)
Cassava cookies	7(11.7%)	11(18.1%)	5(12.6%)

Table 9 shows the impact of the intervention: Among the stunted subjects, after intervention the percentage who became non- stunted was 33.8% in the FSBM group, whereas it was 19.9% in the UFSBM group and less, 11.7% in the CC group. The percentage of subjects who changed from being underweight to normal was 32.6% in the FSBM group whereas it was 21.6% in the UFSBM group and 18.1% in the CC group. We can see the impact of intervention on the reduced percentage of low MUAC as shown: 28.6% in the FSBM group, 27.4% in the UFSBM group and only 12.6% in the CC group.

The reason for the significantly higher increase of anthropometric data in the FSBM and UFSBM than in the cassava cookies group could be related to the effect of food supplementation and nutrients interaction. The vitamin A status improves, in theory, could have effect to improve of physical growth status. Also iron status improve lead to improve of physical growth status through the immune system. In the present study the children in the FSBM group increased in average 1.13 kg, 0.33 kg higher or 140% of the median weight gain rate (NCHS reference of WHO 1983).[70] In the UFSBM group, the weight gain was 1.05 kg, 0.25 kg higher or 131% of a median weight gain rate. Whereas the weight gains in the cassava cookies group was 0.94 kg or 118% of the median weight gain rate. Normally, a child of 39 months of age with a height similar to the median of NCHS reference would need to grow 3.2 cm in 5 months.[70] An average increase in height of the FSBM group in this study was 3.8 cm or 0.4 cm taller or 119% of the median growth rate. In the UFSBM group the increase of height was 0.3 cm taller or 116% of a median growth rate. Whereas the increase in height of children in the cassava cookies group was 3.4 cm, 0.2 cm taller than or 106% of a median growth rate. These results indicate a significant effect of supplementary feeding. The food supplementation effect was similar to that found in Columbia, India,[11] Jamaica and Indonesia.[14]

In the present study, the impact of food supplements was shown at any age interval of preschool children. However the younger children got more benefit from the food supplement. This in agreement with the finding of Walker et al, Gopalan et al.[11] Lutter et al[12] reported that the responsiveness to food supplement was greatest between ages 9 and 12 months (the period of peak diarrheal prevalence), followed by age 3-6 months (the period of weaning). The report by Schroeder et al[13] showed there was no impact of nutritional supplementation on growth between 3- 7 years of age.

This improvement may have been positively influenced by the deworming treatment before intervention study. A study by Stephenson et al[6], Kruger et al[17] showed the improvements in the Albendazole treatment group were associated with large decreases in the prevalence and intensity of helminth infestation. This positive effect can be expected with reduction in diarrhea, anorexia, increased food intake, better absorption, and decreased loss of nutrients caused by control of parasitic infestations.[71,72]

Thus supplementary feeding by using soybean milk fortified with a combination of vitamin A and iron or without fortification also improve the physical growth of preschool children but the effects are better in the fortified SBM than the unfortified SBM.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The study done on preschool children attending day-care centers in the peri-urban and rural area, one month after the national vitamin A supplementation in Vietnam showed that the nutritional status of the rural preschool children was worse. Thus the 5 months intervention using soybean milk (SBM) fortified with vitamin A and iron pyrophosphate was done on the rural area which showed the following :

1. One month after a single high dose of vitamin A capsule supplementation, the vitamin A status of rural Hatay preschool children was lower than the peri-urban Hanoi preschool children and 57.5% of the rural children had serum retinol below the cut-off point 1.05 μmol/L. The iron status was similar and the prevalence of anemia was about 20% in both studied areas. The physical growth of preschool children in both the peri-urban and rural area was poor. The nutritional status of the rural children was worse than that of the peri-urban children.
2. The soybean milk fortified with a combination of 300 μgRE and 5 mg of iron pyrophosphate (≈1.2 mg of elemental iron) was well accepted by the preschool children and the relative bioavailability of iron in the fortified soybean milk was low (45%).
3. Comparison of the daily supplementation of fortified soybean milk with unfortified soybean milk and cassava cookies for 5 months showed that the intervention improved the mean serum retinol concentration and kept it at the absolute level. The mean serum retinol of those given fortified soybean milk was the highest followed by the unfortified soybean milk and cassava cookies group (**Hypothesis 1**).
4. The fortified soybean milk increased the hemoglobin and serum ferritin significantly. An increase in hemoglobin was also observed in those given unfortified soybean milk and cassava cookies, although less than the group given fortified soybean milk which may be related to serum retinol and age increase. (**Hypothesis 2**)
5. Supplementation with fortified soybean milk, unfortified soybean milk and cassava cookies improved the physical growth status; fortified soybean milk was the best and cassava cookies least (**Hypothesis 3**).
6. In the peri-urban preschool children and the rural preschool children before intervention the serum retinol levels parallel the hemoglobin levels.

Recommendations

1. The bioavailability of iron pyrophosphate used in this study was low, further research is needed to find and iron compound suitable for fortification with higher bioavailability.
2. Soybean milk fortified with vitamin A and iron could be considered as fortified supplementary food to improve the nutritional status of Vietnamese preschool children.
3. Higher levels of iron for fortification of soybean milk than that used in this trial (3 mg of lemental iron/30 g soybean milk powder) in combination with vitamin C should be investigated for feeding programs to improve the iron status of preschool children better.
4. Long-term supplementation programs using vitamin A and iron fortified SBM should be combined with a nutritional education package program. The package should emphasize on the production and consumption of food rich in vitamin A, beta-carotene, and food with high bioavailability of iron.
5. Deworming should be a part of feeding and/or vitamin A and iron supplementation program to increase its effectiveness.

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