

## Original Article

# Impact of milk consumption on performance and health of primary school children in rural Vietnam

Do Thi Kim Lien PhD<sup>1,2</sup>, Bui Thi Nhung PhD<sup>1</sup>, Nguyen Cong Khan PhD<sup>1</sup>, Le Thi Hop PhD<sup>1</sup>, Nguyen Thi Quynh Nga MD<sup>2</sup>, Nguyen Tri Hung MD<sup>2</sup>, Jeroen Kiers PhD<sup>4</sup>, Yamamoto Shigeru PhD<sup>3</sup>, Rob te Biesebeke PhD<sup>5</sup>

<sup>1</sup>National Institute of Nutrition, Hanoi, Vietnam

<sup>2</sup>Dutch Lady Vietnam, Ho Chi Min City, Vietnam

<sup>3</sup>Ochanomizu University Graduate School of Humanities & Sciences, Tokyo, Japan

<sup>4</sup>Current address: NIZO Food Research, Ede, The Netherlands

<sup>5</sup>FrieslandCampina, Pieter Stuyvesantweg 1, Leeuwarden, The Netherlands

This is a follow-up study to an investigation on the prevalence of malnutrition and micronutrient deficiencies among Vietnamese primary schoolchildren. A total of 454 children aged 7 to 8 years attending three primary schools in the Northern delta province of Vietnam were either provided with regular milk, milk fortified with vitamins, minerals and inulin or served as a reference control group. Children were monitored for anthropometrics, (micro)-nutritional status, faecal microbiota composition, school performance, and health indices. Both weight-for-age (WAZ) and height-for-age (HAZ) significantly improved during 6 months of milk intervention; and underweight and stunting dropped by 10% in these groups. During intervention the incidence of anemia decreased and serum ferritin levels increased significantly in all groups. Serum zinc levels increased and consequently the incidence of zinc deficiency improved significantly in all three groups. Serum retinol levels and urine iodine levels remained stable upon intervention with fortified milk whereas in the control group the incidence of iodine deficiency increased. Bifidobacteria composed less than 1% of the total faecal bacteria. After three months of milk intervention total bacteria, bifidobacteria and *Bacteroides* sp. increased significantly in both milk and inulin fortified milk groups. Children in the milk consuming groups had significantly better short-term memory scores. Parent reported that health related quality of life status significantly improved upon milk intervention. In conclusion, (fortified) milk consumption benefited the children in rural Vietnam including lowering the occurrence of underweight and stunting, improving micronutrients status and better learning indicators as well as improving the quality of life.

**Key Words:** malnutrition, micronutrients, Vietnam, children, health

## INTRODUCTION

A considerable part (30-50%) of children in the developing world suffers from underweight or stunting. In Vietnam, protein-energy malnutrition and micronutrient deficiencies among children are still a major health concern.<sup>1</sup> Malnutrition leads to numerous problems such as impaired growth and cognitive development.<sup>2,3</sup> School age is the period of life when children grow rapidly, both mentally and physically, whereas they receive generally less care than they used to have at home. As far as children in rural areas are concerned, they also help their parents with housework and farming. Although the prevalence of malnutrition, including stunting, has significantly declined in the past decade,<sup>4</sup> recent investigations in the rural area of Vietnam still showed high prevalence of underweight and stunting.<sup>5</sup> It was concluded that the prevention of malnutrition and micronutrient deficiencies should be given serious attention. Earlier nutritional intervention initiatives have been shown to be effective. A nutrition program based on home garden food production and nutrition education for mothers of preschool children was associated

with significant reductions in morbidity from acute respiratory infection and diarrhoeal disease in preschool children in Viet Nam's Vinh Phu province.<sup>6</sup>

Several micronutrients are required for adequate growth among children. The lack of key vitamins and minerals brings anaemia, cretinism and blindness to tens of millions of people, but the news of the last decade is that these manifestations are but the tip of a very large iceberg.<sup>7</sup> Levels of mineral and vitamin deficiency that have no clinical symptoms can and do impair intellectual development, cause illness and early death on a very large scale, and condemn perhaps a third of the world to live life below their physical and mental potential. Perhaps

**Corresponding Author:** Dr Rob te Biesebeke, FrieslandCampina, Pieter Stuyvesantweg 1, Leeuwarden, The Netherlands

Tel: 0031612504012; Fax: 0031582992540

Email: tebiesebeke@yahoo.com

Manuscript received 17 January 2009. Initial review completed 11 June 2009. Revision accepted 2 July 2009.

40% of the developing world's people suffer from iron deficiency, probably 15% lack adequate iodine, and as many as 40% of children are growing up with insufficient vitamin A.<sup>7</sup>

It has been unclear as to which nutrient deficiencies contribute most often to growth faltering in populations at risk for poor nutrition and poor growth. If children are deficient in multiple micronutrients, it can be difficult to interpret the effect of single micronutrient supplementation trials. Rivera et al. reviewed evidence from community based, randomized, placebo-controlled, micronutrient supplementation trials to determine which micronutrient deficiencies have been found to be causal in growth faltering and identified especially zinc but also vitamin A and iron deficiency as causative factors.<sup>2</sup> Interestingly, these authors also reviewed trials on animal food sources and showed that skimmed milk powder alone also resulted in a positive growth response. A combined approach consisting of multiple micronutrient fortification of foods would therefore seem very promising. A number of trials have been published and demonstrated the potential of the concept of fortifying beverages, biscuits, and milk.<sup>8-10</sup> Food-based interventions may have several advantages above supplement use, including the provision of additional nutrients in a familiar form that can be integrated in the usual diet and the provision of additional sources of energy and high-quality protein. We decided to study the effects of regular milk with or without fortification with minerals, vitamins and fibre (inulin) on anthropometrics, (micro) nutritional status, faecal microbiota composition, school performance and health indices among Vietnamese primary school children.

## MATERIAL AND METHODS

### *Subjects and Design*

The study was carried out over six months between November 2004 and April 2005 and included 454 children aged 7-8 years, 217 boys and 237 girls from three primary schools in the Yen Phong District, Bac Ninh Province, Northern Vietnam. The research project has been approved by the Ethics Committee of the National Institute of Nutrition in Hanoi, Vietnam.

Children in two schools were randomly assigned to either the group consuming regular milk or the group consuming fortified milk. Before participation in the study, one of the parents (or the caregivers) of each child gave informed consent and anonymity was preserved. The intervention was double-blind. The children consumed two servings of 250 ml milk in the morning of each class day (6 days a week) for six months, except for holidays. Milk provision as well as consumption was carried out under supervision of teachers and health care workers. A third group of children served as a control and, for ethical reasons, attended the third school. The third group did not receive any compensation for energy and protein.

### *Food intake and Milk products*

In a subset of children dietary intake levels were assessed (not shown). Commercial milk obtained from Dutch Lady Milk Industries was used as regular milk. Fortified milk contained (partly) vegetable fat, inulin (Frutafit® HD, Sensus, The Netherlands) (1%), milk calcium (Vivinal

MCA 9, FrieslandCampina DOMO, The Netherlands), iron pyrophosphate, sodium selenite, zinc sulphate, manganese sulphate, potassium iodide, vitamins A, D<sub>3</sub>, E, K1, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>6</sub>, B<sub>12</sub>, folic acid, calcium D-pantothenate, biotin, sodium ascorbate, and taurine. Milks were UHT treated and packed in single portion carton bricks without prints. Milk products were stored at 4°C until 3-4 weeks before consumption in order to minimize taste deviations between regular and fortified milk. The composition of the regular and fortified milk is shown in Table 1. Two servings of milk provided approximately 20% of daily energy and 40% of daily protein requirements according to the Vietnamese RDA for children aged 7-9 years.<sup>5</sup>

### *Anthropometrics*

At the start of the intervention (base-line survey), and after 3 and 6 months of intervention, length and weight of all children was determined.

### *Biochemical analysis*

At the start of the intervention, and after 3 and 6 months of intervention, blood and urine samples were taken from all children. Blood samples were analyzed for haemoglobin, serum ferritin, retinol and zinc concentrations. Fasting venous blood and urine samples were collected, stored at 4°C and transported to the National Institute of Nutrition within 4 hours. Haemoglobin (Hb) was measured by the cyanmethaemoglobin method within 12 hours (Boehringer kit, Germany). Serum was prepared and stored at -70°C until analyses. Ferritin was measured by ELISA. Zinc was determined by flame atomic absorption spectrometry (Perkin-Elmer Model 5000, Norwalk, USA) and serum retinol was measured with High performance Liquid Chromatography (Shimadzu LC 10ADvp, Japan). Urinary iodine was analyzed based on alkaline digestion using the Sandell-Kolthoff reaction.<sup>11</sup>

Deficiencies and anaemia were defined following WHO guidelines. Anaemia was defined as Hb below 115 mg/L, iron deficiency was defined as serum ferritin levels below 15 µg/L, zinc and vitamin A deficiency were de-

**Table 1.** Analyzed composition of 100 g milk products

		Regular milk	Fortified milk
Energy	Kcal	77	75
Protein	g	3.0	3.2
Fat	g	3.0	3.0
Carbohydrates	g	14.9	15.1
Calcium	mg	111	156
Phosphate	mg	260	350
Sodium	mg	41	46
Potassium	mg	153	165
Magnesium	mg	10	13
Iron	mg	0.04	1.3
Zinc	mg	0.4	1.1
Iodine	mg	0.015	0.037
Manganese	mg	<0.01	0.26
Copper	mg	<0.1	<0.1
A	IU	1.5	4.4
D <sub>3</sub>	IU	0.12	0.70
E	mg	0.16	2.6
B <sub>1</sub>	mg	0.03	0.12
B <sub>2</sub>	mg	0.16	0.23
C	mg	0.7	33

defined when serum levels were below 10.8  $\mu\text{mol/L}$ , and 0.70  $\mu\text{mol/L}$  respectively. Iodine deficiency was defined as below 99  $\mu\text{g/L}$  urine.

### Faecal bacteria analysis

In one school ( $n=150$ ) faecal samples were collected at base-line and after 3 months of milk intervention. Faecal samples were frozen at  $-20^{\circ}\text{C}$  within 2 hours after collection. Samples were shipped frozen to The Netherlands and in a subset determination of total bacteria, bifidobacteria, clostridia and *Bacteroides* sp. by using quantitative real-time PCR as described previously.<sup>12</sup>

### Health and Mental performance surveys

A validated quality of life questionnaire was used specifically for children of 8-15 years old.<sup>13</sup> The questionnaire was translated to Vietnamese, and health related quality of life problems were monitored at baseline and after 6 months of intervention by self reporting of the children, with the help of teachers and health care workers. Children were included in an assessment of their mental working capacity performed by associate Professors Dinh Ngoc Bao and Vo Thi Minh Tri of the Hanoi National University of Education, Vietnam. The assessment provided exercises to determine the speed, accuracy, and efficiency of working by making use of Bourdon alphabets, as well as exercises in memorizing words and numbers in order to judge the short-term memory.

### Statistics

Data presented as mean  $\pm$  SD. Z-scores of weight-for-age (WAZ), height-for-age (HAZ), and weight-for-height (WHZ) were calculated using Epi Info version 6.04. Independent t-tests were used to test differences of normal distribution of variables between groups. For the analysis of baseline parameters and the outcome measures we used ANOVA.

$P$ -value of  $<0.05$  was considered statistically significant. All data were analyzed using SPSS software (SPSS version 11.0, Chicago, USA).

## RESULTS

### Food intake

Average daily nutrient intake is shown in Table 2. All intake levels were similar between the three groups at base-line. In the control group all intakes remained stable over the six months period. By consuming two cartons of milk the daily energy intake increased with more than 20%, and daily protein intake increased with 14 grams (almost 40%). Milk fortification led to very significant increased intakes of iron (4.9 mg/day) and vitamin A (0.41 mg/day).

### Anthropometrics

Weight and height did not differ between the three groups at base-line, and increased significantly upon the six months period in all groups (Table 3). Although weight and height gain in children consuming either regular or fortified milk increased more than in the control group children (on average 0.5 kg and 0.4 cm), these differences were not statistically significant over the six months intervention period.

Both weight-for-age (WAZ) and height-for-age (HAZ) significantly improved during six months of milk intervention and as a consequence the incidence of underweight and stunting dropped by roughly 10%.

### Anaemia

The incidence of anaemia occurred in 45% of the children in schools where the milk intervention took place and 29% of the children in the control school (Table 4). It was shown that more than half of the children appeared to be infected by round and whip worm, whereas the

**Table 2.** Average daily intake ( $\pm$ SD) of energy, macronutrients, iron and vitamin A at base-line (T0) and after 6 months of intervention (T6) as assessed via interviews in a subset of the subjects.

	Time	Fortified milk ( $n=70$ )	Regular milk ( $n=75$ )	Control ( $n=71$ )	$p$ †
Energy (kcal)	T0	1240 $\pm$ 371	1245 $\pm$ 259	1279 $\pm$ 277	0.70
	T6	1513 $\pm$ 277*	1537 $\pm$ 241*	1293 $\pm$ 224	<0.01
	Change	273	292	14	<0.05
Protein (g)	T0	37.2 $\pm$ 10.7	37.5 $\pm$ 9.8	38.7 $\pm$ 11.2	0.73
	T6	51.3 $\pm$ 10.7**	51.4 $\pm$ 9.8*	39.2 $\pm$ 10.4	<0.01
	Change	14.1	13.9	0.5	<0.01
Fat (g)	T0	17.6 $\pm$ 8.9	16.2 $\pm$ 9.1	17.1 $\pm$ 9.7	0.65
	T6	28.9 $\pm$ 11.1**	29.1 $\pm$ 10.9**	15.4 $\pm$ 11.0	<0.01
	Change	11.3	12.9	-1.7	<0.05
Sugar (g)	T0	225 $\pm$ 69.7	229 $\pm$ 48.3	234 $\pm$ 48.1	0.61
	T6	261 $\pm$ 45.7**	269 $\pm$ 37.4**	240 $\pm$ 33.2	<0.01
	Change	36.4	40.2	6.6	<0.01
Iron (mg)	T0	6.9 $\pm$ 2.4	6.5 $\pm$ 1.9	6.4 $\pm$ 1.8	0.33
	T6	11.8 $\pm$ 2.0**	6.9 $\pm$ 2.0	6.7 $\pm$ 2.3	<0.01
	Change	4.9	0.4	0.3	<0.05
Vitamin A (mg)	T0	0.07 $\pm$ 0.14	0.07 $\pm$ 0.19	0.08 $\pm$ 0.28	0.94
	T6	0.48 $\pm$ 0.32**	0.28 $\pm$ 0.38*	0.09 $\pm$ 0.12	<0.05
	Change	0.41	0.21	0.01	<0.01

\*  $p<0.05$ , \*\*  $p<0.01$ : between T0 and T6 within one group

†  $p$ -value for comparison of the three groups with ANOVA

**Table 3.** Anthropometrics (mean  $\pm$ SD), z-scores (WAZ, HAZ, WHZ) and nutrition status (%) of children before (T0) and after intervention (T6).

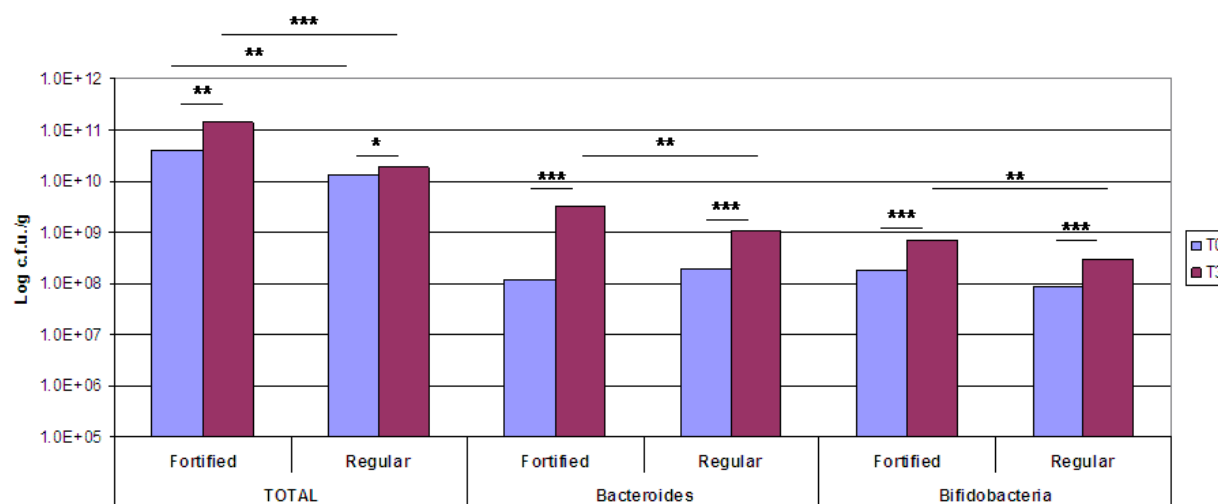
	Time	Fortified milk (n=150)	Regular milk (n=151)	Control (n=143)	<i>p</i> †
Weight (kg)	T0	18.9 $\pm$ 2.2	18.8 $\pm$ 2.1	19.0 $\pm$ 2.4	0.45
	T6	20.5 $\pm$ 2.4*	20.3 $\pm$ 2.4**	20.1 $\pm$ 2.6**	0.55
	change	1.6 $\pm$ 2.2	1.5 $\pm$ 2.3	1.1 $\pm$ 2.4	0.15
Height (cm)	T0	117.3 $\pm$ 5.2	117.4 $\pm$ 4.9	116.8 $\pm$ 5.8	0.25
	T6	120.9 $\pm$ 5.2**	121.0 $\pm$ 5.0*	120.0 $\pm$ 5.9**	0.28
	change	3.6 $\pm$ 5.1	3.6 $\pm$ 4.8	3.2 $\pm$ 5.6	0.23
WAZ	T0	-1.85 $\pm$ 0.61	-1.95 $\pm$ 0.64	-1.71 $\pm$ 0.65	<0.05
	T6	-1.68 $\pm$ 0.64*	-1.76 $\pm$ 0.61**	-1.50 $\pm$ 1.49	0.06
	change	-0.17 $\pm$ 0.75	-0.19 $\pm$ 0.76	-0.21 $\pm$ 1.59	0.93
Underweight (%)	T0	42.4	49.6	36.0	<0.05
	T6	32.4*	36.6*	35.4	0.45
	change	10	13	0.6	<0.01
HAZ	T0	-1.64 $\pm$ 0.78	-1.70 $\pm$ 0.83	-1.52 $\pm$ 0.87	0.19
	T6	-1.49 $\pm$ 0.81*	-1.52 $\pm$ 0.79**	-1.27 $\pm$ 1.56	0.11
	change	-0.16 $\pm$ 0.87	-0.18 $\pm$ 0.92	-0.25 $\pm$ 1.67	0.79
Stunting (%)	T0	32.4	40.5	33.3	0.31
	T6	23.0**	30.7*	26	0.30
	change	9.4	9.8	7.3	0.64
WHZ	T0	-1.17 $\pm$ 0.65	-1.29 $\pm$ 0.69	-1.07 $\pm$ 0.72	<0.01
	T6	-1.05 $\pm$ 0.75	-1.14 $\pm$ 0.68	-0.92 $\pm$ 1.47	0.16
	change	-0.12 $\pm$ 0.97	-0.15 $\pm$ 0.95	-0.15 $\pm$ 1.66	0.98
Wasting (%)	T0	9.9	13.8	11.3	0.55
	T6	8.6	9.2	9.4	0.98
	change	1.3	4.6	1.9	0.15

\*  $p < 0.05$ , \*\*  $p < 0.01$ : between T0 and T6 within one group†  $p$  level for comparison of the three groups with ANOVA**Table 4.** Serum and urine biochemicals (mean $\pm$ SD) at the start (T0) and after six months (T6) and deduced deficiency levels.

	Time	Fortified milk (n=150)	Regular milk (n=151)	Control (n=143)	<i>p</i> †
Hemoglobin (g/L)	T0	116.9 $\pm$ 21.1	114.8 $\pm$ 10.3	118.3 $\pm$ 6.7	1.03
	T6	126.1 $\pm$ 7.2*	123.7 $\pm$ 10.5*	122.5 $\pm$ 7.4*	<0.01
	change	9.1 $\pm$ 21.8	8.9 $\pm$ 11.0	4.2 $\pm$ 6.9	<0.05
Anemia (%)	T0	46.7	43.7	29.4	<0.01
	T6	9.3*	19.2**	17.5**	<0.05
	change	-37.4	-24.5	-11.9	<0.05
Ferritin ( $\mu$ g/L)	T0	82.3 $\pm$ 50.3	76.1 $\pm$ 57.2	77.9 $\pm$ 34.6	0.52
	T6	108.3 $\pm$ 69.4**	104.2 $\pm$ 64.1**	84.2 $\pm$ 45.7	<0.05
	change	25.9 $\pm$ 60.4	28.1 $\pm$ 70.1	6.3 $\pm$ 38.3	<0.01
Vitamin A ( $\mu$ mol/L)	T0	0.97 $\pm$ 0.24	1.07 $\pm$ 0.63	0.96 $\pm$ 0.33	<0.01
	T6	0.99 $\pm$ 0.21	1.01 $\pm$ 0.49	0.92 $\pm$ 0.33	0.54
	change	0.02 $\pm$ 0.24	-0.06 $\pm$ 0.28	-0.04 $\pm$ 0.21	<0.01
Vitamin A deficiency (%)	T0	12.7	9.9	16.8	0.21
	T6	5.3*	8.6**	6.9*	0.53
	change	7.4	1.3	9.9	<0.05
Zinc ( $\mu$ mol/L)	T0	11.7 $\pm$ 2.1	11.3 $\pm$ 1.9	10.5 $\pm$ 1.5	<0.05
	T6	12.6 $\pm$ 2.6*	12.2 $\pm$ 2.0*	11.0 $\pm$ 1.9**	<0.01
	change	0.9 $\pm$ 3.2	0.9 $\pm$ 2.3	0.5 $\pm$ 2.0	0.25
Zinc deficiency (%)	T0	35.3	41.7	58.7	<0.01
	T6	19.0*	21.0*	47**	<0.01
	change	16.3	20.7	11.7	<0.05
Iodine in urine ( $\mu$ g/dL)	T0	26.5 (2.0; 152) ‡	27.0 (2.7; 352) ‡	23.5 (3.4; 93.5) ‡	<0.05
	T6	28.3 (3.0; 95.9) ‡	23.1 (2.0; 114) ‡	19.5 (3.8; 164) ‡	<0.01
	change	1.8	-3.9	-4.0	0.12
Iodine deficiency (%)	T0	6.7	13.7	10.6	<0.05
	T6	5.4	12.3	14.7	<0.05
	change	1.3	1.4	-4.1	0.14

\*  $p < 0.05$ , \*\*  $p < 0.01$ : between T0 and T6 within one group†  $p$  level for comparison of the three groups with ANOVA

‡ median (lowest; highest)



**Figure 1.** Levels of fecal bacterial groups before and after 3 months of intervention with (inulin) fortified milk (n=32) and regular milk (n=39)

percentage of children infected with at least one of the three types of worms was 68-77% and this did not differ between the three groups (results not shown). During intervention, the incidences of anaemia decreased significantly in all groups. This was strongest for the fortified milk group, from 47% to 9%.

#### Other micronutrient deficiencies

Serum vitamin A levels remained stable upon intervention with fortified milk, whereas these levels decreased slightly in the regular milk and the control group (Table 4). Notwithstanding these decreases, more children had serum vitamin A levels above 0.70  $\mu\text{mol/L}$  and vitamin A deficiency improved significantly in all groups.

The serum ferritin levels increased significantly in both groups consuming milk. Serum zinc levels increased and consequently the incidence of zinc deficiency improved significantly in all three groups, although changes were more profound in the milk treated groups.

Urine iodine levels increased only upon intervention with fortified milk, whereas a decrease was seen in the other two groups. The incidence of iodine deficiency slightly decreased (not statistically significant) in the milk treated groups, whereas in the control group the incidence of iodine deficiency increased.

#### Faecal microbiota

Base-line faecal bacteria levels for total bacteria differed significantly in the subsets of the two intervention groups studied (Figure 1). Only 13 out of 71 children included in the faecal microbiota analysis contained demonstrable levels of clostridia ( $> 10^6$  CFU/g) in their faeces, and therefore these findings are not being discussed further. At baseline, *Bacteroides* species only account for less than 1.5% of the total bacteria, whereas bifidobacteria amount to only 0.7 % of the total number of bacteria.

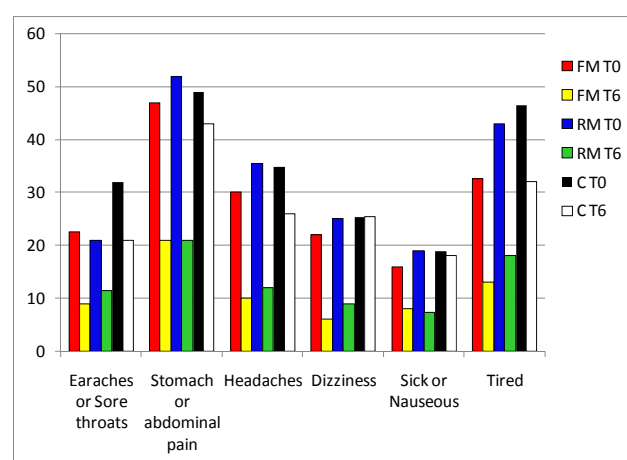
Total bacteria counts increase significantly ( $p<0.01$ ) after 3 months intervention in both the regular and fortified milk group. Also, *Bacteroides* species and bifidobacteria increase significantly ( $p<0.001$ ) after intervention in

both groups. After 3 months of intervention, the faeces of the children in the fortified milk group contains significantly higher numbers of total bacteria, bifidobacteria and *Bacteroides* species compared to that of the children consuming regular milk.

#### Health and mental performance indicators

The self-reported health related quality of life showed a considerable decrease in the parameters: stomach-aches or abdominal pain, headaches, dizziness, and sick or nauseous in the children consuming either fortified or regular milk (Figure 2). Earaches or sore throats and tiredness also decreased during the six months period, but this also was observed in the control group to a significant extent.

The performance of children consuming milk for six months was significantly higher than that of children in the control school. The milk drinking children achieved significant higher work volume (speed) and higher work



**Figure 2.** Health related quality of life parameters as reported by the children in the three study groups (FM: fortified milk; RM: regular milk; C: control) before (T0) and after six months of intervention (T6). Percentage of children that expressed either occasional or often occurrence of the complaints (earaches or sore throat, stomach or abdominal pain, headaches, dizziness, sick or nauseous, tired) is shown.

efficiency in the later minutes of the exercise (results not shown). Children drinking milk had significantly higher recall of memorized words and numbers compared to children in the control group (results not shown). Children consuming fortified milk showed superior performance compared to the regular milk drinking group.

## DISCUSSION

### *Nutritional status*

Recent observations showed that incidences of malnutrition and micronutrient deficiencies were high among rural school children in Vietnam.<sup>1</sup> Our study that provided intervention with regular and fortified milk showed increased weight and height gain, and both the incidence of underweight and stunting decreased by roughly 10% upon milk consumption. Also, recently a school nutrition program demonstrated significant impacts on height and weight gains by supplementing the diet of Vietnamese primary school children with fortified milk and biscuits.<sup>9</sup> Typically, growth faltering begins at about six months of age, as children transition to foods that are often inadequate in quantity and quality, and increased exposure to the environment increases their likelihood of illness.<sup>14</sup> The food consumption surveys of the Vietnamese population in 1985 showed that inadequate energy intake occurred in 15% on average, and protein intake was low.<sup>15</sup>

The dietary macronutrient pattern of primary school girls in rural South Vietnam showed a deficiency of energy, fat, animal protein and fibre content.<sup>16</sup> Dietary micronutrient pattern of rural primary schoolgirls showed deficiency of iron, calcium, phosphorus, potassium, magnesium, beta-carotene, vitamin A and vitamin C.<sup>17</sup> Supplementing the diets of primary schoolchildren in rural areas with dairy products would therefore have potential beneficial effects, because of the supply of high quality proteins as well as fats and a number of essential micronutrients that are lacking in the regular diet.

### *Micronutrients*

Iron and vitamin A deficiencies have been demonstrated to cause growth faltering, but only when the deficiency state is severe.<sup>2</sup> Iron supplementation resulted in a beneficial effect on linear growth only in anaemic children, whereas vitamin A trials have reported little or no benefits.<sup>18</sup>

Although anaemia (low Hb levels) decreased in the control group as well as in the treated groups, this was not the case for low ferritin levels. Regular milk hardly contains any iron, and the improvement of anaemia in the group receiving regular milk might reflect another phenomenon, as an improvement is seen in the control group as well over the same period of time. Fortified milk showed greater improvements in anaemia prevalence, possibly related to the increased iron intake.

Vitamin A deficiency was much less common than iron deficiency and improved over the six months period in all groups, demonstrating that although fortification of milk with vitamin A slightly improved serum retinol levels it was not directly possible to correlate this to improvements in vitamin A status. A beverage fortified with 10 micronutrients at physiologic doses improved haematologic and anthropometric measurements and signifi-

cantly lowered the overall prevalence of anaemia and vitamin A deficiency in rural primary school children in Tanzania.<sup>8</sup> Fortified biscuits (beta-carotene, iron and iodine) improved serum retinol, ferritin, haemoglobin, transferrin saturation and urinary iodine during the first 12 months of the biscuits intervention.<sup>10</sup>

Serum zinc concentrations are generally maintained within the normal range with small or moderate reductions in zinc intake. Brown et al. assessed the effect of zinc supplementation on the physical growth and serum zinc concentrations of pre-pubertal children.<sup>19</sup> Zinc supplementation produced highly significant, positive responses in height and weight increments, and caused a large increase in the children's serum zinc concentrations. Population mean serum zinc concentration appeared to be a useful indicator of the successful delivery and absorption of zinc supplements in children.<sup>19</sup> In our study milk consumption appeared to increase mean serum zinc concentrations beyond the increase observed in the control group. Regular milk, containing moderate zinc levels, showed equal improvements as the fortified milk, indicating that a relatively moderate increase in zinc intake would already suffice in optimizing serum zinc concentrations and consequently improving zinc status and related health benefits. Meta-analysis of zinc supplementation trials confirmed that zinc has a significant but small impact on length increases in children 0-13 years of age, and there appears to be strong evidence for the contribution of zinc deficiency to growth faltering among children.<sup>2,18</sup> So, the observed improvements in height gain could be the combined result of increased intake in energy, protein, iron and zinc as well.

Although the importance of several indicators of iodine status are debatable it was shown that urinary iodine concentrations is an indicator most influenced by a changing iodine supply and can be indicative for iodine deficiency.<sup>20,21</sup> In our study, only iodine fortification of milk showed an increase in urinary iodine levels, but improvement of the prevalence of iodine deficiency was not different between the regular and the fortified milk group. Significantly lower baseline iodine deficiency in the fortified milk group, might have camouflaged part of the treatment effect or possibly milk provision alone could positively influence the iodine uptake from dietary sources and/or iodine handling in the body.

### *Faecal microbiota*

Numbers of total bacteria in the two subsets at baseline differed significantly. This makes actual comparison between the groups rather difficult. It is remarkable that the *Bacteroides* group is only making up less than 1.5 percent of the total population, suggesting that a large number of the faecal microbiota of Vietnamese children remains unidentified. Also total bifidobacteria numbers (0.7%) are very low compared to levels reported for Western breast-fed infants (63-91%), children 2-3 years (4-27%) and adults aged 20-52 (1.2-7.7%).<sup>22</sup> Earlier studies using the qPCR technology for faecal bacteria quantification in Western infants have been able to detect these high *Bacteroides* sp. and bifidobacteria levels.<sup>12</sup>

It is generally believed that low levels of bifidobacteria are indicative for a suboptimal microbiota resulting in

possible decreased immunity. Although generally prebiotics such as inulin are capable of increases in especially low levels of specific intestinal bacteria such as bifidobacteria,<sup>23,24</sup> the inulin fortified milk demonstrated a low increase in faecal bifidobacteria counts. Surplus protein and/or lactose not completely digested in the upper part of the gastro-intestinal tract could have entered the large intestine, served as substrate for bacterial growth and hence caused the overall increase in bacterial numbers.

#### **Physical and mental performance indicators**

Malnutrition and susceptibility to infections are closely related and could result in a vicious cycle. Among the children in this study malnutrition was abundant and also the reported health related problems were high. Incidence of parents diagnosed earaches and sore throats decreased during the study in all groups and could have been related to changing of the seasons since the study started in winter and ended in spring, which also might explain the decrease in tiredness reported in all three groups. Milk consumption clearly demonstrated improvements in physical constraints such as stomach-aches and abdominal pain, headaches, dizziness and feelings of nausea and sickness. Enhanced supply of macro- and micronutrients clearly showed its benefits in countering the vicious cycle of malnutrition and illness.

Results of test scores in both mathematics and Vietnamese language were significantly negatively correlated with z-scores of height-for-age and weight-for age, but not with weight-for-height.<sup>25</sup> In this study it was also shown that (fortified) milk consumption resulted in improvements in mental performance, possibly reflecting improved nutrient status. Children consuming fortified milk showed a superior performance regarding short-term memory. For Indonesian schoolchildren, it was shown that urinary iodine excretion correlated significantly with cognitive performance (IQ).<sup>20</sup> Hence, the availability of high levels of iodine and/or iron might have contributed to this observed difference, although a four month iodine intervention in school-aged children in Bangladesh increased urinary iodine levels but failed to demonstrate a significant treatment effect of iodine supplementation on cognitive function.<sup>26</sup> Also iron-, iodine-, and beta-carotene-fortified biscuits failed to improve cognitive function after 12 months of intervention in South African school children.<sup>10</sup>

#### **Future perspectives**

Fortification of dairy products requires understanding on interactions between the fortified nutrients, these nutrients and the product matrix, as well as the processing of the fortified product. Recent reviews have been compiled addressing important factors to take into account.<sup>27-29</sup> It has been shown for example that chocolate milk is a poor vehicle for iron fortification unless sufficient amounts of an iron-absorption enhancer are added,<sup>30</sup> and food processing can influence the relative absorption of chocolate milk iron fortification.<sup>31</sup>

#### **Conclusion**

Milk consumption reduced underweight and stunting by 10% because of the increased energy and protein supply.

In addition, multiple micronutrient supplementation of milk has good potential in increasing serum levels and decreasing deficiencies of micronutrients known to impair growth, cognition, and immune responses. Preliminary faecal microbiota quantification revealed remarkable deviations compared to the Western population, and outlined potential for improvement of the intestinal microbiota of Vietnamese children. As a consequence, fortified milk consumption clearly demonstrated improvements in physical constraints and mental functioning. Fortified dairy products have therefore a very strong potential for further reduction of the prevalence of malnutrition and consequently improving the quality of life of school age children in rural areas of Vietnam.

#### **ACKNOWLEDGEMENT**

Deputy Professors Dinh Ngoc Bao and Vo Thi Minh Tri of the Hanoi National University of Education are greatly acknowledged for their excellent work in mental performance testing. Dr Diederick Meyer of Sensus is greatly acknowledged for the fruitful discussions on childhood microflora composition.

#### **AUTHOR DISCLOSURES**

Dutch Lady Vietnam and FrieslandCampina Innovation International contributed financially to realize the study. The financial contribution of Sensus to realize the fecal microbiota composition analysis is greatly appreciated. Do Thi Kim Lien, Nguyen Thi Quynh Nga, and Nguyen Tri Hung are employed for Dutch Lady Vietnam and Rob te Biesebeke is employed by Royal Friesland Campina.

#### **REFERENCES**

1. Van Nhlen N, Khan NC, Ninh NX, Van Huan P, Hop le T, Lam NT Ota F, et al. Micronutrient deficiencies and anemia among preschool children in rural Vietnam. *Asia Pac J Clin Nutr.* 2008;17:48-55.
2. Rivera JA, Hotz C, González-Cossío T, Neufeld L, García-Guerra A. The effect of micronutrient deficiencies on child growth: a review of results from community-based supplementation trials. *J Nutr.* 2003;133:4010-20.
3. Black MM. Micronutrient deficiencies and cognitive functioning. *J Nutr.* 2003;133:3927-31.
4. Khan NC, Tuyen LD, Ngoc TX, Duong PH, Khoi HH. Reduction in childhood malnutrition in Vietnam from 1990-2004. *Asia Pac J Clin Nutr.* 2007;16:274-8.
5. Lien DTK, Giay T, Khoi HH. Development of Vietnamese recommended dietary allowances and their use for the National Plan of Action for Nutrition. *Nutr Rev.* 1998;56:25-8.
6. English RM, Badcock JC, Giay T, Ngu T, Waters AM, Bennett SA. Effect of nutrition improvement project on morbidity from infectious diseases in preschool children in Vietnam: comparison with control commune. *BMJ.* 1997;315:1122-5.
7. Adamson, P. Vitamin and mineral deficiency – a global progress report. The Micronutrient Initiative and UNICEF. 2004; <http://www.micronutrient.org/reports/>
8. Ash DM, Tatala SR, Frongillo EA Jr, Ndossi GD, Latham MC. Randomized efficacy trial of a micronutrient-fortified beverage in primary school children in Tanzania. *Am J Clin Nutr.* 2003;77:891-8.
9. Hall A, Hanh TT, Farley K, Quynh TP, Valdivia F. An evaluation of the impact of a school nutrition programme in Vietnam. *Public Health Nutr.* 2007;10:819-26.
10. Van Stuijvenberg ME, Kvalsvig JD, Faber M, Kruger M, Kenoyer DG, Benadé AJ. Effect of iron-, iodine-, and beta-carotene-fortified biscuits on the micronutrient status of

- primary school children: a randomized controlled trial. *Am J Clin Nutr.* 1999;69:497-503.
11. Dunn JT, Crutchfield HE, Gutekunst R, Dunn AD. Two simple methods for measuring iodine in urine. *Thyroid.* 1993;3:119-23.
  12. Fenicia L, Anniballi F, De Medici D, Delibato E, Aureli P. SYBR green real-time PCR method to detect *Clostridium botulinum* type A. *Appl Environ Microbiol* 2007 73:2891-6.
  13. Grootenhuys MA, Kopman HM, Verrips EG, Vogels AG, Last BF. Health-related quality of life problems of children aged 8-11 years with a chronic disease. *Dev Neurorehabil.* 2007;10:27-33.
  14. Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE. Stunting, wasting, and micronutrient deficiency disorders. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, Jha P, Mills A, Musgrove P, editors. *Disease control priorities in developing countries*. New York: Oxford University Press; 2006. p. 551-68.
  15. Hop LT. Programs to improve production and consumption of animal source foods and malnutrition in Vietnam. *J Nutr.* 2003;133:4006-9.
  16. Tuyet Mai T, Kim Hung N, Kawakami M, Nguyen VC. Macronutrient intake and nutritional status of primary school-aged girls in rural and urban areas of South Vietnam. *J Nutr Sci Vitaminol.* 2003;49:13-20.
  17. Ta TM, Nguyen KH, Kawakami M, Kawase M, Nguyen C. Micronutrient status of primary school girls in rural and urban areas of South Vietnam. *Asia Pac J Clin Nutr.* 2003; 12:178-85.
  18. Bhandari N, Bahl R, Taneja S. Effect of micronutrient supplementation on linear growth of children. *Br J Nutr.* 2001; 85:2:131-7.
  19. Brown KH, Pearson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2002;75:1062-71.
  20. Pardede LV, Hardjowasito W, Gross R, Dillon DH, Totoprajogo OS, Yosoprawoto M, Waskito L, Untoro J. Urinary iodine excretion is the most appropriate outcome indicator for iodine deficiency at field conditions at district level. *J Nutr.* 1998;128:1122-6.
  21. Van den Briel T, West CE, Hautvast JG, Vulsma T, de Vijlder JJ, Ategbo EA. Serum thyroglobulin and urinary iodine concentration are the most appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply in schoolchildren in Benin. *J Nutr.* 2001;131: 2701-6.
  22. Welling GW, Wildeboer-Veloo L, Raangs GC, Franks AH, Jansen GJ, Tonk RHJ, Degener JE, Harmsen HJM. Variations of bacterial populations in human faeces measured by FISH with group-specific 16S rRNA-targeted oligonucleotide probes. *Bioscience Microflora.* 2000;19:79-84.
  23. Kim S-H, Lee DH, Meyer D. Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. *Asia Pac J Clin Nutr.* 2007;16:172-7.
  24. Yap WKW, Suhaila M, Jamal MH, Meyer D, Manap YA. Changes in infants faecal characteristics and microbiota by inulin supplementation. *J Clin Biochem Nutr.* 2008;43:159-66.
  25. Hall A, Khanh LNB, Son TH, Dung NQ, Lansdown RG, Dat DT, Hanh NT, Moestue H, Khoi HH, Bundy DAP. An association between chronic undernutrition and educational test scores in Vietnamese children. *Eur J Clin Nutr.* 2001; 55:801-4.
  26. Huda SN, Grantham-McGregor SM, Tomkins A. Cognitive and motor functions of iodine-deficient but euthyroid children in Bangladesh do not benefit from iodized poppy seed oil (Lipiodol). *J Nutr.* 2001;131:72-7.
  27. Huma N, Salim-Ur-Rehman, Anjum FM, Murtaza MA, Sheikh MA. Food fortification strategy-preventing iron deficiency anemia: a review. *Crit Rev Food Sci Nutr.* 2007;47: 259-65.
  28. Mannar MG. Successful food-based programmes, supplementation and fortification. *J Pediatr Gastroenterol Nutr.* 2006;43:47-53.
  29. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. *Lancet.* 2007;370:511-20.
  30. Davidsson L, Walczyk T, Morris A, Hurrell RF. Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *Am J Clin Nutr.* 1998;67:873-7.
  31. Hurrell RF, Reddy MB, Dassenko SA, Cook JD. Ferrous fumarate fortification of a chocolate drink powder. *Br J Nutr.* 1991;65:271-83.



## Original Article

# Impact of milk consumption on performance and health of primary school children in rural Vietnam

Do Thi Kim Lien PhD<sup>1,2</sup>, Bui Thi Nhung PhD<sup>1</sup>, Nguyen Cong Khan PhD<sup>1</sup>, Le Thi Hop PhD<sup>1</sup>, Nguyen Thi Quynh Nga MD<sup>2</sup>, Nguyen Tri Hung MD<sup>2</sup>, Jeroen Kiers PhD<sup>4</sup>, Yamamoto Shigeru PhD<sup>3</sup>, Rob te Biesebeke PhD<sup>5</sup>

<sup>1</sup>National Institute of Nutrition, Hanoi, Vietnam

<sup>2</sup>Dutch Lady Vietnam, Ho Chi Min City, Vietnam

<sup>3</sup>Ochanomizu University Graduate School of Humanities & Sciences, Tokyo, Japan

<sup>4</sup>Current address: NIZO Food Research, Ede, The Netherlands

<sup>5</sup>FrieslandCampina, Pieter Stuyvesantweg 1, Leeuwarden, The Netherlands

## 越南農村小學生牛奶攝取對於表現和健康的影響

這是一個追蹤調查，研究越南小學生營養不良和微量營養素缺乏的盛行率。總共有 454 名兒童，年齡 7 至 8 歲，來自越南北方三角洲的三所小學，分別接受一般牛奶、牛奶強化了維生素、礦物質和菊糖、或做為參考對照組。監測學童的體位、（微量）營養素狀況、糞便菌落的組成、學校表現和健康指數。經過六個月的牛奶介入，學童按年齡的體重（WAZ）和按年齡的身高（HAZ）有顯著改善，體重不足和發育不良比率下降 10%。介入期間，貧血的發生率下降和血清鐵蛋白顯著增加。在所有三組中，血清鋅升高，因而鋅缺乏有顯著改善。介入強化牛奶後，血清視網醇和尿碘水平維持穩定，但是對照組碘缺乏發生率卻增加。介入前，雙歧桿菌在總糞便細菌組成中小於 1%。經三個月的一般牛奶或強化牛奶介入，總菌數、雙歧桿菌和類桿菌均顯著增加。兒童攝取牛奶顯著提高短期記憶分數。在牛奶介入後，家長陳述健康相關生活品質狀況有顯著改善。結論，（強化）牛奶的攝取有益於越南農村兒童，包括減少體重不足和發育不良，改善微量營養素狀況和提高學習指標以及改善生活品質。

**關鍵詞：**營養不良、微量營養素、越南、兒童、健康