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Food and Nutrition Bulletin

Food and Nutrition Bulletin, vol. 20, no. 2

United Nations University Press
The United Nations University
53-70 Jingumae 5-chome, Shibuya-ku, Tokyo 150-8925, Japan
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ISSN 0379-5721

Design and Production by Desktop Publishing & Design Co., Newton, MA USA
Printed by Rivkind Associates, Stoughton, MA USA

Maternal dietary intake and pregnancy outcomes in Baghdad, Iraq

H. I. Tawfeek, Jwameer Nihad Abdulla, and Amna Hameed Rasheed

Abstract

A survey was conducted among 103 pregnant women attending a maternal and child health centre in Rasaf, Baghdad, to study the relationships between maternal dietary intake and pregnancy outcome. Dietary intake values were not significantly correlated with birthweight and birth length except for caloric intake, which was significantly correlated with pregnancy weight gain, delivery weight, pre-pregnancy weight, birthweight, and birth length ($r = +.33$, $r = +.30$, $r = +.26$, $r = +.38$, *and* $r = +.32$, *respectively). The results showed that the pregnant women who delivered small-for-gestational-age infants had diets that provided less energy than those who delivered full-term infants (*$p < .05$*).*

Introduction

Since pregnancy is a time in the life cycle when nutrition is of special importance, and maternal nutrition has an important influence on the course and outcome of conception [1–3], there is ample evidence from many countries that there is a good correlation between maternal nutritional deprivation and the birthweight of the infant [4]. Since 1990, the nutritional status of Iraqi pregnant women has deteriorated, largely as a result of the long economic sanctions. Many recent investigations have shown that the incidence of low birthweight (LBW), defined as less than 2,500 g, among Iraqi newborns is as high as 13.3% [5].

We studied the relationship between dietary intake and pregnancy outcomes among Baghdadi pregnant women under conditions of economic hardship.

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Materials and methods

Between November 1991 and December 1994, 103 pregnant women were chosen for the study, regardless of their age, parity, and residence. All attended the maternal and child health centres in Baghdad during the first trimester of pregnancy.

Socio-demographic data were collected by trained interviewers. One 24-hour dietary intake value was obtained each month by the same interviewer. Portions were estimated by standardized food models along with visual aids, such as posters with photographs of food. The diets were scored for energy, protein, and other nutrients using a food composition table for the Middle East [6]. Nutrient intakes were evaluated against the 1989 recommended dietary allowances (RDA) [7].

The anthropometric examination included measurement of height and body weight during each visit. The pre-pregnancy weight and height were obtained from self-reports. Pregnancy outcome data, including birthweight, length, and gestational age, were obtained during the first 48 hours after delivery by trained medical staff at the Teaching Hospital in Baghdad. Trained home visitors obtained the pregnancy outcome data from those women whose infants were delivered at home.

The gestational age of the neonates was assessed from the date of the last menstrual period. LBW infants were defined as infants with birthweight less than 2,500 g. Premature infants were classified as those with birthweight less than 2,500 g and gestational age less than 37 weeks. Infants were defined as small-for-gestational-age (SGA) if they had a birthweight less than 2,500 g with a gestational age of 37 weeks or greater. Student's *t* values and correlation coefficients were calculated as appropriate.

Results

Descriptions of the 103 pregnant women are given in table 1. The mean age was 28.6 years. The overwhelming majority of the subjects (96.1%) were married, and

few were separated or divorced (0.9% and 2.9%, respectively). The mean pre-pregnancy weight was 52.6 ± 16.3 kg, and the mean pregnancy weight gain was 13.0 ± 3.5 kg. Sixty-six percent had a high school education or lower, and 33.8% had college or advanced degrees. Household incomes were generally low.

Table 2 summarizes the dietary intakes for the 103 pregnant women. The mean energy consumption during pregnancy was 1,990 ± 340 kcal/day. Protein intake averaged 57 ± 11.2 g/day and was below the 1989 RDA. During the entire pregnancy, the intakes of calcium, iron, thiamine, vitamin B_6, and niacin were below the 1989 RDA. The intakes of vitamins A and C were adequate.

On the other hand, these women had lower intakes before pregnancy than during pregnancy, but the difference was not significant. The correlations between dietary intake and maternal anthropometric measurements are shown in table 3. Energy intake was significantly correlated with pregnancy weight gain and delivery weight ($p < .001$) and was weakly, but significantly, correlated with pre-pregnancy weight ($p < .05$). On the other hand, protein intake was not significantly correlated with pre-pregnancy weight, pregnancy weight gain, or delivery weight.

Women who had low dietary intakes generally had LBW infants. Information on the outcome of pregnancy is given in table 4. All pregnancies resulted in single live births. The mean birthweight and height were 3,146 ± 44 g and 49.5 ± 0.2 cm, respectively; 14% of the infants weighed less than 2,500 g. The mean gestational age at delivery was 39.2 weeks (range, 30 to 43 weeks).

The correlation coefficients between maternal dietary intakes and infant birthweight and length are shown in table 5. The positive correlation between caloric intake and birthweight was highly significant ($p < .001$).

Discussion

The average intakes of calories, protein, calcium, iron, thiamine, and riboflavin were below the RDA. There was no significant relationship between the intakes of these nutrients and pregnancy outcome, except for the relation between caloric intake and birthweight. The negative correlations that have been reported between

TABLE 1. Socio-demographic characteristics of the 103 mothers

Characteristic	Value
Age (yr)—mean ± SD	28.6 ± 1.3
Age (yr)—%	
16–19	15.5
20–24	24.2
25–29	33.9
30–34	16.5
35–39	4.8
≥ 40	4.8
Marital status—%	
Married	96.1
Divorced	2.9
Separated	0.9
Maternal anthropometric measurements (kg)—mean ± SD	
Self-reported pre-pregnancy weight	52.6 ± 16.3
Weight gain in pregnancy	13.0 ± 3.5
Education—%	
High school or less	66.2
College degree	24.2
Advanced degree	9.6
Annual household income (Iraqi dinars)—%	
< 2,500	15.5
2,500–4,999	42.9
5,000–6,999	21.3
7,000–9,999	6.5
≥10,000	13.8

TABLE 2. Nutrient intakes of the 103 mothers (means ± SD)

Nutrient	Before pregnancy	During pregnancy (average of all measurements)		
		All mothers	Mothers of SGA infants	Mothers of premature infants
Energy (kcal)	1,984 ± 280	1,990 ± 340	1,630 ± 356	1,774 ± 780
Protein (g)	56 ± 18.3	57 ± 11.2	41 ± 31	49 ± 23
Calcium (mg)	694 ± 110.2	700 ± 80.3	716 ± 81.5	694 ± 113.4
Iron (mg)	11.2 ± 2.1	11.2 ± 3.1	11.6 ± 4.1	10.3 ± 2.8
Vitamin A (IU)	4,593 ± 807	4,586 ± 902	4,008 ± 607	4,579 ± 1,030
Thiamine (mg)	1.12 ± 0.1	1.21 ± 0.2	1.04 ± 0.3	1.19 ± 0.4
Riboflavin (mg)	1.43 ± 0.1	1.53 ± 0.2	1.43 ± 0.2	1.51 ± 0.3
Vitamin C (mg)	85 ± 16.4	87 ± 17.3	81 ± 20.3	86 ± 20.4

TABLE 3. Correlation coefficients (*r*) between anthropometric measurements and nutrient intakes

Nutrient	Pre-pregnancy weight	Pregnancy weight gain	Weight at delivery
Energy	.26*	.33**	.30**
Protein	.09	.18	.13

* $p < .05$; ** $p < .001$.

maternal intakes of calories, protein, and calcium and infant birthweight and length [2] were not observed in this study.

Although there was a general agreement that the incidence of prematurity increased when the dietary intake was poor, there was a disagreement about the relationship between dietary intake and the size of the infant at birth [2].

The results from the population survey showed that the pregnant women who delivered SGA or premature infants had lower dietary intakes than those women who delivered full-term infants. These results are in keeping with observations by others who studied risk factors for SGA births in India [8].

A good relationship of maternal status to risk factors for SGA was found. Thomson reported that the influence of current diet on birthweight was very small [9]. These conclusions have been confirmed by others [10].

In a study in Cairo, slight but significant differences were observed in birthweight, height, and skull circumference between infants of well-nourished and moderately nourished mothers [11]. Since the differences were slight, it was concluded that moderate malnutrition did not seriously affect the health of the infant.

The relationships between dietary intake and pregnancy outcome have been reported with conflicting findings and inconsistent results. Worthington-Roberts and Klerman reported that the inconsistencies may be due in part to methodological problems in evaluating dietary intake accurately and in part to variations in daily nutrient intake among the women in many populations studied [12].

The interaction between nutrition and other environmental and biological factors frequently interferes with the proper interpretation of the results. The interrelation between the mother and her growing foetus and the extent to which the foetus depends on the maternal diet are problems that need more investigation [3]. Clearly there is a need for more such studies in the future.

TABLE 4. Pregnancy outcomes

Outcome	Value
Full-term birth—%	74.7
Premature birth—%	3.9
SGA infant—%	7.8
LBW infant—%	13.6
Birthweight (g)—mean ± SD	3,146 ± 44
Length (cm)—mean ± SD	49.5 ± 0.2
Gestational age (wk)—mean ± SD	39.2 ± 0.3

TABLE 5. Correlation coefficients (*r*) between nutrient intakes and pregnancy outcomes

Nutrient	Outcome	
	Birthweight	Birth length
Calories	.38**	.32*
Protein	.17	.04
Calcium	.21	.20
Iron	.21	.22
Vitamin A	.16	.08
Thiamine	–.18	.09
Riboflavin	.12	.14
Vitamin C	.07	.08

* $p < .05$; ** $p < .001$.

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Erratum

In the article "New issues in developing effective approaches for the prevention and control of vitamin A deficiency," by Martin W. Bloem, Saskia de Pee, and Ian Darnton-Hill (*Food Nutr Bull* 1998;19:137-48), on p. 144, column 2, in the sentence that begins on line 10, the authors intended to say, "A recent study in Nepal showed an impact of both vitamin A and carotene separately on maternal mortality [5]." The authors regret the misleading phrasing of the sentence as published.

Interventions to improve intake of complementary foods by infants 6 to 12 months of age in developing countries: Impact on growth and on the prevalence of malnutrition and potential contribution to child survival

Laura E. Caulfield, Sandra L. Huffman, and Ellen G. Piwoz

Abstract

To evaluate programmatic efforts to improve dietary intake and growth in 6- to 12-month-old infants in developing countries, we reviewed the results of 5 efficacy trials and 16 programmes conducted in 14 countries. Efficacy trials were able to improve infant dietary intakes by 65 to 302 kcal/day and infant growth by 0.04 to 0.46 SD. Programmes reported large improvements in maternal knowledge and practices concerning infant feeding. Four programmes that provided information reported improvements in dietary intakes of 71 to 164 kcal/day and changes in growth of –0.08 to 0.87 SD. Despite variability in the results, the majority of research and programmatic efforts improved growth rates by 0.10 to 0.50 SD. In absolute terms, this range of improvement in growth would reduce prevalences of malnutrition (< –2 SD) at 12 months of age by 1% to 19% and could reduce deaths due to malnutrition by 2% to 13%, depending on the underlying prevalence of malnutrition in the community.

Introduction

Childhood malnutrition is a major public health problem throughout the developing world and is one of the principal underlying causes of death for many of the world's children [1, 2]. Research conducted over the past 20 years throughout the world in a variety of settings has demonstrated that post-natal growth faltering begins at around six months of age, just as infants begin to receive foods to complement their breastmilk intake [3].

How should babies be fed during their first year of life? For the first four to six months of life, it is recommended that infants receive no food or liquid other than breastmilk (not even water). From the age of about six months onward, infants should continue to receive breastmilk and, in addition, should be fed safe and adequate amounts of local foods frequently throughout the day. A recent review [3] suggests that on the assumption of an average intake of energy from breastmilk, infants 6 to 8 months of age should receive 270 kcal/day and those 9 to 11 months of age should receive 450 kcal/day from complementary foods. The variability in energy requirements is wide at any age; thus, some infants will need considerably more, whereas others will need considerably less than these average values. The total length of time that a child receives breastmilk is left open for the mother and child to decide, but it is recommended that mothers continue breastfeeding throughout the second year of life and for as long as possible.

The provision of safe and adequate amounts of local foods appropriate for 6- to 12-month-old infants who are just learning to eat is not as simple as it seems. Complementary foods fed to infants in the second six months of life (and beyond) are often inadequate in energy density, protein, and micronutrient concentration or quality and are often prepared, stored, or fed to children in ways that increase their risk of illness. Because of the complexity of the nutritional and behavioural issues involved, careful, detailed, interdisciplinary work must be undertaken to define the nutritional problems of older infants and identify appropriate and effective interventions to improve complementary feeding and infant growth and development in country settings.

Many nutrition programmes have been implemented over the past 20 years to improve complementary feeding for young children throughout the world, and there is renewed interest in strengthening this component of

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Funding for this activity was provided by the United States Agency for International Development (USAID) under Cooperative Agreement HRN-A-0097-0007-00 and by field support from USAID/Bolivia. The contents of this document do not necessarily reflect the views or policies of USAID.

child survival programmes. Therefore, it is a timely point to review the scientific and programmatic literature to identify what has been accomplished and what can be learned from earlier programmes and applied in future efforts to improve complementary feeding.

The purpose of this review is to evaluate the extent to which research and programmatic efforts over the last 20 years have been able to improve the dietary intakes of 6- to 12-month-old infants in developing countries. First, we review the results of efficacy trials conducted to improve the growth of these older infants, and then the information available on programmes designed to increase dietary intakes and reduce malnutrition in developing countries. The following questions are addressed: What improvements in dietary intakes and growth of infants have research projects (with efficacy designs) been able to achieve? What improvements in dietary intakes and growth of infants have developing-country programmes been able to achieve? What are the features of successful programmes to improve complementary feeding of infants in developing countries? What is the likely impact of improvements in growth rates (brought about by such programmes) on malnutrition rates? On deaths due to malnutrition?

Methods

A search was undertaken to identify scientific papers and programme project documents with data pertinent to this analytic review. The search was not restricted to work on older infants; rather, we sought to identify research or programmatic efforts conducted with preschool children and then to determine whether pertinent data were available on the 6- to 12-month age group. To identify efficacy studies, we conducted a computer-based search for the years 1970 to 1997 and examined all citations in the identified studies. We also examined the references in reviews on dietary intakes and growth of infants and children in developing countries. The final list is probably complete with respect to published reports of controlled trials to improve dietary intakes and growth of non-hospitalized 6- to 12-month-old infants in developing countries. To identify relevant programme projects, we reviewed the citations of published reviews as well as other documents (published by ACC/SCN or the World Bank) detailing programmatic efforts to reduce child malnutrition. We also enlisted the help of public health professionals working in the area of child feeding and growth in developing countries to identify relevant documents. In all, 5 efficacy trials were identified for review, as well as 16 programmes conducted in 14 countries. Complete information (design, coverage, and impact on outcomes of interest) was not always available for all 16 programmes; however, because we felt the information available would be useful to researchers and programme planners, we have made maximal use of the information available.

One important goal of the review was to describe the impact of the interventions on the growth rates of children. To do so, we converted (when necessary) the reported nutritional status data into standard deviation (SD) units. This was accomplished in a variety of ways, depending on how the published data were reported. For example, the pre-supplementation nutritional status of each treatment group was converted to *Z* scores or SD units by subtracting the mean weight or length at six months of age in each group from the gender-averaged median of weight or length from the international growth reference [4], divided by the gender-averaged reference SD of weight or length at that same age. The differences in the post-supplementation average *Z* scores of weight or length describe the impact of the intervention on growth rates in SD units. The treatment effect on growth in SD units could also be calculated by taking the difference in the simple post-supplementation means (in kilograms or centimetres) and dividing by the gender-averaged reference SD for children of that age.

Calculating nutritional impact in terms of shifts in the distribution of nutritional status in SD units is useful, because the results allow us to project reductions in the prevalence of malnutrition (% < –2 *Z* score) associated with various levels of programme impact. This can be done if we assume that the post-intervention *Z* scores are normally distributed and have a given SD (in our case, we chose 1.0), and we utilized information on the area under a normal curve at selected cut points [5]. As a simple example, suppose that the average weight-for-age or length-for-age of children in a community is 3.0 ± 1.0. In this case, about 83% of children would be considered underweight or stunted, respectively, because –2 *Z* score falls at +1 SD on the community *Z*-score distribution, and the proportion of the area under the normal curve [5] to the left of +1 SD is 0.83 (or 0.50 + 0.33). Similarly, if the average growth rates of infants in the community were improved (greatly) by +1.0 SD with the variance of growth unaffected, we would expect the post-intervention *Z* scores in the community to be –2.0 ± 1.0 and the prevalence of malnutrition to be reduced to 50%.

To examine the potential impact of improvements in growth on child survival, we used the method developed by Pelletier et al. [6] for calculating the proportion of deaths attributable to malnutrition in children in developing countries. The population attributable risk (PAR), or the number of deaths due to malnutrition as a proportion of all deaths, is calculated as follows:

$$\mathrm{PAR} = \frac{\sum\left[P_i \times \left(RR_i - 1\right)\right]}{1 + \sum\left[P_i \times \left(RR_i - 1\right)\right]}$$

where P_i is the prevalence of malnutrition of a given degree of severity and RR_i is the relative risk of mortality associated with that degree of malnutrition [6]. On the basis of the analysis of data from eight studies, the relative risk of dying for children of a given weight-for-age (as a percent reference median) as compared with children with weight-for-age of 90% can be calculated as $RR = 10^{-0.0264\,(WA - 90)}$, where *WA* is weight-for-age [6]. This information can also be used to calculate the expected reduction in deaths due to malnutrition given improved growth rates of children. Pelletier et al. [6] determined that the method is valid across the age range of 6 to 36 months and thus is appropriate for the reduced age range of 6 to 12 months that is of interest here. Further, although the method is based on nutritional status calculated as percent reference median, their analyses support the application of their method to nutritional data calculated as *Z* scores. For our purposes, the distributions of weight or length *Z* scores were converted to units of percent reference median at 12 months of age and then used to calculate the PAR depending on the theoretical prevalences of varying degrees of malnutrition and associated *RR*. By calculating the PAR for different theoretical *Z*-score distributions, the impact of improving growth rates of children on reducing malnutrition and therefore the PAR could be quantified.

Results

Efficacy studies to improve dietary intakes and growth

It is widely held that the largest improvements in the intakes of complementary foods of older infants will be achieved in scientific research trials because of technical, financial, and other factors that differentiate research from programmes. Because of this, it is helpful to review the achievements of small-scale randomized intervention trials in this area to provide us with an idea of what optimally might be achieved. From the scientific literature, five studies [7–13] were identified for review. The studies, conducted in Indonesia, Bangladesh, Colombia, Guatemala, and Jamaica, are summarized in table 1.

The interventions varied with respect to design and precise objectives. However, most of them were randomized controlled trials with the common goal of improving the growth of children through improvements in their dietary intakes. None of the trials focused exclusively on 6- to 12-month-old infants. For this reason, most of the information on programme impact was derived from the results reported for the subsample of study participants of the appropriate age. In Colombia, however, it was not possible to separate out the impact of the programme on 6- to 12-month-old infants, and therefore we report the impact on infants 3 to 12 months of age. Further, in Bangladesh the intervention involved 8-month-old infants who were followed up until they were 13 months of age. The study is included here under the assumption that the results obtained are appropriate to what would be observed in 6- to 12-month-old infants. Most of the trials gave food as supplements to the usual dietary intakes of the infants, but the studies varied with respect to the foods offered and the delivery mechanism. The exception to this was the study conducted in Bangladesh [13], in which specific feeding advice and cooking demonstrations were provided to caretakers in their homes. Although our focus is not on the statistical significance of the findings, it is important to note that each of the five studies found that the nutritional interventions significantly improved the growth rates of participating children.

With respect to our objectives, there are four conclusions that can be drawn from these published studies. Each of these points is described below.

The studies were largely successful in identifying foods that were liked and accepted by children in this age group and their caretakers in each cultural setting

The foods offered to the children to enhance their energy intakes from non-breastmilk foods varied across the settings. The foods were either offered daily as ready-to-eat foods [7–9] at feeding centres [8, 9] or day-care settings [7], or provided weekly as raw ingredients to the family to be prepared and fed to the study children [10–12]. Of the foods offered, many provided one or two nutritious foods to be added to the diet. However, in Indonesia [7] local foods were used to develop 20 distinct snack foods to augment the dietary intakes of children while they attended a day-care centre. In several settings, drinks as opposed to semi-solids or solids were offered. In Guatemala atole, a high-energy, moderate-protein, micronutrient-fortified drink, was provided [8, 9]. Whole powdered milk was distributed to the families of the study children in Colombia [10, 11], and a milk-based formula and skim milk powder were distributed in Jamaica [12]. Again, only in the study in Indonesia [7] were combinations of semi-solids and solids offered to increase the energy intakes of 6- to 12-month-old infants.

In general, the foods were well liked and consumed by infants in this age group. In three sites [7–9, 13], part of this success can be attributed to the formative research conducted before the intervention to identify infant-feeding issues, foods appropriate for young children, and the level of energy required from complementary foods in the population. In Guatemala [8, 9] infants were offered either of two different drinks, atole and fresco, a low-energy, no-protein, micronutrient-fortified drink. Consumption of atole was good, because mothers thought it was a good food for infants, whereas consumption of fresco was very low, because mothers did not consider it an acceptable food.

TABLE 1. Efficacy trials to improve dietary intakes and growth of infants 6–12 months of age in developing countries

Site	Subjects	Duration	Food/nutrient profile	Net increase (kcal/d)[a]	Baseline WAZ and HAZ	Change in WAZ and HAZ
Guatemala [8, 9]	150–200 per group or village, 4 villages	From birth, 60–80% participation Atole: 40–60% d Fresco: 10–30% d	Two drinks: atole (high energy, moderate protein) and fresco (low energy, no protein), each with added vitamins and minerals. Offered in unlimited amounts	+83		+0.25 WAZ +0.17 HAZ
Colombia [10, 11]	70 per group	From about 3 mo to 3 yr	Whole powdered milk + commercial high-protein vegetable mix. Provided 428 kcal/d. Also gave 10 mg/d iron and 1,552 IU/d vitamin A	At 18 mo: +178	At 6 mo: –0.21 to –0.50 WAZ –0.16 to –0.36 HAZ	3–12 mo: +0.40 WAZ +0.35 HAZ
Jamaica [12]	128 stunted children 9–24 mo old	12 mo	1 kg/mo milk-based formula and 1 kg/mo skim milk powder and cornmeal for the family, delivered weekly to home. 750 kcal/d with 20 g/d protein	+106	–2.5 WAZ –3.0 HAZ	+0.44 WAZ +0.35 HAZ
Indonesia [7]	112 children 6–20 mo old	90 d	20 snacks of local foods containing bread, rice, wheat flour, sweet potato, coconut milk, cassava, potatoes, sugar, and oil. 400 kcal/d on average with 5 g/d protein in a day-care setting	+ 317 (9–23 mo old) children	–1.6 WAZ –2.4 HAZ	+0.29 WAZ +0.04 HAZ
Bangladesh [13]	120 infants 8 mo old	5 mo	In home demonstrations to enrich meals with energy and protein, using oil, molasses, fish, lentil flour, fruits, and vegetables Frequent and persistent feeding of chop-chop: wheat flour, oil, and brown sugar. Feed more snacks (not meals). No information on kcal/d or nutrient profile	+65	–1.94 to –2.11 WAZ	+0.46 WAZ

Abbreviations: HAZ, height-for-age *Z* score; WAZ, weight-for-age *Z* score.

a. Net increase refers to the difference in energy intakes between treatment and control infants net of home and breastmilk energy intakes. In Guatemala, the intake estimate is also net of the intake of fresco.

Regardless of the acceptability of the supplemental foods, an important drawback in some of the studies was related to the texture (liquid) and uniformity of the supplements chosen [8–12]. Offering a variety of textures and flavours to 6- to 12-month-old infants is important, because two of the developmental goals of complementary feeding are to introduce the infant to varying food textures in preparation for mastication [14, 15] and to begin the transition of the infant from a single-source (liquid) diet to one characterized by diversity and variety. Many psychologists believe that delays or failures to introduce textures to older infants may lead to later feeding problems [14, 15]. Whether this happened in any of the published studies is not known. However, the implication for programmes is that the addition of single-source or liquid supplements to the diets of older infants may not be the best approach to improving the dietary intakes and well-being of children living in underprivileged environments in the long term.

Energy intakes from complementary foods were improved by the interventions

It is important to note that these studies did not compare the outcomes of infants who did or did not re-

ceive complementary foods, but rather the outcomes of infants who had higher versus lower intakes of complementary foods in addition to breastmilk—differences created by the intervention itself. As shown in table 1, the study infants were offered at least 400 kcal/day in addition to their usual energy intake from both breastmilk and non-breastmilk foods given them at home.

Humans, including infants, self-regulate their energy intake. At all sites, study infants did not consume all of the food offered. Although the calculation is difficult to make for all studies, it appears that the study infants consumed 15% to 75% of the extra energy offered from non-breastmilk foods. Thus, the intervention strategies were able to increase energy intakes from non-breastmilk foods by 90 to 500 kcal/day, with most efficacy studies reporting intakes in the range of 150 to 300 kcal/day.

Replacement of energy from breastmilk occurred in many of the studies, and thus the net improvements in total energy intake were somewhat smaller but still important

As stated by Brown et al. [3], optimal complementary feeding in this age range involves adding energy and micronutrients while maintaining high energy intake from breastmilk. One disturbing finding reported in some of these studies is that additional intake of non-breastmilk foods replaced some of the energy previously provided by breastmilk. Thus, the total energy intake (breastmilk energy plus complementary food energy) was lower than expected. Information is not available for all studies, but the decrease in the amount of energy obtained from breastmilk was as much as 200 kcal/day in infants offered additional complementary foods. Across the studies, the net increase in total energy intake due to the interventions ranged from 65 to 300 kcal/day.

The growth of infants was improved by the interventions

The nutritional status of the infants at enrollment in the studies was generally poor, with average *Z* scores of –2.5 to –0.21 for weight-for-age and –3.0 to –0.5 for height-for-age.

To assess the impact of increasing total energy intake by 65 to 300 kcal/day, most of the studies compared rates of growth between infants in the intervention and control groups. All five studies reported statistically significant positive impacts of the interventions on somatic or linear growth, or both. Each of the studies provided evidence that the intervention and control groups of infants had similar nutritional status at baseline. At the end of the interventions, which lasted from five to nine months, differences in nutritional status due to the intervention in the five studies ranged from –0.25 to +0.46 SD units for weight-for-age and from –0.04 to +0.35 SD units for height-for-age.

In summary, the results suggest that improvements in complementary food intakes of 6- to 12-month-old infants in developing countries can be made, and that such improvements in dietary intakes will translate into improved rates of growth. However, these were research projects in which the delivery of and compliance to the intervention were tightly controlled. Further, the projects were small in scale, involving only several hundred infants. The next question is, "How effective have programmes been for improving complementary food intakes of older infants?"

Programmes to improve complementary feeding

This section summarizes the experiences of 16 programmes in 14 countries to improve the complementary food intakes of 6- to 12-month-old infants. The approach taken in the majority of programmes has been comprehensive, involving formative research for the development of specific complementary foods as well as complementary feeding education and counselling and some form of mass-media communication strategy. During implementation, most programmes conducted some form of monitoring or evaluation in order to modify or enhance the impact of the programme. Many of these programmes were implemented under the umbrella of growth-monitoring programmes, and most of them were large in scale, reaching populations of 1,000 or more potential beneficiaries. It should also be noted that these programmes were not focused solely on the feeding of 6- to 12-month-old infants; rather, they were designed to improve feeding practices in children up to 3 years of age, including appropriate feeding during illness. The programmes were conducted in Peru [16–18], the Dominican Republic [19], Indonesia [20, 21], the Philippines [22], Mali [23, 24], Burkina Faso [25, 26], Nigeria [27, 28], the Gambia [29], Cameroon [29], Tanzania [29], Swaziland [29], Ghana [29], Niger [29], and Senegal [29].

Most of the programmes used a combination of qualitative and quantitative research techniques in five stages in order to formulate the behavioural change strategies [29]. The stages were the following:

1. Review of pre-existing information on feeding practices and diet, *inter alia*;
2. Ethnographic study of health and nutrition beliefs and practices of the health providers, community members, and mothers, and their sources of information;
3. A nutritional assessment of existing diets and practices, their potential for enrichment or improvement, and possible resistances or obstacles to improving diet quality and feeding practices;
4. Individual and group trials of new feeding practices, foods, and recipes to determine the most feasible alternatives for improving dietary intake and people's reactions to new products and behaviours;
5. Development of an overall strategy for improving child feeding in the population, based on the findings from steps 1 to 4.

Because of the nature and complexity of the endeavours undertaken, it is important to consider the achievements of these programmes at two stages. The first stage considers the results of step 4 above, in which the feasibility of new feeding practices and recipes was tested on a relatively small scale with intensive motivation and counselling. Following that, we will consider the overall impact of these programmes on feeding behaviours, dietary intakes, and infant growth.

Trials of new feeding practices, foods, and recipes

Studies of the complementary foods currently fed to older infants in developing countries have highlighted the problems of low energy density and poor nutritional quality [30, 31]. To address these problems, many programmes have attempted to develop new complementary food recipes, building largely on what mothers were already "doing right." An example we have already seen is from the study in Bangladesh [13] in which a complementary food called *chop-chop* was promoted. The food—a mixture of wheat flour, oil, and brown sugar—was already being fed to children when they had diarrhoea; the project sought to promote its use for healthy children as well. To determine the nature and feasibility of a new (or improved) food product, household trials were conducted with small numbers of individual mothers or groups of mothers. In these trials, programme planners and mothers worked together to develop and evaluate new food products and feeding behaviours.

Shown in table 2 are the results obtained from formative research involving recipe development and testing with the purpose of designing nutritionally improved complementary foods for young children. Three points can be drawn from these results.

Nutritionally improved complementary foods could be developed in diverse cultural settings

Eleven of the programmes cited above involved the development of new or improved complementary foods. As shown in table 2, most of the foods developed were grain-based porridges to which protein-, energy-, and micronutrient-rich ingredients were added. It is interesting to note the similarity of the approaches taken in very diverse settings. Throughout sub-Saharan Africa, a twofold strategy was followed. First, protein and micronutrients were added to a staple porridge using peanuts, peanut butter, or cowpea flour. Second, in some settings, fermented flour was added to reduce the viscosity of the porridge, thus enhancing energy density as well as the acceptability to children. In Indonesia [20, 21] and the Philippines [22], fish flakes, vegetables, and oil were added to rice.

It should not be concluded from these results that formative research is no longer a necessary step in the development of projects to improve complementary feeding. Rather, experience thus far suggests that some commonality exists in the nature of the problems in complementary feeding as well as in the means of addressing them. This commonality will probably streamline the formative research necessary for the development of new projects.

From a nutritional standpoint, the foods developed in these recipe trials represented clear improvements over the traditional foods offered to children in each setting. However, little information is available to quantify the extent to which regular consumption of these foods (as well as others offered to children) would translate into overall improvements in the energy, protein, and micronutrient intakes of older infants and children.

Poor mothers are willing to prepare new foods and their children are willing to eat them

Are poor mothers in developing countries willing to try new ways of preparing complementary foods for their infants? As shown above, the overriding conclusion to be drawn from the recipe trials is "yes!" The majority of women participating in the trials were willing to prepare new foods and feed them to their children and were active participants in their development. However, the trial results suggest that although mothers were generally willing to try new foods, they were less able to feed the foods regularly and to incorporate (adopt) them into their usual feeding repertoire because of time and resource constraints. Children liked the foods and were willing to eat them, and this was a major motivation for mothers to change and continue new foods and practices.

There are common barriers to using new foods and food preparations

Despite the willingness of mothers to try something new, several common barriers to using the new foods were identified. In a variety of settings, mothers stated a willingness to try the new foods and to continue to feed them as long as their children liked them. Second, the foods for enrichment had to be affordable; a key strength of several projects was the development of options for mothers at varying levels of poverty. For example, in Senegal [29] mothers who could not afford to add peanuts were willing to add milk or butter to their infants' porridge. Time is an important constraint to many women; to be successful, enriched foods must involve minimal changes in maternal time spent in preparation and feeding. Finally, it was reported in several sites that mothers would discontinue giving the food if their infants became sick or had other negative reactions that the mothers attributed to the food. This is a difficult barrier to overcome, because infants in developing countries will invariably get sick, and some may happen to get sick after consuming the enriched complementary food.

Although these results appear optimistic, it is impor-

TABLE 2. Household and recipe programme trials to improve complementary food intakes of infants 6–12 months of age in developing countries

Site	Enriched weaning food	Nutrient profile	Time period	Acceptance
Peru [16]	*Sanquito:* wheat flour, toasted pea flour, brown sugar, oil, carrots, water	237 kcal/100 g, recommended to feed 1 cup/d, providing 550 kcal, 11.9 g protein, and 206 μg RE	Small groups of women in 10 rural highland communities	88% (on 1st try, accepted 4 mouthfuls); compared with pudding, children consumed 6 times more kcal/kg/12-h day
Nigeria [27, 28]	*Eko ilera,* a fortified *ogi,* containing maize or sorghum *ogi* paste, cowpea flour, red palm oil, sugar, water, sorghum malt flour	85 kcal/100 g (+50 kcal/100 g from traditional *eko*); designed for 12% increase in net energy intake/d		Good acceptance, but no estimates available
Cameroon [29]	*Bouillie enrichie,* the traditional pap enriched with milk, egg, or peanut butter			Good acceptance, but no estimates available
The Gambia [29]	Millet pap *ogi,* enriched with peanut paste; alternative additional ingredients were bean flour, butter, milk, and dried fish		31 women visited 4 times at home over 2-wk period	68% liked the peanuts, an additional 13% added alternative ingredients
Tanzania [29]	Maize peanut gruel *ugi* with germinated sorghum flour ("power flour") or *kimea*		40 children aged 5–60 mo in 3-mo trial	28% prepared gruel with *kimea* regularly; 85% used gruel about 25% of the time
Ghana [29]	Traditional cereal porridge, thickened and enriched with legumes, fish powder, or oil; frequency of feeding also increased		About 50–70 mothers over 4–7 d	79% willing to thicken; 82% willing to enrich; 82% willing to increase feeding frequency
Swaziland [29]	Maize porridge *liphalishi* with germinated sorghum malt, enriched with relish, oil, or peanut butter		28 families over 1 wk	86% were able to enrich; 90% added the malted grains
Niger [29]	Millet flour-based porridge with sugar and peanut solids (*kulikuli),* sour skimmed milk, or a fried bean, millet, or wheat cake	83 kcal/100 g enriched with cakes; 51 kcal/100 g enriched with peanut solids or milk	116 mothers of mostly ill children 4–24 mo old in a 1-wk trial	73% used food at least once a week; 11% used it for 3 or more days; 4% never tried it; 26% modified the recipe slightly; 91% would continue to give the food to their child

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TABLE 2. Household and recipe programme trials to improve complementary food intakes of infants 6–12 months of age in developing countries *(continued)*

Site	Enriched weaning food	Nutrient profile	Time period	Acceptance
Senegal [29]	Porridge enriched with peanut butter, milk, butter, or oil		31 mothers	77% tried food; 68% would continue; milk and butter were preferred; butter for infants 6–11 mo old. Milk most likely to be continued
Indonesia [20, 21]	*Nasi tim bayi*, rice enriched with fish, vegetables, and oil		22 mothers [21]	86% [21] made it as milk; 55% continued to make it with slight changes; 45% were willing to add fat source to the recipe
Philippines [22]	Rice or *lugao* enriched with oil plus flaked fish plus a vegetable from the family pot			Good acceptance, but no estimates available

tant to keep in mind that several key features inherent in the design of these recipe trials influence the type of results obtained. First, the approach involves small numbers of women or other target audiences. Second, the approach is participatory; women are asked to provide suggestions regarding the ingredients, methods of preparation, and ways to overcome barriers to their use. Thus, the dynamic created during these sessions is a one-on-one partnership between the programme planners and the target beneficiaries. Third, the nature of the process is to begin with a list of possible options (in this case, recipe variations), with the goal of the research being to refine the list. Thus, the results presented necessarily reflect the most successful approaches, and details on everything that was tried but not found to be successful are often not described. It is therefore important to consider whether the new foods or promoted feeding practices will be adopted when the intervention is conducted on a larger scale with less intensive interaction and participation among all mothers in the population.

Programme messages and communication strategies

How did these programmes try to improve complementary feeding? Presented in table 3 are brief descriptions of the content and communication strategies employed in nine of the programmes. Several features of these programmes are evident from the table.

Improving complementary feeding practices requires a comprehensive approach

The majority of the programmes used a comprehensive approach for improving complementary food intakes of older infants. First, the design of the programme content indicates that optimal complementary feeding begins at birth and that what constitutes optimal feeding changes as the infant grows and develops. Thus, for mothers to feed their infants optimally, they need simple, action-oriented information that is age-appropriate and that changes as their infants grow and mature. Second, the content is built upon current local practices, beliefs, and concerns—just as the food products or recipes that are developed are built upon local beliefs and practices. Third, the messages used reflect the fact that mothers need advice and information not only on what to feed, but also on how to feed their infants.

As described in table 3, the key features of the educational strategies are the following:

Promote exclusive breastfeeding at birth as the critical first step. Promoting exclusive breastfeeding until four to six months of age delays the introduction of complementary foods until the appropriate time. Breastmilk is a critical component of an optimal feeding strategy for infants: the more optimal the breastmilk intake, the lower the amount of energy required from complementary foods. The programmes differed with respect to the recommended duration of exclusive breastfeeding because of changes in the international recommendations over time and because of variations among national policies. Besides promoting exclusive breastfeeding, several of the programmes had messages that attacked the reasons given by women for not exclusively breastfeeding their infants for the first four to six months of life. For example, in many countries water

TABLE 3. Programmes to improve complementary feeding of infants 6–12 months of age in developing countries: Messages and communication strategies

Site	Messages and interventions	Communication strategies
Dominican Republic [19]	Growth monitoring Only breastmilk until 4 mo At 5–8 mo, also feed 3 times/d if child is growing well; feed 4 times/d + fruit (banana, orange, or mango) if child is not growing well Give thick foods (add little water), since "Water fills but does not nourish" Feed 9- to 24-mo-olds 3 times/d + 2 snacks of family food, continue breastfeeding, if not growing well—give family foods (don't add water), feed 4 times/d + 2 snacks, continue breastfeeding	Mass marketing and individual and group education (community growth chart); also community development education, counselling cards, individualized growth monitoring, and counselling in mother's home
Peru [17, 18]	Booklet: "You can learn to produce enough breastmilk" Breastfeeding helps keep an infant *sano* Breastmilk only until 6 mo; if the baby is thirsty, the mother should drink water At 6 mo feed thick, easy-to-prepare foods, feed thick foods before soup General messages on "How to feed your child during the first year of life"	Posters, mobile loudspeakers, radio spots, 3- to 8-min instructional videos in health clinics, leaflets, recipe booklet and infant-feeding guide, nurse-led lactation classes, nutritionist-led cooking demonstrations
Peru [16]	Feed 1 cup/d of *sanquito* to children 6–18 mo old when ill, 2 cups/d if > 18 mo old Continue breastfeeding Feed other foods as well Do this during and for 1–2 wk after episode of diarrhoea	Radio messages, flip chart, calendar with recipe on it, training materials for health professionals
Nigeria [27,28]	Exclusive breastfeeding to 4 mo At 4 mo, prepare *eko ilera* instead of traditional *eko* to help your child be "active and powerful" Feed other solids as well, starting at 6 mo Continue breastfeeding at least until child's 1st birthday Hygienic methods of food preparation Encourage spoon feeding (as opposed to hand feeding) Strongly discourage bottle-feeding of any kind	In each village, 10 village-selected "teaching mothers" trained by community health worker each taught 10 mothers via 2 cooking demonstrations; mothers were given flyers to take home and 3-d supply of ingredients
Cameroon [29]	Prepare and offer *bouillie enrichie* Advice on feeding frequency, meal composition for healthy and sick children, 0–3, 4–9, 10–15, and 16–36 mo old	*Animateurs* led monthly discussion groups, held cooking demonstrations, performed growth monitoring, gave individualized nutrition counselling, made home visits for those moderately malnourished
Burkina Faso [25, 26]	Breastfeed exclusively to 4–6 mo Give enriched *bouillie* and mashed fruits at 4–6 mo, continue breastfeeding, give 3 bowls/d plus fruit or other snack once child begins chewing food Increase well-baby clinic attendance	Drama, story-telling, print materials, counselling, handouts, flip charts, songs and cassettes; in counselling, negotiate rather than educate

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TABLE 3. Programmes to improve complementary feeding of infants 6–12 months of age in developing countries: Messages and communication strategies *(continued)*

Site	Messages and interventions	Communication strategies
Mali [23, 24]	Growth monitoring Only breastmilk until 4 mo, no water until after 4 mo Promotion of vitamin A–rich foods Use small feeding bowl, supervise feeding, feed patiently and persistently	5-step counselling approach, live drama, radio programmes, flip charts, counselling cards, training in negotiation
Indonesia [20]	Growth monitoring Give calorie- and nutrient-dense mashed family foods at 4 mo; mix with fat for infants 6–9 mo old (*nasi tim bayi*); add adult foods to provide complete meal at 10 mo Feed 6- to 9-mo-olds 4–6 times/d, plus breastmilk; feed 10- to 24-mo-olds 5 times/d (meal + snack) plus breastmilk; feed children ≥10 mo old larger portions and get more variety in diet Children don't know what is best for them: mothers do Feed patiently Enhance parental aspirations for child	Mass media (jingles, posters, cassettes, leaflets) Age-specific counselling cards with messages, feeding schedule insert for growth card
Indonesia [21]	Growth monitoring Breastmilk only until 4 mo, feed from both breasts In addition, at 5–8 mo, feed enriched rice porridge *bubur campur* 4 times/d, feed patiently At 9–24 mo feed adult food 4 times/d, including *tempe, tahu*, or fish, and green vegetables; offer snacks Continue breastfeeding as long as possible	Radio education spots, *Kader* (community health worker) training, posters for families to have in house

is given to young infants to curb their thirst, and several programmes developed specific messages to counter this idea. In several sites, messages suggested that mothers should drink more to quench their infants' thirst [17, 18, 27, 28]. In one of these programmes, the decision was made not to try to dissuade mothers from giving infusions (teas), because the beliefs in the population regarding this practice were so tightly held. Throughout the world, "insufficient milk" is the principal reason mothers begin introducing complementary foods before the appropriate time [32]. Given that this is likely to be true only rarely, the projects sought to overcome this barrier by increasing mothers' self-confidence with respect to breastfeeding, discouraging the use of pacifiers and bottles, and teaching women behavioural strategies for optimizing breastmilk production, such as increasing frequency, breastfeeding at night, and feeding from both breasts each time. In Peru a booklet titled "Como tener bastante leche" (How to have enough milk) was produced and disseminated [17, 18].

Begin complementary feeding with small amounts of mashed foods; texture is important. Although a five- or six-month-old infant is beginning to need more energy than that provided by breastmilk alone, the need develops slowly over a few months, depending on the mother's milk production and the infant's individual growth pattern. Thus, the goal of complementary feeding at the beginning is to introduce new flavours and textures to the infants, prepare them to begin chewing, and encourage their interest in food. Mothers throughout the world, however, are worried about giving infants semi-solids or solids before they can handle them. For this reason, many of the programmes recommended that mothers start by giving mashed or pureed foods. Two of the programmes also recognized maternal concerns regarding the time involved in preparing special complementary foods; one programme provided recipes or food ideas that were "fast and easy," and the other promoted the mashing of the foods fed to the rest of the family. By focusing the issue around texture and ease of preparation, the mothers' initial concerns are validated, and this is likely to be a key factor influencing maternal acceptance of the recommendation.

Provide energy and nutrient-dense complementary foods on a daily basis. As described earlier, most of the food products or recipes recommended in the programmes

were designed to provide affordable, energy- and nutrient-dense foods suitable for daily consumption by older infants. These foods, along with breastmilk, form the base of the diet of older infants as they make the transition to the solid foods consumed by the family. As described earlier, the energy- and nutrient-dense foods tended to be protein- and micronutrient-enriched versions of local complementary foods, enriched porridges with malted flour added to decrease the viscosity, adaptations of local foods not typically provided to older infants, or local enriched foods usually fed only during illness that are promoted for everyday use.

Offer foods frequently throughout the day. As the infant's energy needs increase, the need to consume more non-breastmilk foods increases; however, the infant's stomach cannot handle large amounts of food at one meal. Therefore, it is recommended that smaller amounts of foods be offered several times throughout the day. How frequently should they be offered? That depends on the energy density of the foods offered: the more energy-dense the foods, the less frequently infants need to be fed in order to meet their energy requirements [3]. The projects made decisions regarding feeding frequency based on the energy density of the local complementary foods, with most programmes recommending that infants between 6 and 12 months of age be offered three to five meals each day. As shown in the programme in the Dominican Republic [19], mothers of children identified as not growing well were advised to increase feeding frequency and variety, and were reminded that foods should not be watered down.

Feed patiently and persistently. Feeding older infants can be difficult and therefore requires patience and persistence. Such messages about feeding style were incorporated into several of the projects. Mothers were also encouraged to take a more proactive feeding style and to keep trying to feed the infants even when they did not appear to be hungry or when they rejected foods. In Indonesia mothers were told that mothers and not infants know what is best. In Mali [23, 24] the programme encouraged mothers to feed their children from small bowls and to supervise them while they ate.

Increase variety in the infant's diet. A well-accepted principle in nutrition is that a diverse diet is a healthful diet. Children need to be offered a variety of foods so that they can develop tastes and preferences and move away from a one-food liquid diet (breastmilk) to the variety of foods that will characterize their diet for the rest of their life. One programme in Indonesia [20] explicitly incorporated the concept of variety in their educational messages to caretakers of infants 10 months of age and older. Other programmes stressed variety implicitly by encouraging the consumption of foods from distinct food groups, particularly foods rich in vitamin A.

Continue breastfeeding for as long as possible. Even though the infant is consuming other foods, breastmilk is still a nutritious food and should continue to be offered. Recognizing the multiple benefits of breastfeeding for both the mother and the child, the programmes supported continued breastfeeding. It should be noted, however, that none of the projects developed messages about maintaining high levels of breastmilk intake during the first year of life as complementary foods are introduced and their consumption increases, or about how to do it.

Mass media and one-on-one counselling approaches were used jointly to improve complementary feeding

Each of the programmes used a variety of communication approaches to impart its messages to the population. First, mass-media techniques were used to reach the target population of mothers with infants as well as the larger population. Such approaches are necessary to change cultural norms regarding optimal complementary feeding. Second, one-on-one interactions between community health workers and mothers were used to provide individualized information and support to mothers of infants. In several of the programmes, the counselling component was explicitly integrated with growth-monitoring programmes, and community health workers were provided with training in growth monitoring and promotion, complementary feeding, and specific counselling techniques. Importantly, the approach to counselling was both process- and action-oriented. For example, in Mali [23, 24] a five-step counselling approach was followed that stressed negotiation with mothers as opposed to education *per se*. Finally, each programme produced printed materials such as counselling cards, recipe booklets, leaflets, and posters for mothers to take home and use.

Impact of programmes on maternal knowledge and practices

How effective were the educational strategies used in these programmes? Presented in table 4 are the reported impacts of the interventions on maternal knowledge and practices related to complementary feeding. However, before examining programme impact, it is important to consider the coverage achieved in these projects as well as the evaluation strategies.

Most of the projects achieved good coverage of the target population, but the coverage rates varied depending on the communication strategy. As expected, mass-media strategies reached a greater percentage of the target population than did strategies involving individualized or one-on-one interaction. A word of caution should be added here, because little documentation was available that described how coverage was calculated. Nevertheless, the data provided suggest that the interventions did in fact take place and that a sizeable proportion of the potential beneficiaries were exposed to one or more messages.

TABLE 4. Programmes to improve complementary feeding of infants 6–12 months of age in developing countries: Impact on maternal knowledge and feeding practices

Site	Evaluation design	Exposure or coverage	Appropriate knowledge	Improved feeding practices
Dominican Republic [19]	Cross-sectional surveys of 18 randomly selected programme communities and 18 matched non-programme communities. Also followed programme families through the 3-yr programme	70% overall contact with programme	18% ↑ belief in exclusive breastfeeding for < 4-mo-olds	30% ↓ introduction of complementary foods < 4 mo 70% ↓ use of cow's milk and other foods instead of breastmilk 0–4 mo 200% ↑ breastfeeding on demand; no difference in no. of feeds or snacks for 4- to 12-mo-olds 200% ↑ 3 food groups (diversity)
Peru [17, 18] 1-yr programme divided into 2 6-mo phases, conducted in 9 communities	Pre-post cross-sectional survey of ~200 infants 0–12 mo old from the 9 communities conducted 1 yr apart. Interview, 24-h recall, structured observations, weighed intakes, and anthropometry. Ethnography based on maternal adoption post-programme	98% any exposure 80% posters 29–45% had booklets 57% knew of courses 29% attended courses 61–88% heard breastfeeding messages 44–58% heard weaning food messages	73% "Every mother can produce enough milk" 88% "Don't give other milks" 61% "Don't give teas" 86% "Begin complementary foods at 6 mo" 44–55% "Purees and puddings are more nutritious than soup" 58% "Add 1 teaspoon oil to complementary foods"	8% ↑ breastfeeding 6–12 mo 24% ↓ giving water < 4 mo 50% ↓ complementary foods at 3 mo 32% ↓ complementary foods at 5 mo 83% ↑ feeding thick foods 2 times/d No change in specific foods 400% ↑ use of purees, but not puddings No change in no. of foods per day
Peru [16] 5-mo intervention	30 cluster survey of 648 representative families conducted after intervention	87% radio 25% home clubs 19% print material 16% friends 5% health personnel	82% knew of *sanquito*	16% tried food in class, 12% tried it at home (19% and 15% of those who knew, respectively) 12% overall adoption (15% of those who knew, 76% of those who ever tried, 98% of those who tried at home)
Nigeria [27, 28] 1-wk intervention	Post-intervention survey of 295 participants and 301 non-participants conducted 2–8 wk after intervention	937 mothers from 11 communities were exposed	57% of participants could accurately describe *eko ilera* 2% dissemination to non-participants	48% tried *eko ilera* 17–24% adopted the recipe and had the necessary ingredients at home

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It should also be noted that significant exposure to programme activities (contamination or crossover) occurred in the comparison communities in some sites. In several programmes, the level of exposure was minimal (2% to 3%). However, in Mali [23, 24] 18% of respondents in the comparison (non-programme)

TABLE 4. Programmes to improve complementary feeding of infants 6–12 months of age in developing countries: Impact on maternal knowledge and feeding practices *(continued)*

Site	Evaluation design	Exposure or coverage	Appropriate knowledge	Improved feeding practices
Burkina Faso [25, 26] 2.5-yr intervention	Baseline and final surveys of programme communities	52% received nutritional advice from community health workers 60% recalled the counselling card 21% radio dramas	Overall low retention of messages 50% reported that children should be fed 3 times/d with variety	20% ↑ correct timing of introduction of solids
Mali [23, 24] 3-yr intervention involving multiple non-governmental organizations and private voluntary organizations	Baseline, mid-term, and final evaluation surveys in intervention and comparison communities	45% recalled a programme activity 18% cross-treatment		50% ↓ giving water < 4–6 mo Consumption at 4–6 mo: 29% ↑ porridge 140% ↑ fruit 57% ↑ cow's milk 300% ↑ greens 150% ↑ meat and liver
Cameroon [29] Varying durations of programme activities	Post-intervention survey in 23 intervention (n = 463) and comparison (n = 302) communities after 6–12 mo of programme implementation		41% ↑ "Feed more after illness" 8% ↑ "Continue feeding during diarrhoea" 11% ↑ "Can encourage a sick child to eat"	13% ↑ *bouillie enrichie*
Indonesia [20] 1-yr programme	Baseline population-based survey and final survey conducted 1 yr after programme implementation in 3 programme and 3 matched-comparison *kecamatan*. Also followed a subsample of programme children longitudinally	50% recalled message correctly 25% recalled message but not correctly	50% ↑ exclusive breastfeeding at 3 mo At 6–9 mo child should receive: animal protein 15% ↑ vegetable protein 22% ↑ vegetables 21% ↑ oil 51% ↑ feed at least 3 times/d 16% ↑ fish 15% ↑ 1 spoon/mo of age 9% ↑ feed 4 times/d at 12 mo 51% ↑	6% ↓ complementary foods before 4 mo 21% ↑ *nasi tim bay* overall 288% ↑ *nasi tim bay* prepared correctly 266% ↑ maternal advice sharing

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communities had been exposed to one or more programme activities or messages. Exposure of non-programme communities to the programme complicates the evaluation process and leads to an underestimation of programme impact. It also speaks well of the intervention itself, in that it was diffused beyond the limits of the target population.

A variety of approaches were taken to evaluate the impact of these programmes. In a few of them, pre- and post-intervention surveys were conducted in programme communities. The principal problem with this approach is that it may overestimate the impact of the programme because it does not separate the impact of the programme from secular improvements in knowledge and

TABLE 4. Programmes to improve complementary feeding of infants 6–12 months of age in developing countries: Impact on maternal knowledge and feeding practices *(continued)*

Site	Evaluation design	Exposure or coverage	Appropriate knowledge	Improved feeding practices
Indonesia [21] 3-yr programme	Baseline survey of project communities, plus final cross-sectional survey of 600 project and 400 matched-comparison households with infants < 24 mo of age	88% got advice from *kadar* (health worker) 78% attended growth monitoring regularly 85% growth monitoring education 37% personal counselling 79% received at least 1 poster 44% heard radio spots High rate of contamination of comparison group	Knowledge scores (% correct): 75% vs 45% overall. For mothers of infants 0–4 mo: 70% vs 48% correct; of infants 5–8 mo: 58% vs 10% correct; of infants 9–24 mo: 90% vs 85% correct.	Infants 5–8 mo old consuming on previous day: greens 200% ↑ coconut milk 253% ↑ oil 54% ↑ fish 200% ↑ *tempe* 290% ↑ negative effect on *tahu bubur campur* 350% ↑ + effects also seen at 9–24 mo

practices. Two projects compared outcomes among participants with those among non-participants in the same community. The potential problem with this approach is one of selection bias; that is, an undocumented process exists through which individuals become participants or remain as non-participants, and this process can lead to a lack of comparability between these two groups of individuals within the community. This lack of comparability may lead to either overestimates or underestimates of programme impact. Other projects examined impact by comparing outcomes between programme and comparison communities. To improve comparability, the comparison communities were matched to programme communities on the basis of their socio-economic and demographic characteristics, and in a few cases, both baseline and final evaluations were conducted. The strongest design would have been to randomize individuals or communities to receive the programme activities. However, to the best of our knowledge, none of the projects used randomization procedures to identify programme and comparison communities.

Maternal knowledge and practices were assessed using knowledge, attitude, and practice (KAP) survey techniques. Mothers were asked to recall specific messages of the programme, and the accuracy of their responses was noted. They were also asked whether they agreed with a series of belief statements regarding breastfeeding and complementary feeding. Most projects compared persons in programme and non-programme communities with respect to changes in their knowledge of specific intervention messages over time. Only one project created knowledge composite scores based on the percentage of correct responses [21]. In general, the results indicate that the interventions resulted in large shifts in maternal knowledge regarding optimal feeding during the first year of life.

The impact of the programme on infant-feeding practices was assessed via interviews or structured observations and weighed intakes. Most of the projects used survey techniques that provided information on infant-feeding practices as reported by the mother. Mothers were asked how they were currently feeding their infants and were administered a 24-hour recall test to describe what their infants had eaten on the day before the interview. The 24-hour recall also provided quantitative information on dietary intakes. The results suggest large differences in the frequency with which recommended feeding practices were reported in programme versus comparison communities. In the following section, we consider whether the changes in maternal knowledge and reporting practices translated into measurable improvements in the dietary intakes and nutritional status of programme participants.

Impact of programmes on complementary food intake and nutritional status

Changes in the adequacy of the dietary intake of participating infants were reported for only three programmes. This information was converted to intake in kilocalories per day for comparison with the results of efficacy trials. As shown in table 5, the results suggest that the projects were able to increase the energy intake of 6- to 12-month old infants by about 70 to 165 kcal/day. This change is impressive, because it covers the lower range of net improvements in dietary intake reported by the efficacy studies described earlier (65–300 kcal/day). It is important to note, however, that separate information on energy intakes from breastmilk and non-breastmilk foods was not provided, and thus we do not know whether increased consumption of complementary foods reduced breastmilk intake. On the basis of the results of the efficacy studies, this cer-

TABLE 5. Programmes to improve compleentary feeding of infants 6–12 months of age in developing countries: Impact on infant dietary intakes and nutritional status

Site	Change in dietary intakes	Baseline WAZ, HAZ	Change in WAZ, HAZ
Dominican Republic [19]		–1.0 WAZ, 0–11 mo –1.4 WAZ , 12–23 mo	+0.24 WAZ, 0–11 mo +0.36 WAZ, 12–23 mo
Peru [17, 18]	30% ↑ energy density: +19 kcal/100 g at 6–12 mo	–0.23 WAZ, 6–12 mo	–0.08 WAZ
Mali [23, 24]		–1.18 WAZ[a], 12 mo –1.75 HAZ, 12 mo	+ 0.41 WAZ + 0.87 HAZ
Indonesia [20]	No change at 4–5 mo ↑ 71 kcal/d at 6–9 mo[a]	–2.0 WAZ[a], 12 mo –1.6 HAZ[a], 12 mo	+0.50 WAZ +0.40 HAZ
Indonesia [21]	↑ 79 kcal/d, 5 mo[a] ↑ 141 kcal/d, 9 mo[a] ↑ 164 kcal/d, 12 mo[a]	–1.7 WAZ[a], 12 mo –1.8 HAZ[a], 12 mo	+0.35 WAZ[a] +0.30 HAZ[a] (corrected for differences at baseline)

Abbreviations: HAZ, height-for-age *Z* score; WAZ, weight-for-age *Z* score.
a. Extrapolated estimates based on data available in reference.

tainly seems likely. Thus, the actual improvements in complementary food intakes may be larger.

Also presented in table 5 are estimates of the improvements in nutritional status of infants achieved in the project area. This information was not presented uniformly across all programme reports; therefore, the information was transformed to make it comparable to that presented in the efficacy studies. The methods used were similar to those described earlier; exact details on the transformations used are available from the authors. With the exception of the programme in Peru [17], all of the projects with available data showed positive programme impacts on nutritional status. The lack of impact of the project in Peru may be related to the evaluation strategy, which consisted of cross-sectional nutritional status surveys in the communities before and after the intervention. When using this strategy, one cannot separate programme impact from secular trends in nutritional status over the life of the project. The range of positive impacts was on the order of +0.24 to +0.87 SD for both weight-for-age and height-for-age. For several reasons described above, however, it is likely that these estimated impacts are somewhat overstated. Thus, the actual improvements in nutritional status are probably smaller. However, even if the true impact was overestimated by 50% (i.e., infants of 0.10–0.40 SD), the impact would still probably be large enough to translate into tangible reductions in malnutrition of older infants in developing countries.

Discussion

The results of this analysis provide evidence that increasing complementary food intakes of 6- to 12-month-old infants is both an efficacious and an effective approach to reducing early childhood malnutrition in developing countries. The first section reviewed five efficacy studies that provided an additional 65 to 300 kcal/day, resulting in improvements in growth of 0.1 to 0.5 SD. These studies used randomized designs, and confounding issues were addressed. Overall, they were well conducted and provide causal evidence that improving dietary intakes of infants living in impoverished environments will improve their growth.

In the next section, we reviewed programme projects that attempted to reduce malnutrition by improving infant-feeding practices. The information is less comprehensive, because several of the projects examined were not formally evaluated for impact. The evaluation designs, when available, were not optimal, although most at least conducted pre-post evaluations of maternal knowledge, infant-feeding practices, and infant nutritional status in both programme and comparison communities. One way to strengthen these types of designs would be to randomly assign individuals or communities to programme or comparison groups. Programme planners and policy makers, however, are reluctant to adopt such strategies. If randomization is not done, demonstration of comparability of the groups and an analysis of congruence of the findings become more important. Such analyses were performed to a greater or lesser extent in these evaluations. Thus, the results provide plausible evidence that comprehensive, multifaceted intervention approaches involving breastfeeding promotion and improved complementary feeding can identify affordable and acceptable means for caretakers to improve infant feeding, improve caretaker knowledge

and beliefs regarding optimal infant feeding, improve infant-feeding practices, increase total energy intake, and improve the nutritional status of older infants.

The evaluations provide evidence that improvements in dietary energy intake of 70 to 165 kcal/day were associated with changes in growth rates on the order of –0.04 to +0.87 SD. Thus, it would appear that the programme projects were able to produce equivalent or greater improvements in nutritional status with about one-half the improvement in dietary energy intakes. How can this be? The answer probably lies in the fact that whereas the efficacy trials used one method to improve infant feeding (direct provision of additional foods), the programmes intervened in multiple ways and at multiple moments, beginning at birth and continuing throughout infancy and beyond. Thus, it is likely that some of the improvements in nutritional status are a direct result of improved breastfeeding practices (particularly improvements in the duration of exclusive breastfeeding) as well as improved means and motivation for improved complementary feeding. It is also likely that evaluations that provide plausible evidence of programme impact are more subject to small levels of positive confounding that lead to a slight overestimation of programme impact.

Despite the variability in the impact of programmes on growth rates of infants, most efficacy studies as well as programmatic efforts increased the growth rates of children by 0.10 to 0.50 SD. What do these rates of improvement in growth mean for reducing malnutrition in a population? Assuming that the distributions of *Z* scores post-intervention are normal, with an SD of 1.0, we can calculate the impact of an upward shift in the average *Z* score of 0.10 to 0.50 SD and the expected prevalence of malnutrition (% < –2 *Z* score). This is done in table 6A and B for various levels of nutritional status in the population and intervention impact. As shown, improvements in growth on the order of 0.10 SD imply absolute reductions in the prevalence of malnutrition of 2% to 4%, whereas improvements on the order of 0.50 SD imply reductions of 5% to 19%, depending on the underlying prevalence of malnutrition in the population (assumed to be between 7% and 69%). If we consider the average positive impacts on growth from both studies and programmes to be on the order of 0.2 to 0.3 SD, then we can expect absolute reductions in the prevalence of malnutrition on the order of 3% to 12%, depending on the underlying prevalence in the population.

It is well known that poor nutritional status contributes to more than half of child deaths in developing countries. For this reason, interventions to improve nutritional status are integral components of child survival efforts worldwide. Therefore, it is likely that improvements in nutritional status will also translate into reductions in mortality rates for infants and young children. Recently, Pelletier et al. [6] provided a method of quantifying the proportion of deaths among children attributable to malnutrition, based on the traditional statistic, population attributable risk (PAR). We used this technique to provide an indication of the potential reduction in mortality attributable to malnutrition that would result if programmatic efforts were able to improve the growth rates of older infants by 0.10 to 0.50 SD. These results are shown in table 6C. The underlying PAR in the community is a function of the underlying prevalence of malnutrition (characterized here by the average *Z* score), and the change in PAR depends on the improvement in nutritional status. These extrapolations indicate that programmatic efforts that improve the nutritional status of children by 0.10 to 0.50 SD might reduce the proportion of deaths attributable to malnutrition by 2% to 13%, depending on the underlying prevalence of malnutrition in the community.

Several words of caution must be said regarding the review and the analytic results extrapolated from these data. First, it is likely that positive publication bias exists for both types of interventions, meaning that studies not able to improve the growth rates of older infants were less likely to be published in the scientific literature, and that few evaluations of ineffective programmes may have been available for review. Thus, the overall findings presented here may be overstated to an unknown degree. Second, in the evaluation we have emphasized the validity and magnitude of the effect sizes reported rather than the statistical significance (or lack thereof) of specific estimates. In fact, some of the results from programme evaluations were not statistically significant at traditional alpha values of 0.05. Statistical significance is arguably less important in this type of analysis, given the relative dearth of information that was available *a priori* for designing optimal evaluations.

Despite these limitations, nutritionists, health professionals, and policy makers should take note of the likely magnitude of the positive contribution that complementary feeding interventions can make towards ensuring the growth and survival of young children in developing countries. The results presented here with respect to reductions in malnutrition and mortality are not proven, but are testable within a programmatic context. Thus, it is our intention that the analyses presented here provide guidance as well as starting values for designing programmes with the greatest potential for improving nutritional status, and with improved evaluation designs that will allow us to demonstrate directly the contribution of such programmes to child survival worldwide.

TABLE 6. Likely impact of improvements in complementary feeding on prevalence of malnutrition and deaths attributable to malnutrition among infants 6–12 months of age in developing countries, estimated from the range of improvements in growth reported from efficacy studies and programmatic interventions (tables 1 and 5).

A. Post-intervention prevalence of malnutrition (% < –2 Z score)

Average *Z* score in population	Population prevalence of malnutrition[a]	Programme impact (SD)				
		+ 0.10	+ 0.20	+ 0.30	+ 0.40	+ 0.50
–2.5	69	66	62	58	54	50
–2.0	50	46	42	38	34	31
–1.5	31	27	24	21	18	16
–1.0	16	14	12	10	8	7
–0.5	7	5	4	4	3	2

a. Calculated as described in the text.

B. Absolute change in prevalence of malnutrition (%)

Average *Z* score in population	Population prevalence of malnutrition	Programme impact (SD)				
		+ 0.10	+ 0.20	+ 0.30	+ 0.40	+ 0.50
–2.5	69	–3	–7	–11	–15	–19
–2.0	50	–4	–8	–12	–16	–19
–1.5	31	–4	–7	–10	–13	–15
–1.0	16	–2	–4	–6	–8	–9
–0.5	7	–2	–3	–3	–4	–5

C. Absolute change in deaths attributable to malnutrition (%)

Average *Z* score in population	Deaths attributable to malnutrition[a] (%)	Programme impact (SD)				
		+ 0.10	+ 0.20	+ 0.30	+ 0.40	+ 0.50
–2.5	62	–2	–4	–7	–9	–11
–2.0	51	–3	–5	–8	–11	–13
–1.5	38	–3	–6	–8	–11	–13
–1.0	24	–3	–5	–7	–9	–11
–0.5	13	–2	–4	–5	–7	–8

a. Calculated as described in the text, following the method developed by Pelletier et al. [6].

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Successful start of salt iodization in Laos

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Abstract

An assessment of the severity of iodine-deficiency disorders and of the iodized salt programme in Laos was undertaken in 1996 through a field study among 957 schoolchildren in seven schools of different regions in Laos. Urinary iodine concentrations in 225 samples were remarkably lower in schools from the north than in those from the south, a finding similar to that from a national survey in 1993. However, in 1996 the median concentrations of urinary iodine were much higher than in 1993: 85 versus 5 μg/L in the northern sites and 195 versus 22 μg/L in the southern sites. The prevalence of consumption of iodized salt, introduced only six months to one year before, had increased from 69% to 91%, levels that could explain the nearly normal and normal urinary iodine concentrations. The wide range of mild to very severe iodine-deficiency disorders found by palpation of goitre most likely reflected a delay in the regression of the thyroid gland due to the rather recent introduction of iodized salt and due to the varying severity of iodine-deficiency disorders in the different sites before the start of the programme. In one of the six salt production plants with moderate capacity in Laos, the consistency of the salt-iodization process was checked and found to be good. The iodine concentration was 15 to 45 ppm in 90% of the salt lots sampled, as compared with the criterion of 30 ppm. At consumption 82% of the salt samples were also found to be adequately iodized, with an iodine concentration of 15 ppm or more. These data reflect the initial success of the iodized salt programme in Laos, but they also emphasize the need for an evaluation of the iodine-deficiency disorder situation in different demographic and social settings, as well as for the systematic monitoring of the iodized salt programme at the production, distribution, and consumption levels, accompanied by sufficient social marketing to maintain attention on this public health problem.

Introduction

Iodine deficiency manifests as goitre and a range of other physical and mental handicaps, collectively included in the term iodine-deficiency disorders. Iodine-deficiency disorders are primarily caused by an environmental deficiency of iodine in the soil and hence in locally grown food. Control of iodine deficiency is best achieved by salt iodization, but complementary methods, such as slowly resorbable iodized oil, were sometimes used as emergency measures before the start of iodized salt programmes [1–8].

South-East Asia and the Western Pacific together account for more than 50% of the world's population at risk for iodine-deficiency disorders [1–3]. Laos, with an area of 235,000 km^2 and a population of around 4 million, has a low population density of 17/km^2. The problem is aggravated by accelerated deforestation and soil erosion. The country is landlocked and shares 4,000-km borders with China, Viet Nam, Thailand, Cambodia, and Myanmar. The country has a very low gross national product, and access to medical care and other social services is restricted for most of the largely rural population [9].

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Iodine-deficiency disorders have been recognized in Laos for a long time [10], but it was only in the early 1990s that the health authorities, together with different multilateral agencies and consultants, began to plan a countrywide control strategy [9, 11–14]. This was part of a drive by UNICEF, the World Health Organization (WHO), and the International Council for the Control of Iodine-Deficiency Disorders (ICCIDD) to virtually eliminate iodine-deficiency disorders from the world before the year 2000. In 1993 a UNICEF-supported national survey recognized widespread iodine-deficiency disorders throughout Laos. At that time six main sites of salt production from underground salt brines of moderate capacity and six other sites with low capacity were identified [14]. The equipment to convert the underground salt brines into salt by evaporation of brine in open vats was old, extremely simple, and consumed large quantities of wood. The salt produced was of unknown quality but appeared as very white, clean, small crystals.

After the national survey, equipment for salt iodization with potassium iodate was specified and provided to all six major plants through an agreement between UNICEF and various governmental ministries. Money to buy the plants and the potassium iodate came from a donation from the German government. Later, medium-sized salt-iodization machines and hand mixers were provided to most small-scale producers and are still being tested. It is difficult to accurately assess the amount of salt that is imported, but it has been estimated that the local annual production reaches over 90% of the total demand of about 25,000 tons.

Most of the iodized salt was packed in 12-kg polyethylene bags, and small quantities were packed in 1-kg and 0.5-kg bags. At the production site, the packages were well labelled with the production site and date as well as the iodination level. However, in the market and shops, the salt is transferred into simple, unlabelled 500-g plastic bags, making it impossible to trace the specifications at the consumer level. Furthermore, legislation on universal salt iodization was initiated by a decree on 20 May 1995 that gave a new impetus to the programme for the control or elimination of iodine-deficiency disorders [15].

At the end of 1996, in a few selected schools, we conducted a brief epidemiological, biochemical, and operational assessment of the severity of iodine-deficiency disorders, as well as the level of implementation of the iodized salt programme one year after its start and six months after full implementation of this national preventive programme at the major salt factories. These data were analysed taking into consideration other information gathered during the fieldwork and through discussions with many authorities. We also compared our data with those obtained in 1993 during the national survey [Gutekunst R, personal communication, 1993].

Materials and methods

Status of iodine-deficiency disorders

The areas for the survey were selected in order to document the prevalence of iodine-deficiency disorders both in the northern mountainous area, previously known as a region of severe iodine-deficiency disorders, and in the south, where only moderately low urinary iodine concentrations had been recorded earlier. They were also chosen for their relatively easy access by air or road. Seven schools were surveyed. Two of the schools were located in Luang Prabang Province in the northern mountains. Three were in Champasak Province and two in Salavan Province, both of which are situated in the southern tropical lowland plain near the Bolaven plateau.

The epidemiological and biochemical evaluation of iodine-deficiency disorders included goitre palpation and determination of urinary iodine. Goitre palpation was performed in 957 schoolchildren by the same investigator (M. Coppens). According to the new guidelines recommended by WHO/UNICEF/ICCIDD [16], the assessment of goitre prevalence was confined to 6- to 11-year-old children in equal numbers from the different schools. The total goitre rate (TGR) was defined as the sum of cases of grades 1 and 2 goitre according to the new goitre classification [16]. For this work, the prevalence of clinically visible goitre (CVG) was also assessed, including grades IB, II, and III of the former classification [17].

At least 30 urine samples were collected at random from each school (except for one school in Champasak). Iodine concentrations were measured at the laboratory of the National Iodine-Deficiency Disorders Control Programme in the Hospital of Endocrinology in Hanoi, using the method of Wawchinek as modified by Dunn et al. [18]. The median urinary iodine concentrations were calculated, and the distribution of urinary iodine concentrations was compared with international targets for the elimination of iodine-deficiency disorders as a public health problem (at least 80% of the samples should have more than 50 μg of iodine per litre of urine, and at least 50% should have more than 100 μg of iodine per litre of urine).

Iodized salt

The teachers in the schools instructed the children to bring salt from their homes for testing. In all, 318 salt samples were examined in the field with a rapid qualitative test kit for iodated salt (MBI Chemicals, Madras, India). The test results were used to calculate the amount of iodine in salt used at the consumer level. Salt was considered adequately iodized if at least 15 ppm was indicated on the colour chart.

For 132 children, the iodine content of the salt used in their homes was also determined by quantitative titra-

tion. These results were related to the urinary iodine concentrations of the corresponding children. The median urinary iodine concentrations were calculated in four groups of these children according to the iodine content of the salt consumed in their homes: non-iodized (< 5 ppm), insufficiently iodized (5–14 ppm), adequately iodized (15–45 ppm), and over-iodized (> 45 ppm).

To assess the range of the iodine content in salt at the production level, 30 paired samples were taken from a production line (lot of 4 tons) at an iodination plant near Vientiane and analysed both with the qualitative test kit and by quantitative titration.

Analysis of data

EPI-Info 6.3 and StatSoft were used for data analysis. The chi-squared test was used to test the difference of proportions. To test the difference between means, the non-parametric Mann-Whitney U test was used; for the difference between medians, the non-parametric Kruskal-Wallis test for medians was used. The results were presented as ppm (parts per million) of iodine in salt and micrograms of iodine per litre of urine.

Results

The median urinary iodine concentrations in the various regions for the baseline national survey in 1993 and for the present study are shown in table 1. The percentages of urine samples with iodine concentrations above 100 and 50 μg/L were also compared with the usual target defined for iodine deficiency elimination (table 1). In the 1993 survey, the median urinary iodine concentrations were 5, 15, and 22 μg/L in the northern, central, and southern regions, respectively. The overall median urinary iodine concentration for the whole country was 11 μg/L, with quite a small percentage of the samples (5% and 13%) above 100 and 50 μg/L, respectively. In 1996 the median urinary iodine concentrations were 85 and 195 μg/L in the northern and southern sites, respectively, which were significantly higher than those found in 1993 ($p < .001$). There were also much higher percentages of samples above 100 and 50 μg/L, reaching or almost reaching the targets for iodine deficiency elimination.

Table 2 shows the median urinary iodine concentration, the total goitre rate (TGR), and the prevalence of clinically visible goitre (CVG) in children 6 to 12 years old for all schools surveyed, together with the percentage of adequately iodized salt samples. The median urinary iodine concentrations reached near normal values in the two schools of the north and in one in the south (85, 85, and 97 μg/L). In the three other schools in the south, they were clearly adequate, being well above 100 μg/L (293, 242, and 166 μg/L). By contrast, the goitre prevalence was still rather high as compared with both the near normal and the normal urinary iodine concentrations. In one village of the northern region (Ban Kiou Mak), goitre prevalence was very high, with a TGR of 71% and a CVG of 39%. Not shown in the table are the high prevalence of large goitres among women and men and the presence of a neurological cretin in this village. For the other six schools studied, TGR ranged from 9% to 40% and CVG from 3% to 13%. The percentage of adequately iodized salt samples was above 80% in all villages except one, where it was 69%. This exception was Ban Kiou Mak, where goitre prevalence was the highest (fig. 1).

Figure 2 presents the median urinary iodine concentration for 132 children from all sites together,

TABLE 1. Median urinary iodine concentration and distribution of urinary iodine values in 1993 and 1996[a]

Year and region	No. of sites	No. of samples	Median urinary I (range within sites)—μg/L)[b]	> 100 μg I/L urine		> 50 μg I/L urine	
				% of samples[c]	No. of samples	% of samples[d]	No. of samples
1993							
Overall country	31	2,454	11	5	128	13	312
Northern Laos	14	900	5 (2–13)[e]				
Central Laos	6	375	15 (2–33)				
Southern Laos	9	675	22 (7–56)				
1996							
Northern Laos	2	83	85 (85–85)	41	34	78	65
Southern Laos	4	142	195 (97–293)	77	110	94	133

a. 1993 values were obtained by Gutekunst et al.[14], and 1996 values were obtained in the present study, one year after the start of the universal salt-iodization programme in Laos and six months after full implementation of salt iodization at the major salt factories.
b. Target ≥100 μg/L.
c. Target ≥50%.
d. Target ≥80%.
e. Two survey sites were excluded, one where the use of iodized oil had been recently begun and one where iodized salt was imported.

TABLE 2. Median urinary iodine concentration, total goitre rate (TGR), prevalence of clinically visible goitre (CVG), and coverage of iodized salt at household level by schools

Region	Province	Commune	Median urinary I (μg/L)		TGR		CVG		Coverage[a]	
			%	No.	%	No.	%	No.	%	No.
North	Luang Prabang	Ban Kiou Mak	85	31	71[b]	52	39	52	69	54
		Ban Tha Pen and Som	85	52	20	128	5	128	90	22
South	Champasak	Champasak	–	–	40	216	13	216	90	39
		Nong Phane	97	40	19	191	4	191	83	41
		Don Talat[c]	293	38	9	113	3	113	84	99
	Saravan	Saravan Town	242	34	41	79	13	79	91	30
		Ba Chieng	166	30	33	30	10	30	90	30

a. Percentage of adequately iodized salt samples (by kit).
b. A single nodule was present in 10% of the children.
c. Some iodized salt samples originated from Thailand.

grouped according to the level of iodine in the salt samples brought from their homes. The levels of iodine in salt used to define the groups are those defined in Materials and methods. A clear positive correlation (r = .98) was observed between the median urinary iodine concentrations of the four groups in order of increasing salt iodine levels: 58, 110, 172, and 270 μg/L (p < .01).

FIG. 1. Visible goitre in a postpartum woman in the village of Ban Kiou Mak

Among the salt samples collected at the schools that were defined as iodized according to the test kit, 82% reached 15 ppm iodine by titration, a level set as the lower limit at retail and consumption levels. Among these salt samples containing more than 15 ppm iodine, 71% had between 15 and 45 ppm, and 11% had high iodine contents of 45 to 105 ppm.

Not shown in the tables or figures is a slight difference in urinary iodine concentrations as a function of TGR and CVG: the mean urinary iodine concentration was 134 μg/L in children with clinically visible goitre versus 204 μg/L in those with either no goitre or only a small palpable goitre (p < .05). There were no differences in urinary iodine concentration in relation to age or sex. Thyroid size was slightly larger in girls than in

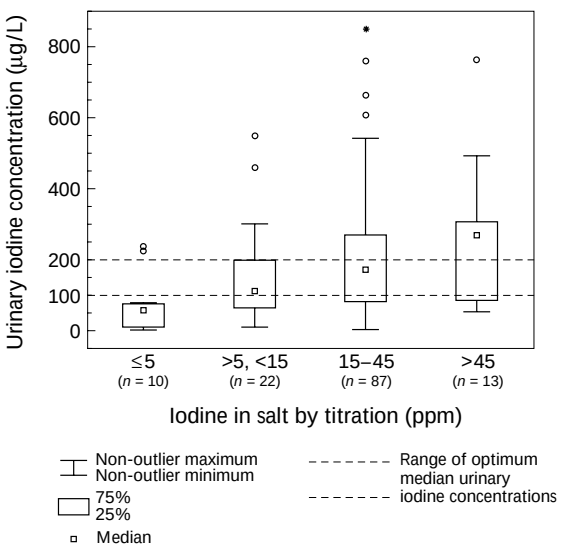

FIG. 2. Relation between median urinary iodine and salt iodine content

boys. TGR was 58% in girls versus 42% in boys (c^2 = 3.4; p = .06), as is usually seen in early puberty.

Figure 3 shows the consistency of salt iodization during the production of a single lot (4 tons) at the largest salt production site. A relatively narrow range (17–51 ppm; SD = 9) of salt iodine content was observed, with 90% of the samples between 15 and 45 ppm (30 ± 15 ppm) and the other 10% somewhat skewed to higher values between 45 and 55 ppm. The mean (31 ppm) and the median (28 ppm) iodine concentrations were close to each other and were very close to the mandatory production level (30 ppm) set by the government.

Discussion

Six months to one year after the implementation of a national programme for the control and elimination of iodine-deficiency disorders through universal salt iodization, there was a clear impact on iodine status, which was evident from the urinary iodine concentration in samples collected from schoolchildren in seven schools in the north and the south of the country. Indicators of the quality and coverage of iodized salt in the same areas, together with data gathered at one of the production sites, are in line with this conclusion.

The two indicators of iodine-deficiency disorders measured, urinary iodine and goitre prevalence, do not give entirely similar interpretations. Among the seven schools surveyed, the iodine intake, according to urinary iodine concentrations, was adequate in four and revealed the persistence of relatively mild iodine deficiency in three, whereas much lower median urinary iodine concentrations were seen in similar areas during the 1993 survey. The percentage of urine samples with iodine concentrations above 100 μg/L was satisfactory in the southern schools (77%), whereas the target of 50% was not reached in the urine samples from the north (41%). However, both percentages were much higher than the 5% estimated in the national study of 1993 [14].

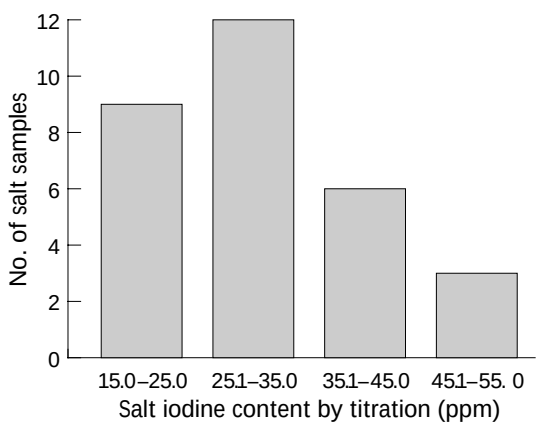

FIG. 3. Frequency distribution of the iodine concentrations of 30 salt samples taken at regular time periods from the production line at the main iodized salt plant of Laos, Khoksa Ath

According to goitre palpation, the prevalence of iodine-deficiency disorders ranged from moderate to severe in the northern sites (TGR 20% and 71%) and from mild to moderately severe in the southern sites (TGR 9% to 41%). The discrepancy in the prevalence of iodine-deficiency disorders according to urinary iodine determinations and according to the prevalence of goitre can be explained by several hypotheses, such as the failure of the goitre to regress in size because the iodized salt was introduced recently [14, 17]; the possibility that in some areas residual salt, which was not iodized, was still being used; the variation in the severity of goitre in different parts of the country before the start of the programme; the presence of goitrogenic factors; and the intraobserver variation of the estimation of goitre size by palpation. Thyroid volume measurements by ultrasonography, now considered as the gold standard, were not available in our survey [17, 19, 20].

In one remote village of the mountainous northern region, we observed a mild iodine deficiency, on the basis of urinary results, together with a frequency of goitre in schoolchildren that was still high, as well as large goitres in both women and men and the presence of one neurological cretin. The relatively high prevalence of single nodules in these schoolchildren (10%) is probably explained by the previous severity of the deficiency [17]. It is, however, unlikely that women in that particular village would still give birth to infants affected by intrauterine iodine deficiency, because of the mild iodine deficiency revealed in the urine of their children and the relatively good coverage of adequately iodized salt, which was observed to be at least 69% in this village.

Most of the data on salt confirm the adequacy of the iodization process and the good coverage of the programme in the sites surveyed [15]. The apparently good quality of the salt, the new salt-iodization plants, and the relatively short time span of salt storage between production and consumption are all factors that may explain in part the excellent start of this programme.

The association between the median urinary iodine concentrations in children and the iodine contents of their household salt suggests the important role of iodized salt as the main source of their iodine intake. The median urinary iodine concentrations of 172 and 270 μg/L noted in the group of children who consumed iodized salt, as defined, indicated that the 15 ppm used is a satisfactory criterion for the lower limit of iodine in salt at the consumption level.

Although consultations and planning of this control programme started three to five years before its active

implementation, it was facilitated by targeted advocacy with the salt producers and awareness raising among health officials and other authorities by WHO/UNICEF/ICCIDD, together with the provision of salt-iodization equipment appropriate for the salt production sites. Two additional elements of the successful launch of this programme have been the passing of a National Decree on compulsory salt iodization and monitoring undertaken by the Food and Drug Laboratory.

However, some programmes that are successful at the start later fail because of different factors [1, 5, 8, 21, 22]. To make these programmes sustainable, it is essential to strengthen the monitoring process; spot sampling of salt with the recommended LQAS (Lots Quality Assurance Sampling) [7, 22, 23] makes it possible to identify the potential failures, bottlenecks, or constraints that limit the effectiveness of a control programme. The advocacy and social mobilization efforts that started this successful action in Laos do not mean that feedback mechanisms and further social marketing will not be needed to make the programme sustainable in the future. Monitoring the iodized salt programme in the schools by testing the salt from the children's homes in front of the children and the teachers is in itself an important advocacy tool, well targeted to the communities at risk. It creates a form of feedback and guides the level of necessary systematic and ongoing monitoring [6, 8, 19, 20, 22].

Connections between the capital, Vientiane, and the provinces are still very limited, and access to social services is a major constraint for the socio-economic development of the country [9]. It is possible that the iodized salt programme may not reach some of the worst-affected areas, such as the northern mountainous regions. More information is needed from these remote, vulnerable populations. Moreover, in a country with such long borders as Laos, salt produced in neighbouring countries will easily penetrate into the country [11, 14]. It is important to monitor intercountry salt flows, which are likely with the increasing exchange and development manifested today in the whole Indochinese peninsula. However, the existence of effective salt-iodization programmes in neighbouring countries, such as Thailand, Viet Nam, and China, may alleviate this concern.

The equipment provided and installed for salt iodization that meets the quantitative and qualitative requirements of the country makes the local production of iodized salt in the six main salt plants cost-effective. It is hoped that the drum iodization machines will enable the remaining 10% of the salt produced in remote small sites to be iodized in a proper and effective way. The increased cost of iodized salt compared with non-iodized salt is small and probably negligible in comparison to family budgets. Consumer education could make this extra cost more acceptable [21].

This study demonstrated that within six months to one year of introduction, salt iodization effectively improved the iodine status of the population. This is especially encouraging, given that Laos is one of the 10 poorest countries in the world, and iodine-deficiency disorders have been recognized as a serious public health problem in the country.

Acknowledgements

The survey benefited from the excellent cooperation of the staff of the Ministries of Vientiane Municipality and Luang Prabang, Champasak, and Salavan Provinces. The programming was assisted by Dr. Y. Renault and Mrs. Konseng of the WHO office in Vientiane. The data were collected with the logistical support of Dr. A. Malayvin and Dr. J. Knowles, UNICEF-Vientiane; Dr. P. Kloppenstein, MSF-Belgium Champasak District Hospital; Dr. Sab, Pakse MSF-Office; and Dr. B. Graz, EMI-Office Salavan. We gratefully acknowledge all the local health services and school staff for facilitating the fieldwork. This work was financially supported by the Belgian Cooperation Service through a cofinanced project with the Public Health School of Brussels, CEMUBAC, ULB University. We thank the Ministry of Public Health, PDR Lao, for permission to publish this work.

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Field tests for iodate in salt

L. L. Diosady, J. O. Alberti, S. FitzGerald, and M. G. Venkatesh Mannar

Abstract

Commercially available field test kits for the determination of iodate in salt were tested to evaluate their performance and to devise improvements in their range and accuracy. Dropper tests based on the blue colour of the starch-iodine complex formed with iodine released by excess potassium iodide gave rapid qualitative indication of the presence of iodate. Because of the dark colour of the complex, the tests were found to be quantitative over only a limited range, typically 0 to 20 μg of iodine per gram of salt. We have developed methods for improving the reliability and colour reproducibility of these types of kits. Instructions for producing reliable kits of this type with improved colour reproducibility are presented. Improved qualitative tests in the range of 0 to 50 μg/g were obtained with a simple liquid-phase colourimetric system, based on the same chemistry. A field test kit was developed and tested in laboratories in India and Zimbabwe, as well as in our laboratory in Toronto. The test gave reproducible results, comparable to those of titration, with a typical standard deviation of ± 5 μg/g. The range of measurements can be extended to 100 μg/g by dilution. Instructions for preparing these kits are presented.

Introduction

Because the diet of more than half of the world's population contains less iodine than is required for developing and maintaining a healthy body, the iodine content of their diet must be supplemented with iodine to prevent iodine-deficiency disorders. Although the salt supplies of most industrialized nations have been iodized with potassium iodide for many years, during the past 20 years there has been a strong effort, led by the United Nations, to iodize all salt for human consumption [1]. It is hoped that by the turn of the century, severe forms of iodine-deficiency disorders will be eradicated through national salt-iodization programmes.

The dosage of iodine is important, because insufficiently high levels in the diet will have no noticeable benefit, whereas excessive doses are wasteful and may actually result in detrimental health effects. In the low-technology setting of most developing countries, it is important that simple, effective tests be available for the determination of fortifying agents. In most developing countries, iodine is added to salt in the form of potassium iodate rather than iodide, because iodate is not oxidized to form volatile free iodine.

PATH Canada contracted with our group to test approaches to rapid field testing of iodate in salt. Despite the superior resistance of iodate to oxidation, iodated salt tends to lose its iodine content over time, and therefore it is important to know the actual amount of iodine present [2, 3]. Accordingly, these methods were designed for use in the field, where they could extend the capability of national iodine-deficiency disorders programmes to verify the potency of iodized salt, both as means of quality control at the manufacturing level and for field monitoring of delivered levels.

Field test requirements

The development of an effective field test depends on a clear understanding of the field conditions and the requirements of the user in terms of the precision and accuracy of the test results. The tests for iodate in salt will be carried out by personnel with limited training, under conditions where laboratory services, utilities, or even simple supplies will not be readily available. As a result, all of the equipment and supplies for the test must be self-contained in a kit that does not require other utilities, such as electric power. Although

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Mention of the names of firms and commercial products does not imply endorsement by the United Nations University.

it would be desirable to require no further component, we realized that better tests could be devised if we accepted the need for water. The field conditions require that the test kit be light, inexpensive, and easy to use. The test must give acceptable results rapidly, and the results must be readily interpreted.

The first step in developing a field test was the survey of the quantitative analytical systems that may be applied to the specific field conditions, sample matrix, and expected concentration range. This was followed by the laboratory testing of selected chemical systems. The laboratory identification of a feasible system must be followed up by the development of a test kit that meets the needs of the client group.

Iodate analysis

There are several standard analytical techniques for the quantitative determination of iodine present in the form of iodate. Iodate analysis is usually based on the reduction of iodate to elemental iodine, which is then determined by titrimetry or colourimetry. The acidulated iodate solution can be reduced by KI, KSCN, a cold aqueous solution of SO_2, $K_4Fe(CN)_6$, Cu_2Cl_2, H_3AsO_3, or $FeSO_4$ to generate elemental iodine. The iodine may be determined colourimetrically directly or by formation of a coloured complex with starch or another reagent [4].

Reduction of iodate to iodine by potassium iodide

The procedure is the reciprocal of the one for the determination of iodide by means of an iodate, also based on the reaction:

$$KIO_3 + 5\ KI + 6\ HCl \rightarrow 6\ KCl + 3\ H_2O + 3\ I_2$$

The reaction liberates iodine quantitatively, and the iodine-starch complex can form the basis of a quantitative or semiquantitative colourimetric correlation suitable for a field test [5, 6].

Visualization of iodine

Starch indicator forms an intense blue-black colour with iodine. In aqueous solution, iodine has the tendency to react with excess iodide anions to form the triiodide ion I_3^- according to the equation:

$$I_2 + I^- \rightarrow I_3^-$$

The triiodide ion can then react with another iodine molecule to form the linear pentaiodide anion:

$$I_3^- + I_2 \rightarrow I_5^-$$

This pentaiodide ion fits inside the helical β-amylose chain of starch. In the presence of excess iodide, iodine in concentrations as low as 0.2 μg/ml will result in the formation of the dark-blue starch-iodine complex. The intensity of the colour varies with the amount of free iodine and can form the basis of a quantitative colourimetric measurement over a narrow range of concentrations. The formation of this complex is reversible, and this is the basis for titrimetric methods. At the end of the titration that removes the iodine, the strong blue complex fades completely to colourless.

Currently available field tests

As the need for rapid field testing became known, a number of test kits were developed and distributed, either commercially or as a part of the national iodine-deficiency disorders strategies of several developing countries. All of the kits that we could obtain through cooperating offices of UNICEF and the International Council for the Control of Iodine-Deficiency Disorders ICCIDD (table 1) work by adding a few drops of a reagent onto the surface of the salt sample and visually comparing the colour developed with a calibration chart. The reagent typically consists of an acidic buffer, a reducing agent, which reduces the iodine in the KIO_3 to I_2, and starch, which forms a deep blue-black complex with the released iodine.

These kits are quite sensitive and readily produce a blue stain on the salt surface, indicating the presence of iodine. It was our understanding, through discussion with experts in the field, that the quantitative measurements made by currently available kits are inaccurate and irreproducible. We tested a large number of kits from several countries in an effort to evaluate their performance and to devise improvements to their range and accuracy.

More sophisticated and accurate test kits are available commercially [e.g., ref. 7]. However, these tests require either a high initial investment in a portable instrument or expensive supplies that make these tests unsuitable for use in developing countries.

Methods

Materials

Food-grade, un-iodized salt was obtained from Toronto Salt Chemical Co., Toronto. All other reagents were analytical grade, obtained from BDH Chemicals, Toronto.

Testing of commercial kits

Iodated salt samples with various levels of iodine were

TABLE 1. Commercially available kits tested

Source	No. tested	Type	Comments
Bangladesh	1	Starch/iodide	Good qualitative kit, may be used quantitatively at 1–15 ppm
China	1	Organic dye	Possibly old kit, saturates above 15 ppm
MBI-India	5	Starch/iodide	Dated July 1994. Nominally 0–100 ppm, saturates at 30 ppm
MBI-India	33	Starch/iodide	Undated. Nominally 0–100 ppm, saturates at 30 ppm
MBI-India	32	Starch/iodide	Dated October 1996. Nominally 0–100 ppm, saturates at 30 ppm
MBI-India	3	Starch/iodide	Undated (new). Nominally 0–100 ppm, saturates at 30 ppm
Indonesia	1	Starch/iodide	Qualitative only: 0 or 30 ppm
Nepal	3	Starch/iodide	Undated. Nominally 0–100 ppm, saturates at 20 ppm
Thailand	1	Starch/iodide	Unable to distinguish between any concentrations. Blank is a lighter blue, not clearly distinguished from iodated samples
Total	80		

prepared by weighing 1 to 3 kg of salt into a laboratory ribbon blender (LeRoy-Somer, Montreal, PQ) and slowly adding the calculated amount of potassium iodate as a 30 g/L aqueous solution, while blending. After the addition of iodate, the mixture was further blended until dry, to obtain a homogeneous, free-flowing solid. These blends were analysed by titration method AOAC 33.147 [6] and neutron activation analysis, using the SLOWPOKE nuclear reactor facility of the University of Toronto [8].

Samples of the analysed salt were spread out in a 5- to 8-mm-thick layer in a petri dish, and their iodine content was measured with the commercially available rapid field test kits, by dropping 2 to 10 drops onto the salt surface, producing wet spots of about 10 mm in diameter. The colour of the spots was compared with those in the colour chart provided with the kit to give the iodine content.

Testing of improved kits

Inter-laboratory tests were conducted on the test kit developed in our laboratory. Test kits were prepared in Toronto and sent to the participating laboratories, together with a series of pre-analysed salt samples and instructions for making up a fresh kit. The salt samples were analysed in Toronto using both the kits and the standard laboratory analytical technique.

The participating laboratories analysed the salt samples with their standard laboratory technique, the kit sent from Toronto, and a fresh kit made *in situ*. They also made up a series of iodated salt samples using local, unpurified salt, and analysed them as described above. Some of the salt was also sent to Toronto, where it was also analysed using both the improved kits and the standard laboratory techniques.

Discussion of results

Commercial test kits

The commercial kits tested are listed in table 1. All but one of these kits used the reaction with potassium iodide and starch for colour development. Initially we believed that the reported reproducibility problem might be due to the variability in the starch supply. If the amylose/amylopectin ratio changed, the colour of the iodine-starch complex would be shifted from the reddish colour of amylopectin to the blue of β-amylose. The problem has turned out to be more substantial.

Actually, at the low pH of these kits (typically pH 2.6), salt with more than 15 or 20 ppm iodine will produce a dark-blue colour, with no further visibly noticeable darkening due to higher concentrations. Thus, concentrations of 15 ppm and higher all give the same dark colour. The deep colour is due to the fact that one molecule of iodate reacts with five molecules of potassium iodide, liberating three molecules of elemental iodine. Thus, the elemental iodine is present at six times the concentration originally in the sample.

One way to overcome this problem is to dilute the salt 1:10 using a similarly coloured material. Iodine-free salt, sand, or flour would be suitable. Unfortunately, solid dilution is difficult to perform evenly and makes the kit bulky, since 10 to 20 g of inert material must be carried for each test.

The kit we were sent from China used a reaction of iodate with an organic dye for colour development. Nevertheless, this kit also saturated at levels as low as 10 μg iodine/g salt. Since we only received one small, old kit, this result may not be representative.

An additional problem was the colour charts supplied with these kits. Rather than colour samples representative

of the granular, irregular surface of actual samples, the charts show clear, homogeneously coloured discs, which are not easily related to the actual appearance of the salt.

We followed three approaches to producing an improved field test system: we tried to improve the currently used iodide-based dropper test, developed tests based on liquid-phase colour development, and developed a crude microtitration method.

Improvement to existing field tests

As indicated above, the kits now manufactured for determining iodate in salt in the 50 μg/g concentration range are based on the development of the starch-iodine complex and visual comparison of the colour obtained with printed standards. These tests worked well qualitatively, but they produced inconsistent colours and the depth of colour saturated at a low level, so that values above 20 μg/g were not readily distinguished.

On the basis of our laboratory experience and theoretical considerations, it is possible that the colour variations are also influenced by the composition of the starch indicator. The deep-blue colour of starch solutions containing iodine is believed to arise from the absorption of iodine into the helical chain of β-amylose, a macromolecular component of most starches. The closely related α-amylose forms a red adduct with iodine, whereas amylopectin produces a blue colour with a different absorption maximum. Thus iodine complexes with α-amylose, β-amylose, and amylopectin all have different absorption spectra and different colour intensities. As a result, it is critical that the type of starch used be well characterized, and the colour intensities be calibrated for it. In our work we used soluble potato starch.

Accordingly, to reduce this problem, we suggest that the starch be well characterized and that only a β-amylose such as soluble potato starch be used. This ensures that the starch-iodine complex formed will have a single absorption maximum and thus a well-defined, reproducible colour. Although this will improve the kits, the problem due to the kinetics of this reaction and its pH dependence remains a major limitation to these simple kits.

Strong oxidizing agents interfere with the development of colour by oxidizing the added iodide to elemental iodine.

Field test apparatus

The field test apparatus consists of the following:

» metal pan
» two 100-ml plastic flasks containing the reaction solutions A and B
» two disposable polyethylene transfer pipettes, capable of delivering 1 ml of solution
» 10-ml plastic sampling vial
» 50-ml plastic sampling vial
» 0–50 ppm iodated salt colour standard chart (photograph), prepared by photographing a set of six standard salt tests and making colour-corrected photographs
» sampling cap (1 ml)
» sampling cap (9 ml)

Reagents

The reagents are prepared as follows:

1. *Solution A.* Starch solution with KI-soluble potato starch (ACS grade, 3 g) is triturated with 10 ml of cold water and poured slowly, with constant stirring, into 100 ml of boiling water. To prevent spoilage, 400 mg of sodium benzoate is added to the solution. The mixture is boiled for about 2 min to obtain a non-viscous translucent fluid. Extensive boiling reduces the sensitivity of the test. The solution is allowed to cool, and the settled solids are removed by decanting and filtering the supernatant using Whatman No. 41 filter paper or the equivalent. Five grams of KI are added to the filtrate, and its pH is adjusted to 7.5 with 0.1% Na_2CO_3.
2. *Solution B.* To 100 ml of distilled water, add 1% HCl to pH 1.2. Transfer the solution to a 100-ml plastic flask or vial.

Field procedure

1. To the 50-ml plastic sampling vial, add one small sampling cup (1 ml) of iodated salt and one large cup (9 ml) of non-iodized salt. Add 2 drops of water and mix the salts by shaking. Transfer to the metal pan. (Salt must be granular, i.e., ground if necessary.)
2. Fill the metal pan with salt, creating a flat surface flush with its edge.
3. Add 1 ml of solution A and 1 ml of solution B to the 10-ml vial and mix.
4. Apply several drops of the mixed reagent solution to the surface of the salt.
5. Compare the developed starch-iodide blue colour with the standard colour chart

Proposed novel iodate field test for salt

On the basis of the observed limitations of the solid-phase test, including several variants with other reducing agents, it became clear that for better reproducibility and accuracy, we must develop a more quantitative test that would also be suitable for in-plant quality control. Although this introduces increased complexity, we felt that a valid field test might be developed.

Working in a liquid medium reduces the problems due to uneven distribution of reagents. We tested the concept of dissolving a set volume of salt in a set volume of water in a flat-bottomed plastic or glass vessel, adding acid, potassium iodide, and starch, and observing the colour thus developed. It is relatively simple to

control volumes, and therefore this test is reproducible. The exact amount of iodine released can be estimated from the colour of the solution.

Field test apparatus

The field test apparatus consists of the following:
» 250-ml tall form plastic vial (glass optional)
» two 100-ml plastic flasks containing the reaction solutions A and B
» two disposable transfer pipettes, polyethylene, capable of delivering 3 ml of solution
» 0–50 ppm iodated salt colour standard chart (photograph), prepared by photographing a set of six standard solutions and making colour-corrected photographs.
» sampling cap (approximately 1 ml)

Reagents

The reagents are prepared as follows:
1. *Solution A.* Starch solution with KI-soluble potato starch (ACS grade, 3 g) is triturated with 10 ml of cold water and poured slowly, with constant stirring, into 100 ml of boiling water. To prevent spoilage, 400 mg of sodium benzoate is added to the solution. The mixture is boiled for about 2 min to obtain a non-viscous translucent fluid. Extensive boiling reduces the sensitivity of the test. The solution is allowed to cool, and the settled solids are removed by decanting and filtering the supernatant using Whatman No. 41 filter paper or the equivalent. Five grams of KI are added to the filtrate, and its pH is adjusted to 7.5 with 0.1% Na_2CO_3.
2. *Solution B.* The pH of 100 ml of distilled water is adjusted to 1.8 with 1% HCl.
3. Solution B is then mixed 1:1 with solution A.

Field procedure

1. One scoop (~1.8 g) of salt is added to the 250-ml tall form plastic (or glass) vial. (Salt must be granular, i.e., ground if necessary.)
2. With the disposable transfer pipettes, 3 ml of solution A and 3 ml of solution B are added.
3. The solution is thoroughly mixed with gentle swirling until a dark-blue colour develops (about 20 seconds).
4. If no colour develops, more solution B is added, to a maximum of 12 ml.
5. Local water, preferably de-aerated by boiling, is then added to the 250-ml mark and the solution is mixed.
6. After the air bubbles entrained by the mixing have cleared, the iodide concentration of the salt is determined by matching the colour of the solution with the colour standards.

Results

The sequence of steps was critical, since the pH at all stages of the process can influence the development of colour. At very low pH values, the starch blue developed a pink tinge, whereas at high pH values, the development of colour was very slow. Therefore, adjustment of the pH of solution A under laboratory conditions must be done carefully.

The effect of water quality available in the field could be compensated for by performing the test without adding a salt sample and measuring the background colour developed. A positive reading could be due to dissolved oxygen in the water. This effect may be reduced by using previously boiled water or may be corrected mathematically by subtracting the blank value from the measurement.

Because the salt is measured volumetrically, a large error in the weight of the salt sampled could be introduced if the salt was not finely granular or if it was very wet.

We found that some highly alkaline salt samples did not develop the blue colour, resulting in an underestimation of the iodine content. This was readily remedied by adding extra acid. In the test kit instructions, we allowed for this by suggesting that extra solution B be added prior to dilution. It could also be remedied by the use of a "recheck solution," but this may lead to a dilution error.

The performance of the kit was checked against the standard laboratory analysis on salt samples iodated to various levels. The results are presented in table 2. The test kits and the laboratory analyses corresponded very well, and the reproducibility of the kit readings was good in the laboratory.

Test validation

Field test kits for the liquid phase test were supplied to two participating laboratories: Dr. Nhari and T. Nyamandi, Government Analyst's Laboratory, Zimbabwe, and Dr. C.S. Pandav and Dr. M. Karmarkar, Regional Coordinator, ICCIDD, New Delhi, India. In all cases, six replicate analyses were performed, and the average and standard deviation were calculated. The results for coded salt samples from the University of Toronto laboratory are summarized in table 3.

The results indicate that the tests worked well within the target range of 0 to 50 μg of iodine per gram of salt. The developed colours were compared with a set of six colour standards set at intervals of 10 μg/g, and the observers interpolated between these intervals. Indeed, reproducible results within 5 μg/g were obtained when six measurements were averaged.

The results from India had a higher standard deviation than those from Zimbabwe. The titration results from India were not corrected for a blank reading for the un-iodated salt, probably because of an impurity in the Indian salt. Thus, the titration results were consistently high. The kits actually gave a more accurate result than their titration method without blank correction.

TABLE 2. Comparison of kit and titration results for salts prepared in the University of Toronto laboratory[a]

Kit			Titration		
Average iodine (μg/g)	SD	CV	Average iodine (μg/g)	SD	CV
0.0	0.0	0.0	0.0	0.0	0.0
5.2	1.0	19.0	5.4	1.0	9.7
10.0	0.0	0.0	10.3	0.1	0.0
13.7	2.2	15.8	12.1	1.0	6.0
15.8	1.2	7.4	15.7	1.3	5.6
19.8	0.4	2.1	20.2	0.3	1.6
28.3	1.5	5.3	28.1	1.1	3.1
29.2	2.0	7.0	30.9	1.5	4.2
34.8	0.4	1.2	35.4	0.4	1.2
38.0	1.9	5.0	37.1	2.0	4.1
46.7	2.6	5.5	46.6	0.5	1.1

a. All results are averages of six measurements.

The kit results obtained in Toronto were closer to the laboratory measurements than those from the other two laboratories. There were two reasons for this discrepancy: the chart colours and sample degradation. Our laboratory could compare the observed colours with the colours of actual standards, not just with photographs of these standard solutions. We found that the quality of the colour charts was critical for the accurate determination of iodine content.

An error was introduced in the determination of iodine in salts prepared by another laboratory by the degradation of the sample during shipping. We found that iodated salts, especially unrefined local salts, lost iodine with time, and therefore the measured iodine content might have been actually different at the time of reading in the three participating laboratories. Despite these problems, the reproducibility of the results was acceptable.

There is no doubt that the liquid-phase test is more cumbersome, and in some applications the extra quantitative information may not be worth the increased cost and complexity. However, the proposed kits provide an inexpensive and simple method of obtaining valid quantitative measures of iodine content useful for testing compliance to regulations and recommendations, safety, and iodine stability in the field.

Conclusions and recommendations

The existing field tests for iodate are small and simple to use, and they readily detect iodate in salt at levels above 5 μg/g. These tests were found to be quantitative only at low levels of iodine, i.e., 5 to 20 μg/g. The range of the tests may be extended by diluting the salt sample with another iodine-free solid of similar colour and texture, such as salt, sand, or flour, but this makes the kits more bulky. The reproducibility of the kit results may be improved by standardizing the starch indicator used and by controlling the pH of the system with an appropriate buffer system.

The liquid-phase colour development gave reproducible results in the range of 0 to 50 μg of iodine per gram of salt. This range can be readily extended to 100 μg/g by

TABLE 3. Comparison of laboratory and field kit measurements of iodine (micrograms of iodine per gram of salt, mean ± SD, from six replicate measurements) from the University of Toronto (Canada), Zimbabwe, and India

University of Toronto		Zimbabwe			India		
Toronto kit	Titration	Titration	Toronto kit	Zimbabwe kit	Titration	Toronto kit	India kit
10.0 ± 0.0	10.3 ± 0.1	10.4 ± 0.9	10.0 ± 0.0	9.2 ± 2.0	13.6 ± 1.0	13.3 ± 2.6	15.0 ± 0.0
19.8 ± 0.4	20.2 ± 0.4	19.8 ± 0.5	20 ± 0	19.2 ± 2.0	27.4 ± 1.2	19.7 ± 2.0	23.3 ± 2.6
28.3 ± 1.5	28.1 ± 0.5	28.9 ± 2.1	30.8 ± 2.0	27.5 ± 4.5	36.6 ± 1.1	24.2 ± 2.0	32.5 ± 2.7
34.8 ± 0.4	35.35 ± 0.4	39.9 ± 3.6	40 ± 0.0	38.3 ± 2.6	51.3 ± 2.0	30.8 ± 2.0	37.5 ± 2.7
46.7 ± 2.6	46.6 ± 0.5	46.0 ± 0.8	50 ± 0.0	42.5 ± 2.7	60.6 ± 1.6	36.7 ± 2.6	43.3 ± 2.6

dilution. The test results are based on a volumetric measure of solid salt. Because large errors can be introduced by salts of large particle size that do not pack well into the sampling thimble, this type of salt should be ground.

The liquid-phase system adds significant complexity to the field test kit for iodate. However, the improved range, accuracy, and precision of these tests justify its use in field situations, where a qualitative determination is inadequate and a laboratory with trained personnel is unavailable.

A more extensive round of inter-laboratory and field tests would be useful before making the kit commercially available.

Acknowledgements

Samples of salt and local test kits were made available for this study by UNICEF field offices in six countries. The authors are grateful for the testing of the kits and for excellent comments and suggestions by Dr. Nhari and T. Nyamandi, Government Analyst's Laboratory, Zimbabwe, and by Dr. C.S. Pandav and Dr. M. Karmarkar, Regional Coordinator, ICCIDD, New Delhi, India.

This work was initiated by Dr. Timothy R. Stone, Executive Director of PATH Canada, who was tragically killed in the crash of the hijacked Ethiopian airplane in the Comoro Islands in November 1996 while on a humanitarian mission.

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Field tests for iodide in salt

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Abstract

Rapid field tests for the determination of iodide in salt were developed, based on the oxidation of iodide to iodine and reaction of the released iodine with starch to produce a coloured complex that could be visually related to the iodide content of the salt. Potassium iodate and ferric chloride were used as oxidizing agents in the two versions of the test that were examined in detail. The reaction with ferric chloride gave a test with a wider useful concentration range. It had the added advantage of not producing false readings for iodine by reacting with reducing agents that were present in the salt as natural impurities or as a result of fraud. A simple test kit suitable for field use was developed and tested with iodized salts produced using salts from Canada and Ecuador. The kit results were compared with results obtained by neutron activation analysis using the SLOWPOKE nuclear reactor facility of the University of Toronto and by standard iodometric titration. The test kits readily analysed for iodine levels between 0 and 50 ppm, with an accuracy of ± 10 ppm and a reproducibility of ± 5 ppm. Inexpensive rapid tests using these kits may be useful in monitoring iodine levels in areas where salt iodization is based on potassium iodide, because of the presence of impurities in the salt supply that reduce the stability of the usual iodizing agent, potassium iodate.

Introduction

At least half of the world's population has insufficient iodine naturally present in their diet. The lack of iodine leads to iodine-deficiency disorders that range from mild thyroid enlargement to major developmental problems, such as cretinism. For many years, the food supply of developed countries has been supplemented with iodine by adding potassium iodide to salt at levels between 30 and 100 mg of iodine per kilogram of salt. With the leadership of the United Nations, salt-iodization programmes have been implemented in most of the countries of the world during the past decade, and it is hoped that all of the salt sold for food use will be fortified with iodine by the turn of the century, leading to the elimination of iodine-deficiency disorders [1].

The dosage of iodine is important, since insufficient levels in the diet will have no noticeable benefit, whereas excessive doses are wasteful and may actually result in detrimental health effects. In the low-technology setting of most developing countries, it is often difficult to produce salt with well-defined iodine content because of the difficulty of evenly mixing in the low levels of iodine required and the instability of iodine during distribution and sale [2, 3]. The problem is compounded by the fact that not all of the salt supply is centrally controlled, and thus a significant fraction of salt at the retail level may not be iodized. It is therefore important that simple, effective tests be available for the determination of iodine in salt.

Potassium iodate is most often used for salt iodization in developing countries because of its resistance to oxidation under tropical conditions. Many simple test kits have therefore been developed for the qualitative determination of iodate in salt. Iodide is used in developed countries and also in several countries where the impurities naturally present in the salt would reduce the stability of potassium iodate (e.g., Ecuador and some plants in China). There are very few kits available for iodide. Although we understand that MBI of India produces an iodide kit, we have been unable to obtain a kit for testing.

PATH Canada contracted with our group to identify feasible approaches to rapid field testing of iodide in salt. These methods were designed for use in the

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field, where they could be used to extend the capability of national iodine-deficiency disorders programmes to verify the potency of iodized salt, both as means of quality control at the manufacturing level and for field monitoring of iodine levels in salt at delivery.

Field test requirements

The development of an effective field test depends on a clear understanding of the field conditions and the requirements of the user in terms of the precision and accuracy of the test results. The field tests for the measurement of iodine will be carried out by personnel with limited training, under conditions where laboratory services, utilities, or even simple supplies will not be readily available.

The field condition requires that the test kit be light, inexpensive, and easy to use. It must be self-contained, without a need for other utilities, such as electric power. The test must give acceptable results rapidly, and the results must be readily interpreted.

The first step in developing a field test is a survey of the quantitative analytical systems that may be applied to the specific field conditions, sample matrix, and expected concentration range. This is followed by laboratory testing of selected chemical systems. The laboratory identification of a feasible system must be followed up by the development of a test kit that meets the needs of the client group.

Our mandate was to develop a test system that would allow the field identification of the iodide levels in salt in the range of 10 to 50 mg of iodine per kilogram of salt, with a sensitivity of about 10 mg/kg.

Techniques of iodide analysis

Many analytical techniques are used for the determination of iodide in a variety of matrices. These include colourimetric methods, amperometric titration, measurements with ion-selective electrodes, ion chromatography, and high-performance liquid chromatography [4–8]. Of these only colourimetric techniques can be inexpensively adapted to field testing.

Five colourimetric methods were reviewed, selected on the basis of their widespread acceptance as official analytical methods.

In the leucocrystal violet method, iodide is selectively oxidized to iodine with potassium peroxymonosulphate. The elemental iodine reacts with the colourless leucocrystal violet indicator, producing the highly coloured leucocrystal violet dye [9, 10]. Unfortunately, chloride interferes with colour development, and therefore this method is not suitable for use in a salt matrix.

Iodide can be determined on the basis of its ability to catalyse the reduction of ceric ions by arsenous acid. The reaction is stopped after a specific time interval by the addition of ferrous ammonium sulphate. The resulting ferric ions, which are directly proportional to the remaining ceric ions, develop a colour complex with KSCN [9, 11, 12]. Unfortunately, the method is slow and time dependent, and thus it requires instrumentation. It is therefore unsuitable for use in an inexpensive field test.

Iodide can be oxidized to iodine by the addition of bromine water. The excess of bromine is distilled (expelled) out of solution. The iodine is then titrated with sodium thiosulphate, with starch used as the end-point indicator [13]. The need for distillation of free bromine and the reactivity of bromine water make the method too dangerous to be used as a field test.

Iodide can also be oxidized to elemental iodine in the reaction with iodate in the presence of an acid such as hydrochloric acid. The free iodine can then be titrated with sodium thiosulphate solution, with starch used as the end-point indicator [14]. This method seemed readily applicable to field testing.

Iodide can be oxidized to iodine in the reaction with ferric ions, e.g., in the form of ferric ammonium sulphate:

$$2\,NH_4Fe(SO_4)_2 + 2\,KI \rightarrow I_2 + (NH_4)_2Fe(SO_4)_2 + FeSO_4 + K_2SO_4$$

The free iodine is titrated with sodium thiosulphate, with starch used as the end-point indicator, as above [14]. This method also seemed applicable to field testing.

Iodine can be liberated from iodides by other oxidizing agents, such as As^{5+}, Sb^{5+}, Bi^{5+}, Cu^{2+}, Cr^{6+}, $K_3Fe(CN)_6$ (potassium ferricyanide), HNO_3, Cl_2, H_2O_2, or ozone. In each of these approaches, the resulting free iodine is titrated and the end point is detected using starch as indicator. The concentration of iodine may be also detected colourimetrically (over a narrow range of concentrations) on the basis of the colour density of the starch-iodine complex. Unfortunately, most of these redox systems are very sensitive to both impurities and the sample matrix.

After preliminary screening, we selected two approaches to the field measurement of iodide based on potassium iodate and ferric chloride as the oxidizing agents.

Field test for iodide based on potassium iodate

Iodide (I^-) as potassium iodide in iodized salt is converted to elemental iodine by potassium iodate in an acidic medium:

$$5\,KI + KIO_3 + 6\,H^+ \rightarrow 6\,K^+ + 3\,H_2O + 3\,I_2$$

The free iodine reacts with starch to form a deep-blue complex. The depth and intensity of the blue colour of the system are directly related to the iodine concen-

tration, and over a narrow range of concentrations, the colour differences are readily differentiated by eye.

The development of colour is sensitive to pH. The optimum pH in terms of colour development and colour differentiation was in the range of pH 1.8 to 3.4. We then devised an appropriate buffer system, keeping in mind the need to stabilize the starch against microbial and chemical degradation.

Initially, an antimicrobial and pH-adjustment system based on salicylic acid was tested. This gave a reddish colour with ferric iron, introduced either as an impurity or as an ingredient in double fortification. The interference of the Fe^{3+} made this test kit vulnerable, and thus we opted for another organic acid buffer system, sodium benzoate/benzoic acid, after testing several potential alternatives.

In the presence of $CaCO_3$, sodium silico aluminate, or other alkaline substances, the reaction mixture may require a small amount of dilute hydrochloric or sulphuric acid (~1%) solution in the reaction mixture to neutralize these alkaline substances. The buffer system in the kit should take care of this problem, unless the quantity of base is very high. If this consistently happens with salts from a specific plant or region, the kits can be altered by adding extra salicylic, hydrochloric, or sulphuric acid to the reagent. The presence of strong reducing agents other than iodide may also give a positive bias with iodate-based methods.

We opted for a simple dropper test and tested it using salt samples iodized in our laboratory, commercially obtained Canadian salt, and impure salt samples received from target countries.

Field test apparatus

In its prototype format, the kit consists of the following:
» metal pan (~8 cm outside diameter)
» 25-ml plastic flask with dropper in cap, containing the prepared reaction mixture
» colour standard chart (photograph), showing colours obtained with 0 to 50 μg of iodine per gram of salt in intervals of 10 μg/g

Reagents

The reagent solutions are prepared as follows:
1. *Benzoic acid buffer* solution is prepared by adding 400 mg of ACS grade sodium benzoate to 100 ml of water. It is dissolved by gentle mixing, and then the pH is adjusted to 3.2 with dilute (~ 0.1 N) HCl to form the benzoic acid–sodium benzoate buffer solution.
2. *Addition of starch.* The buffer solution is heated to boiling. Three grams of soluble potato starch (ACS grade, iodide free) is triturated with 10 ml of cold water and poured slowly, with constant stirring, into the boiling buffer solution. The mixture is boiled until a thin, translucent fluid is obtained. This is a critical step, since excessive boiling may render the solution less sensitive. The solution is allowed to cool and settle, and then is filtered through a fast filter paper, e.g., Whatman No. 41, to obtain a clear filtrate.
3. *Potassium iodate addition.* Approximately 3 g of KIO_3 is dissolved in the buffered starch solution, completing the reaction mixture. After thorough mixing, it is dispensed into 25-ml plastic dropper flasks.

Procedure

1. Fill the metal pan with salt, creating a flat surface flush with its edge.
2. Apply a few drops of the reagent solution to the surface of the salt.
3. Match the developed starch-iodide blue colour with the colour standards on the chart, and determine the iodide concentration of the salt.

Field test for iodide in salt using ferric chloride

Iodide is quantitatively oxidized to iodine by ferric (Fe^{3+}) salts according to the equation:

$$2\ FeCl_3 + 2\ KI \rightarrow I_2 + 2KCl + 2\ FeCl_2$$

The free iodine will react with starch to form a deep-blue complex. Under controlled conditions, the intensity of the colour is proportional to the amount of liberated iodine and hence the iodide content of the salt. Since ferric chloride has a bright yellow colour, the colour of the sample will vary from yellow through green to dark blue-green.

Field test apparatus

The following apparatus is used:
» three disposable transfer pipettes: one for delivering solution A to the mixing bottle, one for delivering solution B to the mixing bottle (both marked with the required volume), and one for delivering drops of mixture to the iodized salt surface from the mixing bottle
» mixing bottle
» petri dish to contain the salt sample
» large scoop for mixing salt
» 100-ml plastic flask with dropper in cap, containing the prepared reaction mixture
» 10–50 ppm iodized salt colour standard chart

Reagents

The following reagents are used:
1. *Solution A: ferric chloride hexahydrate solution.* A

0.1 M ferric chloride solution is prepared by dissolving 2.70 g of ferric chloride hexahydrate in 100 ml of water at room temperature. The solution is allowed to stand and the clear supernatant is decanted.

2. *Solution B: starch solution.* A starch solution is prepared by triturating 3 g of soluble potato starch (ACS grade) with 10 ml of cold water, and pouring slowly, with constant stirring, into 100 ml of boiling water. To prevent microbial degradation, 400 mg of sodium benzoate is added. The mixture is boiled until a thin, translucent fluid was obtained. The solution is allowed to cool and settle and then is filtered to obtain a clear filtrate.
3. *Reaction mixture.* Equal volumes of the two solutions are thoroughly mixed and dispensed into the plastic dropper bottle. This mixture is not stable for long periods of time, and therefore it should be prepared daily.

Procedure

1. Add 1 volume of solution A to mixing bottle with the first transfer pipette and 1 volume of solution B with the second transfer pipette. Mix well.

For salts with less than 50 ppm iodine as iodide

2. Fill the petri dish with salt, creating a surface flush with its edge.
3. Apply several drops from the mixing bottle to the surface of the salt using the third disposable pipette.
4. After 5 to 8 minutes, compare the colour with the standard colour chart.

For salts with more than 50 ppm iodine as iodide

2. Put one large scoop of sample salt and one large scoop of iodine-free salt in the petri dish. Mix in the petri dish after slightly wetting the salt with water.
3. Apply several drops from the mixing bottle to the surface of the salt, using the third disposable pipette.
4. After 5 to 8 minutes, compare colour with standard colour chart.
5. Multiply results by 2 to obtain the iodide levels.

Results

With pure salt iodized in our laboratory, the iodate-based kit produced reproducible results that readily distinguished different levels of iodide in the range of 10 to 40 μg/g. The colours developed at high concentrations of iodine were dark, and the difference between 40 and 50 μg/g was difficult to distinguish. This may limit its usefulness in quality control, where target levels are typically 50 μg/g.

Since potassium iodate is used as the oxidizing agent, this system will release iodine from the reagent in the presence of many reducing agents, providing a false positive reading for iodine in the salt. Therefore this approach is not recommended for universal application, although it might be useful for testing iodine retention in the field because of its simplicity and portability.

The ferric chloride method was remarkably free from interference. Since the ferric chloride reagent solution does not introduce iodine, the test cannot obtain false iodine readings from strong reducing agents present in the salt as impurities or through deliberate attempts to replace potassium iodide with a less expensive and physiologically useless reducing agent.

Tests in our laboratory produced reproducible results that readily distinguished different levels of iodide in the salt in the range of 10 to 50 μg/g (table 1). The photographs of a series of standards that we produced were not of professional quality, despite colour

TABLE 1. Comparison of iodine concentrations in iodized salt measured by kit and by neutron activation

Kit			Neutron activation		
Iodine (μg/g)[a]	SD	CV (%)	Iodine (μg/g)[a]	SD	CV (%)
4.0	1.3	31.6	4.8	2.7	56.4
6.7	1.2	18.2	7.0	3.2	45.2
10.0	0.0	0.0	10.5	0.5	4.9
17.3	2.3	13.0	17.8	5.8	32.5
19.2	1.0	5.1	19.9	3.8	18.9
19.7	0.8	4.2	20.2	0.4	2.0
28.7	1.2	4.2	28.9	0.7	2.5
37.3	2.2	5.8	38.4	0.6	1.6
48.0	2.4	5.1	48.8	0.8	1.6
72.4[b]	2.0	5.6	73.0	5.8	7.9

a. Average of six measurements.
b. Measured after 1:1 dilution.

correction during development. As a result, the readings based on this crude colour chart were not nearly as accurate as those in which the samples were compared with actual salt standards on which the colour was developed by the kit's reagents.

The colour development requires about 10 minutes. Because colour development is catalysed by both acid and the iodide present, samples with higher iodine content reach the final colour intensity faster. Thus samples containing close to 50 ppm developed a deep-blue colour in three to five minutes, which did not fade or darken for an hour.

The best colour clarity was obtained with a clear reagent solution. Unfortunately, particulates such as starch globules promoted the formation of ferric oxychloride, which, in the presence of starch, made the solution cloudy. Although this did not change the colour reaction, the perceived colour changed, making it harder to distinguish between higher iodine levels.

We found that both the ferric chloride and the starch solution were stable and clear for a long period of time. The ferric chloride solution itself is known to be stable for years, and therefore starch stability limited the useful life of the kit. Despite the addition of benzoic acid as an antimicrobial agent, the starch breaks down after several months from microbial degradation and acid hydrolysis, limiting its usefulness to about six months at higher temperatures. The reaction mixture occasionally became cloudy after extended storage because of precipitation of starch. Its storage life could be extended by removing the precipitate by filtration.

A series of samples were prepared in our laboratory and analysed using both the kits and neutron activation analysis with the SLOWPOKE reactor facility at the University of Toronto. The kit and other kits made up on the basis of these instructions were used in the UNICEF Laboratory by Dr. Hans Vanhassel in Quito, Ecuador. The samples prepared in Toronto were tested with locally made kits, and samples of local salt iodized to various levels were also tested in both Quito and Toronto, using both kits and standard laboratory techniques. The results are presented in table 2. All of the kits gave good results at low iodide levels. At higher iodine concentrations, the limitations of our kit due to the poor colour charts became evident. The Ecuadorian laboratory consistently underestimated iodine values by approximately 30 μg/g. Our laboratory obtained good, reproducible results, since we used actual standard salt samples rather than photographs for estimating the colour and hence the iodide content of the samples.

The extended time between the preparation and the testing of salt added to the inaccuracy of the kits. Iodide slowly loses iodine over time, especially in impure salt stored at high temperature and humidity. Thus readings taken at different times may reflect actual differ-

TABLE 2. Kit validation: Comparison of iodine analysis of iodized salt samples

	University of Toronto		UNICEF–Ecuador		
Value measured	Toronto kit	Neutron activation	Titration	Toronto kit	Ecuador kit
Iodine (μg/g)	4.0	4.8	7.8	5.8	5.0
SD	1.3	2.7	0.3	2.0	0.0
CV (%)	31.6	56.4	4.1	35.0	0.
Iodine (μg/g)	6.7	7.0	8.4	10.0	10.0
SD	1.2	3.2	0.2	0.0	0.0
CV (%)	18.2	45.2	2.9	0.0	0.0
Iodine (μg/g)	19.7	20.2	19.6	20.0	20.0
SD	0.8	0.4	0.5	0.0	0.0
CV (%)	4.2	2.0	2.6	0.0	0.0
Iodine (μg/g)	28.7	28.9	28.5	23.3	25.0
SD	1.2	0.7	1.1	4.1	3.2
CV (%)	4.2	2.5	3.8	17.5	12.6
Iodine (μg/g)	37.3	38.4	38.7	29.2	30.0
SD	2.2	0.6	0.6	2.0	0.0
CV (%)	5.8	1.6	1.7	7.0	0.0
Iodine (μg/g)	48.0	48.8	49.9	28.3	29.2
SD	2.4	0.8	0.4	2.6	2.0
CV (%)	5.1	1.6	0.8	9.1	7.0

ences due to loss of iodine between the time of initial analysis and final analysis by the kits. Despite these difficulties, the results were acceptable.

Conclusions and recommendations

Two simple, inexpensive test kits for the analysis of iodide in salt have been developed and successfully tested. The test results obtained were well within the specified accuracy of ±10 μg/g in the range of iodine concentrations from 0 to 50 μg/g.

The ferric chloride-based test gave a visually more readily distinguishable range of measurements because the samples differed not only in colour intensity but also in hue.

For full implementation of these test kits, professionally made colour charts will be required, which would make it possible for untrained field operators to distinguish between salt samples with higher iodide content.

Acknowledgements

Samples of salt were made available for this study by UNICEF field offices in eight countries. The authors are grateful for the testing of the kits and excellent comments and suggestions by Dr. Hans Vanhassel of UNICEF-Ecuador.

This work was initiated by Dr. Timothy R. Stone, Executive Director of PATH Canada, who was tragically killed in the crash of the hijacked Ethiopian airplane in the Comoro Islands in November 1996 while on a humanitarian mission.

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Effect of tempe and sodium metabisulphite on the microbiological quality, development of rancidity, and sensory quality of Nile perch (*Lates niloticus*) sausages

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Abstract

The conventional method of beef and pork sausage manufacture was adopted for processing fish sausage from Nile perch (Lates niloticus) *fillets. Four types of sausage were made: sausages with 0.02% sodium metabisulphite (MCS); sausages with 10% tempe (TCS); sausages with 0.02% sodium metabisulphite and 5% tempe (MTCS); and sausages with neither tempe nor sodium metabisulphite (SWTM). The proportions are given based on the weight of the fish fillet. Each type of sausage was divided into two equal halves. One half was stored at 5°C while the other half was stored at 20°C. The keeping quality was monitored over seven days by determination of the total plate count to assess microbial spoilage and extent of rancidity development as measured by absorbance of their light petroleum (40°–60°C) extract at 269 nm. The organoleptic quality was assessed by 18 untrained panelists and 3 trained panelists. It was found that tempe had antimicrobial effects but little antioxidant activity, whereas sodium metabisulphite had little antimicrobial activity but greatly reduced the content of secondary lipid oxidation products and possibly inhibited lipid oxidation. The overall acceptability of the sausages was high (scores of about five points on a seven-point hedonic scale).*

Introduction

Fish are one of the most important sources of proteins in the world. The other sources of animal protein, red meat and poultry, are more expensive, especially in developing countries. In Kenya most of the fish is exported. The most popular fish are tilapia *(Oreochromis* spp*)* and sardines (*Rastrineobola argentea*), locally referred to as *omena.*

The introduction into Lake Victoria of Nile perch (*Lates niloticus*), which accounts for 60% of the fresh fish caught in Kenya [1], has resulted in a rapid decrease in the tilapia population. Although tilapia is preferred, the Nile perch is the most widely consumed fish in East Africa because of its availability. Large numbers of dead Nile perch, which the fisherman have been unable to sell, can be found on some beaches.

The price of Nile perch varies from Ksh 25.00 to 45.00 per kilogram, which would translate to US$1.02 to $1.87 per kilogram, compared with US$3.80 and $2.25 for chicken and red meat (beef and pork), respectively.

The methods available for preparing fish in East Africa include smoking, sun-drying, deep frying in shortening or fish oil, or frying to make a sauce to eat with *ugali* (a semisolid cake made from cereal flours). Refrigeration is the predominant method of preservation, used by retailers in urban centres and by fish processors who export fish fillets. In Japan and Thailand, fish are used to make sausages, *kamamboko* (meat loaf), and fish protein concentrates [2, 3].

Sausages are readily accepted in many communities. In Japan fish sausages are made by combining fish fillets with seasonings and preservatives, adding binding agents such as starch and rusk, and finally smoking after stuffing in artificial casings and scalding [3].

The major limiting factor in the use of fish is their rapid spoilage at high ambient temperatures, such as those found in the tropics. Fish have inherent proteolytic and lipolytic enzymes in the pyrolic caeca and intestines [4]. The intestines, together with the microbial flora, are responsible for the rapid spoilage of fish after they are caught.

Fish oils are highly unsaturated and are hence susceptible to the development of hydrolytic and autoxidative rancidity [3]. Hydroxyperoxides form the central mechanism for lipid oxidation. According to Farmer's theory, the reactions proceed through a free-radical mechanism [5]. Hydroxyperoxides are themselves nonvolatile and odourless but have a slightly bitter after-

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taste [5]. The secondary products derived from them contribute to the rancid flavours. These compounds include aldehydes, ketones, alcohols, and hydrocarbons [5]. The extent of formation of secondary oxidation products can be assessed by measuring absorbance at 269 nm [6].

Rancidity is normally accompanied by deterioration of flavour and colour changes, loss of vitamins (especially vitamins A and E), and destruction of sulphur-containing amino acids [5]. Side reactions between products of fat oxidation and proteins may cause textural changes as well as lowering the digestibility of protein and hence of amino acids [5].

The shelf-life of fresh fish is limited to 48 hours [7]. It can be prolonged by refrigeration. However, the ambient temperatures under tropical conditions cause rapid melting of ice. The comminution of fish fillets, as in the manufacture of sausage, allows the use of additives for preservation. The most widely used preservatives are nitrites, nitrofurans, sorbic acid, and semicarbazones, all of which inhibit microbial growth [3, 8, 9].

Sodium metabisulphite is an additive known to have fewer health hazards than other preservatives. Metabisulphites are reducing agents and may inhibit lipid oxidation. The antioxidant activity of sulphur dioxide is related mainly to the unbound non-ionic form [8]. Its effectiveness in killing or inhibiting microorganisms is most pronounced at pH values less than 4. Nevertheless, sodium metabisulphite is used in meat products even when the pH would not be expected to be below 5.3 to 5.4.

From a safety point of view, the naturally occurring preservatives in some foods and spices are preferable to chemical additives [9]. Tempe, an Indonesian *Rhizopus* mould-fermented legume, cereal, or cereal-legume mixture, is a low-cost traditional food product. It is one such food that can be used for this purpose. The calculated cost of tempe, taking into account 30% of the total processing cost, is Ksh 44.00 (US$0.73) per kilogram, which is 20% to 50% less than the cost of meat. Thus, tempe is within the reach of members of most socio-economic groups as a major source of protein and vitamins.

Tempe has both antimicrobial and antioxidative properties [10]. Wang et al. reported an antimicrobial compound in tempe [11]. Tempe is also reported to contain 6,7,4-trihydroxyisoflavone, which has antioxidative activity [12].

This paper presents data on the microbial count, pH, lipid oxidation secondary products (as measured by absorbance at 269 nm), and sensory characteristics of four types of Nile perch sausages containing tempe and sodium metabisulphite, during and after seven days of storage at 5° and 20°C. These data are used to compare the antimicrobial effects, extent of development of rancidity, and overall eating quality of the sausages.

Materials and methods

Materials

Refrigerated fish fillets obtained from the Nairobi city market were wrapped in a 200-gauge polythene bag and stored in a freezer at –1°C for preparation the following day. The pre-refrigeration handling history of the fillets was not known. Soya beans were purchased from the Uthiru supermarket in Nairobi. *Kimbo* (a shortening made by East African Industries), starch, and salt were obtained from the Uchumi supermarket in Nairobi. The tempe mould culture (*Rhizopus oligosporus*) was obtained from the Research and Development Centre for Applied Chemistry in Bandung, Indonesia. Casings were purchased from Naturin-Werk, Weinheim, Germany. All chemicals were obtained from Kobian Chemicals in Nairobi.

Preparation of tempe

Whole beans were sorted, washed in clean water, and boiled for 30 minutes to soften the husks. The beans were soaked for 24 hours in an air oven at 35°C in three times their weight of water. The beans were then dehusked by rubbing them between the hands, and the husks were rinsed away with water. The clean beans were cooked in boiling water for 45 minutes. The water was drained, and the beans were cooled to ambient temperature (20°C). The beans were surface-dried in the sun for 3 hours, after which they were inoculated with a starter culture of *R. oligosporus* at the rate of 0.1% based on the weight of the dry beans and packed in 200-gauge polythene bags that had been perforated with a 2-inch nail at intervals of 1 cm. The inoculated beans were incubated in an oven at 35°C for 24 hours, at which time they were evenly covered with white mould mycelia. The tempe was harvested by removing it from the polythene bags and slicing it into $1 \times 2 \times 1$ cm pieces. These were dried in an air-circulating oven at 60°C and then ground in a coffee attrition mill (Dittings, Switzerland). The tempe was moistened to 60% moisture and made into a dough that was blanched for 20 minutes to inactivate the mould and kill any microorganisms present before use.

Preparation of the sausages

Four different types of sausages were made (see table 1): sausages with 0.02% sodium metabisulphite (MCS), sausages with 10% tempe (TCS), sausages with 0.02%

TABLE 1. Recipes for the different types of sausages[a]

Ingredients	SWTM		MCS		MTCS		TCS	
	g	%	g	%	g	%	g	%
Fillet	1,000	100	1,000	100	1,000	100	1,000	100
Starch	100	10	100	10	100	10	100	10
Fat (kimbo)	100	10	100	10	100	10	100	10
Tempe	–	–	–	–	50	5	100	10
NPS[b]	20	2	20	2	20	2	20	2
Sodium tripolyphosphate	–	–	2	0.2	2	0.2	2	0.2
Sodium metabisulphite	–	–	0.2	0.02	0.2	0.02	0.2	0.02
Black pepper	2	0.2	2	0.2	2	0.2	2	0.2
Sage	2	0.2	2	0.2	2	0.2	2	0.2
Nutmeg	2	0.2	2	0.2	2	0.2	2	0.2

Abbreviations: SWTM, sausages without tempe or sodium metabisulphite; MCS, sausages containing 0.02% sodium metabisulphite; MTCS, sausages containing 5% tempe and 0.02% sodium metabisulphite; TCS, sausages containing 10% tempe.
a. Proportions (%) of ingredients shown are based on the weight of the fish fillet.
b. Nitrite pickling salt (99.95% NaCl and 0.05% $NaNO_2$).

sodium metabisulphite and 5% tempe (MTCS), and sausages with neither tempe nor sodium metabisulphite (SWTM).

Increasing the tempe content above 15% decreased the acceptability of the sausages, mainly because the sausages disintegrated instead of holding together. Therefore, no attempt was made to increase the tempe content above 10%. The sausages were processed as follows: 1 kg of fish fillet was cut in a Killia (Zurich) meat cutter three times. Nitrite pickling salt (99.95% sodium chloride and 0.05% sodium nitrite) was added and the mixture was ground two more times. One millilitre of strawberry red colour was added, along with shortening, spices, polyphosphate, and sodium metabisulphite (for MCS and MTCS), and the mixture was ground another three times. Starch was added to all samples, and tempe was added to the MTCS and TCS sausage mixtures, which were ground again to bind them. The sausage mixtures were stuffed into 6-cm, 500-gauge polythene casings. These were then scalded in a Fressman (Zurich) smoking cabinet at 90°C to a core temperature of 75°C for 1 hour. The sausages were cooled with cold water to 20°C.

Storage of the sausages

Each sausage sample was cut in half. One half was stored at 5°C and the other one at 20°C for 7 days for analysis according to the schedules and methods described below.

Microbiological analysis

After 4 days, 11 g of sausage was weighed into 99 ml of saline solution (8.5%) and macerated in a kitchen blender (Croydon, England). Serial dilutions were then made to a dilution of 10^{-6}. Dilutions of 10^{-5} and 10^{-6} were plated in duplicate in plate count agar (PCA) and incubated at 35°C for 48 hours, and the numbers of colonies in plates with visible growth were counted.

Chemical analyses

Determination of pH

A 10-g sample of sausage was weighed out and ground with a mortar and pestle. Boiling water was added to the ground sausage in a 20-ml flat-bottomed flask, which was then shaken at 100 rpm for 30 minutes on a Gerber (Zurich) shaker. Three pH readings were taken with a model 290 MK2 pH meter.

Determination of the extent of production of secondary products of lipid oxidation

A 10-g sample sausage was weighed out in a tared crucible and then dried in an air-circulating oven at 80°C for 6 hours. The sample was crushed with a mortar and pestle and transferred into a 250-ml flat-bottomed flask. One hundred millilitres of light petroleum (40°–60°C) was added, and the contents were agitated on the Gerber shaker at 200 rpm for 1 hour. The extract was filtered through Whatman No. 1 filter paper, and the residue was washed three times with 10 ml of light petroleum. The washings were combined with the extract. The absorbance of the filtrate was then read at 269 nm in a Beckmann spectrophotometer.

Sensory analysis

The sausages that had been held at 5°C for 4 days were evaluated three times at 2-week intervals by a panel of 18 for appearance, odour, flavour, texture, and overall acceptability on a 7-point hedonic scale (7 = liked very much, 6 = liked moderately, 5 = liked slightly, 4 = neither liked nor disliked, 3 = disliked slightly, 2 = disliked moderately, and 1 = disliked very much) accord-

ing to Larmond [13]. The data were subjected to analysis of variance, also as described by Larmond.

Results and discussion

Microbiological quality

TCS had the lowest total microbial count at both 5° and 20°C after 7 days (table 2). At 5°C, the total count (5.0×10^6 cfu/g) was about 20 times lower than that of SWTM, which had the highest count (2.4×10^7 cfu/g). This indicates that tempe has strong antimicrobial effects. This is also clear from the observation that its incorporation in sodium metabisulphite-containing sausages (MCS) decreased the total count by about 53% (from 1.6×10^7 to 7.0×10^6 cfu/g), as shown by the results for MTCS, whereas incorporation of sodium metabisulphite reduced the total count of SWTM by about 33% (from 2.4×10^7 to 1.6×10^7 cfu/g) in comparison with MCS. The total count for sausages containing 10% tempe (TCS) was in fact 31.3% of the total count for sausages containing sodium metabisulphite only (MCS) and about 11% that of MTCS, which had 5% tempe and 0.02% sodium metabisulphite. This indicates that at the acceptable and allowed levels, sodium metabisulphite has a weaker antimicrobial effect than tempe. These results may have been partly due to the high pH of the sausages (table 3). High pH conditions cause dissociation of metabisulphite, which is otherwise most active in the form of undissociated sulphurous acid (H_2SO_3) [5].

That tempe had a very strong antimicrobial effect may be attributed to the presence of an antimicrobial substance produced by *Rhizopus* mould [11]. This compound is a glycopeptide that is very effective against gram-positive bacteria, which include the food-poisoning *Staphylococcus aureus* and *Clostridium* species.

At ambient temperature (20°C), the pattern was similar to that at 5°C. The counts in all the samples reached the slime limit of 10^8 [4], which is the limit indicative of microbial spoilage. At 20°C TCS containing 10% tempe had a microbial count (8.0×10^8) which was 20% of that of SWTM (a reduction of 80.8%). The reduction when 5% tempe and 0.020% sodium metabisulphite were added (50%) was much lower than that observed in TCS. When sodium metabisulphite alone was added, there was practically no reduction (table 2). This could be because sodium metabisulphite is broken down to release sulfur dioxide, which may be bound to carbonyl compounds of oxidized fat. The odour of the sausages suggested that indeed sulphur dioxide was being released. The antimicrobial effect shown by MTCS (containing 5% tempe) is, therefore, mainly due to tempe, which has superior antimicrobial activity.

TABLE 2. Total microbial count (cfu/g) of different types of sausages stored at 5°C and 20°C for 4 days[a]

Sausage type	5°C	20°C
SWTM	2.4×10^7	4.0×10^9
MCS	1.6×10^7	3.0×10^9
MTCS	7.0×10^6	2.0×10^9
TCS	5.0×10^6	8.0×10^8

Abbreviations: SWTM, sausages without tempe or sodium metabisulphite; MCS, sausages containing 0.02% sodium metabisulphite; MTCS, sausages containing 5% tempe and 0.02% sodium metabisulphite; TCS, sausages containing 10% tempe.
a. The values are averages of two readings.

TABLE 3. pH changes of different types of sausages stored at 20°C for 7 days[a]

Sausage type	Day			
	0	1	4	7
SWTM	6.9	6.8	7.2	6.7
MCS	6.9	7.2	7.1	7.1
MTCS	6.7	6.9	6.7	7.1
TCS	6.7	6.8	6.7	6.8

Abbreviations: SWTM, sausages without tempe or sodium metabisulphite; MCS, sausages containing 0.02% sodium metabisulphite; MTCS, sausages containing 5% tempe and 0.02% sodium metabisulphite; TCS, sausages containing 10% tempe.
a. The values are averages of three determinations.

Change in pH

There were no marked changes in the pH of the sausages over the whole storage period (table 3). The pH remained above 6.7 for all the samples at both 5°C and 20°C. However, the incorporation of tempe in both MTCS and TCS resulted in lower pH values than in MCS and SWTM. The pH of all four samples was, however, above the pH for acidic foods and would thus have little inhibitory effect on the growth of microorganisms, which indicates that the lower total microbial count in tempe-containing sausages was due mainly to its antimicrobial effects. The high pH observed is further supported by Eskin's observation that the pH of fish hardly ever falls below 6.0, even at full rigour [14].

Production of secondary lipid oxidation products

The extent of formation of secondary lipid oxidation products, as shown by absorbance at 269 nm, which is indicative of the degree of development of rancidity, is shown in tables 4 and 5. All the sausage types showed a similar pattern during storage both at 5°C and at 20°C. At 20°C, the highest degree of rancidity development was shown by TCS, as indicated by its absorbance (1.23) after 7 days of storage. The next highest absorbance (1.13) was shown by SWTM, which was 92% of that

TABLE 4. Ultraviolet absorption by secondary products of lipid oxidation of sausages stored at 20°C for 7 days[a]

Sausage type	Day		
	1	4	7
SWTM	0.04	0.17	1.13
MCS	0.04	0.11	0.32
MTCS	0.13	0.24	0.57
TCS	0.26	0.49	1.23

Abbreviations: SWTM, sausages without tempe or sodium metabisulphite; MCS, sausages containing 0.02% sodium metabisulphite; MTCS, sausages containing 5% tempe and 0.02% sodium metabisulphite; TCS, sausages containing 10% tempe.
a. The values are averages of three readings.

TABLE 5. Ultraviolet absorption by secondary products of lipid oxidation of sausages stored at 5°C for 7 days[a]

Sausage type	Day		
	1	4	7
SWTM	0.13	0.17	0.27
MCS	0.04	0.15	0.19
MTCS	0.12	0.23	0.39
TCS	0.23	0.43	0.52

Abbreviations: SWTM, sausages without tempe or sodium metabisulphite; MCS, sausages containing 0.02% sodium metabisulphite; MTCS, sausages containing 5% tempe and 0.02% sodium metabisulphite; TCS, sausages containing 10% tempe.
a. The values are averages of three readings.

of TCS. This clearly shows that tempe did not have antioxidant effects in the sausages. In fact, it appeared to increase the rancidity. The next highest level of secondary products of lipid oxidation was shown by MTCS, whose absorbance was 0.57, 46% of that of TCS and 50% of that of SWTM. The observation that MTCS (containing 5% tempe) had lower absorbance than TCS (containing more tempe) or SWTM, which was expected to have the highest absorbance, clearly shows that sodium metabisulphite not only reduced lipid oxidation in tempe-containing sausages but also inhibited their oxidation in SWTM.

The sausage containing sodium metabisulphite only (MCS) had the lowest absorbance (0.32), which was 26% of that of TCS, 36% of that of SWTM, and 56% of that of MTCS. These results further confirm that sodium metabisulphite had antioxidative effects or that it reduced the development of rancidity, whereas tempe increased rancidity or susceptibility to rancidity. This observation may be due to reactions between sulphurous acid ions and carbonyl groups of the secondary oxidation reaction.

Similarly, at 5°C the degree of rancidity increased with time during storage (table 5). The sausages containing 10% tempe (TCS) also had the highest degree of rancidity (as indicated by their absorbance of 0.52) over the storage period. The sausages with 5% tempe (MTCS) had the second highest degree of rancidity, as shown by the absorption by their secondary oxidation products, which was 0.39 (75% of that of TCS). The sausages with neither tempe nor metabisulphite (SWTM), which at 20°C had the second highest absorbance, had the third highest degree of rancidity at 5°C, with an absorbance of 0.27, 52% of that of TCS. The least rancid sausages were those with metabisulphite only, as was the case at 20°C. These had an absorbance of 0.19, which was only 36.5% of that of TCS.

The above results suggest that metabisulphite strongly reduced the development of rancidity, had strong antioxidative action, or both. The fact that MTCS ranked second after TCS clearly shows that tempe enhanced the rancidity in proportion to the amount added to the recipe. The higher absorbance of SWTM than MTCS at 20°C may have been due to the binding of practically all the SO_2 to the carbonyl compounds and at the same time an increase in the amount of carbonyl compounds due to the presence of tempe (which, as shown above, appears to increase the amount of oxidized lipids).

The degree to which the formation of secondary oxidation products was reduced when the temperature was lowered from 20° to 5°C varied according to the type of sausage. After seven days of storage, SWTM showed the highest reduction factor (4.19). This shows that the reduction was due purely to a low-temperature effect. The next highest reduction factor was observed for TCS (2.37). This was followed by 1.68 and 1.46 for MCS and MTCS, respectively, which were not significantly different. The fact that the sausages with metabisulphite had lower reduction factors suggests strong antioxidative action, inhibitory effects, or both on the development of rancidity by sodium metabisulphite. The similarity of the two reductions indicates that the decrease in temperature had little influence.

The higher level of rancidity in sausages containing tempe may have been due to the failure of the antioxidant 6,7,4-trihydroxyisoflavone [12] to be effective in fat, since it would be in the polar phase.

The observed high absorbance in sausages containing tempe supports observations made by others showing that although tempe is resistant to oxidation [9], it has little antioxidant activity in food systems or when mixed with flours.

Sensory quality

Sausages with 10% tempe (TCS) were significantly different from the rest in terms of taste ($p < .05$) and had the highest score (5.57) (table 6). Although MTCS was not significantly different from MCS and SWTM, it had a higher score for taste (5.26). This shows that the presence of tempe in the sausages enhanced taste. SWTM

TABLE 6. Sensory evaluation of different types of sausages

Sausage type	Taste	Appearance	Odour	Texture	Acceptability
SWTM	5.05^a	5.72^a	5.20^a	5.72^a	5.60^a
MCS	4.47^a	4.78^b	4.30^a	4.78^a	4.70^b
MTCS	5.26^a	4.56^b	4.50^a	5.33^a	5.06^b
TCS	5.57^b	5.44^{ab}	5.40^a	5.72^a	5.40^{ab}

Figures in the same column followed by the same letters are not significantly different from each other at $p = .05$. Each score is the average of 54 observations.

had the third highest taste score (5.05) and MCS had the lowest (4.47). From this observation, it is clear that sodium metabisulphite decreased taste scores.

There were no significant differences in odour scores among any of the samples ($p > .05$). As was the case with taste, TCS had the most acceptable odour (with a score of 5.40), followed by SWTM, which had a score of 5.20. The odour scores for MTCS and MCS were generally very low (4.50 and 4.30, respectively) and bordered on rejection. This could be attributed to a faint sulphurous odour, which was noticed in these sausages.

Three trained panelists noticed a weak fish odour in SWTM, but none of them noticed this odour in any of the other sausages. The relatively high preference for the odour score of SWTM indicates that the fish odour and taste are weak in the Nile perch fillet.

It is important to note that tempe appeared to improve the odour of the sausages, since sausages with tempe (TCS and MTCS) had higher scores than those without tempe (MCS).

There were also no significant differences in appearance among MCS, MTCS, and TCS ($p > .05$). The sausages with neither tempe nor sodium metabisulphite (SWTM) had the highest score (5.72) and were significantly different from sodium metabisulphite-containing sausages (MTCS and MCS) ($p < .05$) but had a score which was not significantly different from that of TCS (5.44), with 10% tempe but no sodium metabisulphite. A relatively intense colour was noticed in SWTM, whereas that of TCS was very light. It is important to note that the cost of sausages would be decreased by about 6% by adding tempe at the rate of 10% (7.5% based on the weight of the sausage mass).

The metabisulphite-containing MCS and MTCS (containing 5% tempe) were less preferred and had lower appearance scores (4.78 and 4.56, respectively). These did not have any trace of strawberry red colour. Thus, it appears that sodium metabisulphite bleached the colour, whereas tempe reduced the rate of bleaching, probably by producing reducing conditions. Sulphurous acid is known to reduce pigments. The residual red colour in MTCS is due to the presence of tempe. It is possible that the creation of reducing conditions decreased the destruction of the colour. Absorption of the colour by tempe could also have accounted for decreased colour destruction.

There were no significant differences in texture among the samples ($p > .05$). The texture scores of SWTM and TCS (5.72 in both cases) were the highest. The next highest texture score was that of MTCS (5.33). The sausages containing sodium metabisulphite only had the lowest score (4.78). Incorporation of tempe to a level not exceeding 10% improved the texture of the sausages.

There was no significant difference in acceptability between SWTM and TCS ($p > .05$). However, SWTM had the highest acceptability score (5.60). TCS, which had a score of 5.40, was not significantly different from MCS and MTCS, which scored 4.70 and 5.06, respectively. The least acceptable sausage (MCS) had the lowest scores for the other four attributes: taste, odour, appearance, and texture. It is important to note that with respect to nearly all the attributes except taste, the ranking was similar to that of acceptability. Taste, a factor expected to influence acceptability, had, therefore, little influence on acceptability.

The observation that sausages containing neither tempe nor sodium metabisulphite (SWTM) had the highest acceptability was unexpected. It is possible that a long period of storage, even at low temperature, resulting in increased rancidity could make these sausages less acceptable than those containing sodium metabisulphite (MCS).

Since the sausage did not have a fish flavour, which is disliked by most people of the non-fish-eating communities in Kenya, especially those in the Central and Eastern Provinces, other products, such as "meat loaf," may be consumed in the future. The tempe-containing sausages would be particularly attractive to workers and schoolchildren, for whom sausages and chips are the most popular lunch meal, mainly because of their palatability and their relatively low cost compared with sausages prepared from other meats.

Conclusions

Acceptable sausages can be made from Nile perch (*Lates niloticus*) fillets without the addition of either tempe or sodium metabisulphite. However, the addition of tempe to decrease microbial activity and hence increase keeping quality is recommended. Tempe, apart from having strong antimicrobial effects, enhances the overall eating quality through improved taste and texture when incorporated at a rate of 10% (based on the fish fillet, i.e., 7.5% of the sausage mass). It has, however, little antioxidative effect in fish sausages and lowers their cost. Sodium metabisulphite is recommended to decrease the rate of development of rancidity arising from the accumulation of secondary products of lipid oxidation.

Acknowledgements

We are greatly indebted to the Department of Food Technology and Nutrition of the University of Nairobi, which funded this project. We also thank the 1996 Food Technology fourth year class of the University of Nairobi for participating in the sensory evaluation of our sausages.

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The changing climate of health and nutrition in Thailand: A report from the Institute of Nutrition at Mahidol University

Kraisid Tontisirin

Introduction

The Institute of Nutrition at Mahidol University (INMU) was established in 1977 as a national planning and implementation body for the Thai government. It has become an internationally recognized leader in the field of nutrition and its allied areas of population, health, agriculture, and rural development. The health and nutritional status of the Thai people has improved dramatically over the past 20 years as a direct result of effective programme planning and implementation in health, nutrition, and alleviation of poverty. Rapid socio-economic development, which generated a 14-fold increase in national income and an 8-fold increase in per capita income, also helped to make programmes sustainable. Household income steadily increased in real terms, and there is no doubt that the nation's economic growth "trickled down" some benefits to the poor. National budget allocations for health care have remained quite stable, with a rate of increase parallel to that of the country's economic performance. In times of economic hardship, the Ministry of Public Health has not been the prime target for budgetary cuts. Even while in the grips of the economic downturn that is affecting many Asian nations today, the Thai government's budgetary adjustment efforts raised the proportion of the Ministry of Public Health budget from 7.1% to 7.5% of the total government budget in 1998. The budget for welfare health services increased 9.4%.

Because of such political and economic commitments, virtually all health and social indicators are favourable in comparison to those in other developing nations. Some——such as life expectancy, prevalence of contraceptive use, total fertility rate, and infant and child mortality rates——are at or are quickly approaching those of industrialized nations. Thailand has also experienced remarkable declines in fertility, infant and under-five mortality, and maternal mortality. The increased availability of water and sanitation facilities has reached over 80% of the population. Near universal access to basic education has led to a high adult literacy rate of over 90% for men and women. Transmission rates for sexually transmitted diseases and HIV/AIDS are also declining. For nutrition specifically, dramatic reductions have been achieved in protein-energy malnutrition, including the virtual eradication of severe and moderate protein-energy malnutrition among the general population.

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Challenges

Thailand is now at a crossroads, and it is likely that the nation will carry a double burden in terms of nutritional problems into the next century. An unfinished agenda of nutrition and poverty, particularly among minority groups and the poverty-stricken who live in remote rural areas and urban slums, will coexist side by side with overnutrition and imbalances in nutrition arising from a more affluent society. Chronic degenerative diseases, largely attributable to nutrition, are on the rise in Thailand, fueled largely by drastic changes in personal and family lifestyles and associated food habits.

Undernutrition

Although protein-energy malnutrition is no longer thought to be a significant issue among the general population, it persists as a major public health problem among several vulnerable groups, especially those living along Thailand's borders. In particular, hill tribe people in northern Thailand are the most at-risk group for malnutrition in the nation. Government data from 1995, based on a survey of only 340 hill tribe villages and the weight and height of 33,292 children under five years of age, reported a combined protein-energy malnutrition rate of 23%, almost twice as high as the average for the general population. Without doubt, this

survey underestimated the actual situation, considering that the hill tribes in Thailand number almost a million people and are known for their large family size. For refugee children, a medical team from the Thai Red Cross who examined 400 Karen children aged 0 to 5 years along the Thai-Myanmar border found that 62% of the children were malnourished, some severely [1]. Finally, children living in semi-remote to remote areas along the Thai-Lao border are also at risk for protein-energy malnutrition. A recent survey by INMU of all schoolchildren in 25 primary schools in Ubol Ratchatani Province revealed that 19% were underweight (weight-for-age) and 26% were stunted (weight-for-height).

Studies show that although the national rate of malnutrition has declined dramatically, due largely to rural-focused primary health-care and poverty alleviation programmes, child malnutrition in the Bangkok slums remains unacceptably high. Estimates in the mid-1980s ranged from 42% to 50%. The national health survey in 1991–1992, conducted among low-income families in Bangkok, revealed that third-degree malnutrition affected 11% of children under one year of age and 7% of children under five. Second-degree malnutrition occurred in 3% of children under five, and 10% had first-degree malnutrition. The underlying causes facilitating malnutrition among slum children in particular are restricted maternal time for child caregiving, low parental educational attainment, limited and very uncertain occupational opportunities, low purchasing power, an unsanitary, unsafe, and unstable physical environment, crowded living conditions, unstable marital relationships and family lives, and numerous other social problems [1, 2].

Although clinical vitamin A deficiency is not a public health problem in Thailand, subclinical cases can be found in many rural areas. In north and north-east Thailand, 20% to 27% of pre-school and school-age children exhibited subclinical deficiency [3]; rates for southern Thailand ranged from 4% to 26%, depending upon the province [4]. The main contributing factors are household food insecurity stemming from a reliance on purchased foods, seasonal variations in food availability, unstable consumption patterns, and the limited bioavailability of vitamin A in specific foods; inadequate maternal and child care due to changing lifestyles in which alternative caregivers provide children with inappropriate complementary foods; and inadequate health service coverage and unsanitary environmental conditions that perpetuate the malnutrition-infection cycle. Although extensive programmes in southern Thailand, where clinical deficiency was found in 1991, have attempted to increase the consumption of vitamin A–rich foods, little action has been taken to tackle subclinical problems in the north and north-east.

Iron-deficiency anaemia, as judged by World Health Organization (WHO) standards [5], occurs among 16% of children aged 6 to 14 and 13% of pregnant women (as of 1995). Currently, pregnant women are given daily iron tablets (120 mg/day for four months), but many difficulties obstruct this preventive approach, including side effects, inadequate prenatal attendance, lack of compliance, and maternal fear of delivering high-birthweight infants. To investigate this problem, INMU is evaluating the efficacy of weekly iron supplementation. In addition, a study has shown that a supplemental dose of 120 mg of iron per day may be too high for pregnant women, given Thailand's current situation (better services, less severe anaemia, and difficulties with side effects). The results show that rural Thai women supplemented with 60 and 120 mg of iron per day have similar haemoglobin and ferritin responses. Through school health programmes, iron supplements have also been given to schoolchildren, usually along with deworming. However, the distribution mechanism has not been monitored. As an alternative, the increased consumption of iron-rich foods by children is being promoted.

According to Thai government data, the total goitre rate (TGR) among schoolchildren fell from 19% in 1989 to 10% in 1993 and then to 4% in 1996. However, iodine-deficiency disorders persist in five northern and north-eastern provinces, with rates ranging from 10% to over 20% [1]. In addition, iodine-deficiency disorders exist among disadvantaged groups, some of whom fall outside the endemic areas traditionally classified by the Ministry of Public Health. For example, in some remote rural villages along the Thai-Lao border, local health workers reported that the prevalence of iodine-deficiency disorders or TGR ranged from 20% to 50% among schoolchildren. Urinary iodine data collected as part of an integrated child learning and community development project showed that of 750 schoolchildren in three north-eastern districts, 38% were normal, whereas 36%, 20%, and 6% suffered from mild, moderate, and severe iodine-deficiency disorders, respectively [6]. TGR stood at 11%. Major contributing factors to iodine-deficiency disorders in this area include the limited distribution and use of iodized salt as well as the traditional use of non-iodized rock salt for a multitude of purposes, ranging from food preservation to animal feed.

Overnutrition

Although undernutrition in terms of protein-energy malnutrition has been declining in importance in Thailand, overnutrition has taken its place as a major nutritional concern. Surveys of children in kindergartens in the Bangkok Metropolitan Administration as well as provinces in the south reveal a rising trend in obesity among children three to five years of age and school-aged children. Physicians in medical school hospitals are also observing increasing numbers of overweight

children. Among primary schoolchildren, a survey in a private school revealed an obesity rate of 14%, as compared with 3% for children from lower socio-economic status groups who attended Bangkok Metropolitan Administration schools [1]. It is speculated that modern infant-feeding practices by better-off mothers, including bottle-feeding, contribute to the rising trend in obesity. In addition, the belief that "fat children are healthy children" persists, especially among wealthier families. Other significant contributing factors to child obesity include parental obesity, higher family income, smaller family size, and lower exercise levels. The ready availability and increasing consumption of processed snacks high in energy is also seen as a contributing factor to poor nutrition among urban and rural children [3].

The future

The papers by INMU staff in this issue, as well as others [7–10], represent some of the wide-ranging nutrition issues that are currently at the forefront of concern: nutritional requirements, control and prevention of major micronutrient deficiencies, food product development, food quality and safety, undernutrition, and overnutrition. They range from clinical and laboratory-based studies to community-based interventions.

Yet Thailand's current situation is a complex one, in which solving nutritional problems is not enough to ensure an adequate quality of life. Presently, disadvantaged mothers and children do not suffer from single problems but multidimensional ones that only multisectoral projects and programmes can effectively solve. To reach these target groups effectively, INMU is undertaking integrated projects and programmes that lay equal stress on short-term curative and long-term preventive efforts [6]. As implied in UNICEF's conceptual framework for the causes of malnutrition and death, improvements in food security, maternal and child care, health services, and environmental sanitation cannot be made in isolation from each other or separately from improvements in other dimensions, such as the alleviation of poverty, education, and specific child protection measures.

Finally, despite the challenges facing Thailand, many developing nations within and outside of the region, and particularly those within Indochina, are looking towards Thailand's health, nutrition, and social development strategies and experiences as templates for improving their own developmental situations. Some are even making formal contractual arrangements with research institutions in Thailand, such as INMU, for long-term assistance in planning, education and training, monitoring, and evaluation in such areas as nutrition.

Considering Thailand's progress in health and nutrition, as well as its commitment to providing assistance and expertise to other nations, it now holds a dual position within the region's development. First, Thailand can serve as a resource centre, particularly with regard to using already accepted regional centres and institutes, such as INMU, as sources of technical assistance and technology transfer for nations within the region. Second, Thailand can serve as an example for the future. Thailand's progress, experiences, problems, and lessons learned which arise as it makes the transition from a developing nation to a newly industrialized one should be documented, and assistance should be given for such purposes, in order that these may benefit other developing nations in the future as they make the transition into the industrialized world.

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Nutrifit programme to improve health-related fitness among young Thai schoolchildren

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Abstract

A Nutrifit programme, consisting of a nutrition education and physical training model, was developed by modifying an established Fitnessgram as a tool. The programme was tested among 514 schoolchildren aged eight to nine years who were enrolled in two provincial government schools (A and B) and two private schools (C and D) in the Bangkok Metropolitan Area. Health-related fitness tests using the Fitnessgram were administered in all four schools, followed by the Nutrifit programme intervention in schools B and D. The intervention included 10 nutrition and physical training education sessions during the regular physical education classes. During the seven-month programme period, though the nutritional status of children did not show a significant change, improvement in health-related fitness tests was noted in both non-intervened and intervened schools. It is recommended that the Nutrifit programme be incorporated into the school curriculum as a measure of nutrition and fitness aimed at health promotion and disease prevention.

Introduction

Small-scale studies from kindergartens in the Bangkok Metropolitan Administration as well as provinces in southern Thailand show an increasing trend in obesity among pre-school and school-age children. Paediatricians in medical school hospitals have also observed increasing numbers of overweight children attending well-baby clinics. In a survey of primary schoolchildren in a private school, 14.3% were obese, a figure that is much higher than that among children from lower socio-economic status groups attending Bangkok Metropolitan Administration schools (3.4 %) [1].

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Parental obesity, higher family income, small family size, and low exercise levels are factors contributing to obesity among Thai children. The ready availability and increasing consumption of processed snack ("junk") foods is also adversely affecting the nutritional status of both urban and rural children [1]. In addition, changing trends have been reported in terms of an increase in energy intake (particularly sugar and fat), along with decreased physical activity (from television viewing and adoption of a sedentary lifestyle) [2, 3]. Such findings emphasize the significance of physical activity as part of the population's changing lifestyle. Indeed, nutrition and physical activity should be considered an integral part of fitness and good health.

Physical fitness tests in Thailand

Exercise and sport have long been recognized as tools in preventive medicine. The pioneering efforts of Prof. Dr. Auay Ketsingh, known as the Father of Sports Medicine in Thailand, have been well demonstrated through several "fitness parks" in both rural and urban areas [4].

In schools physical education to enhance children's physical activity and fitness is rather limited. Motor fitness tests proposed by the International Committee for the Standardization of Physical Fitness Tests (speed, muscular power, muscular strength, muscular endurance, flexibility, agility, and endurance) were first given to Thai schoolchildren in 1968, under the mission of the Department of Physical Education, Ministry of Education. They are now conducted every 10 years, with the objective of determining physical fitness norms of randomly selected groups of children aged 10 to 18 years. The ultimate goal is to modify the physical education curriculum in schools suitably [5].

Play is natural behaviour for young children in terms of exploration and social interaction with others. However, children from affluent societies are more exposed to inactive behaviour through video games, watching television, and other indoor activities. Academic achievement has also been overemphasized, to the extent that

children who play on the playground are seen to be wasting their time. This is more pronounced in the more affluent private schools. Although children may be more active than adults, if they do not develop regular exercise habits as children they may not grow up to be active adults.

Child and adolescent overnutrition is associated with increased morbidity and mortality, especially from coronary heart disease, atherosclerosis, and colorectal cancer [6, 7]. Since physical activity has been found to lower the risk of chronic diseases, regular physical activity and fitness should be advocated, beginning at an early age. Either motor fitness or health-related fitness is suggested for use as an index of physical fitness. However, motor fitness generally includes some physical abilities that relate primarily to athletic performance, whereas health-related physical fitness focuses on the aspect of fitness that is related to day-to-day function and health maintenance [8].

To reinforce the new health fitness approach among Thai schoolchildren, a Nutrifit programme consisting of a nutrition education and physical training model was developed. The main purpose was to create awareness of sound nutrition and physical fitness among schoolchildren and promote self-assessment of nutritional status in terms of weight-for-height and acceptable physical fitness with peer support.

Materials and methods

Preliminary background

A one-day workshop was held at the Institute of Nutrition at Mahidol University (INMU) to obtain information from physical education teachers in primary schools about their programmes and how children use their leisure time in school. All schools in Thailand provided 50 minutes of formal physical education per week as part of the school curriculum. The facilities varied depending upon the availability of sports equipment, space, and time for play during the break periods.

In addition, the teachers expressed a need to conduct physical fitness testing to identify their children's fitness status. To meet this need, the Nutrifit programme incorporated the fitness test using the established and widely used Fitnessgram [9], along with nutrition education and physical training to improve schoolchildren's nutritional status and fitness. The Fitnessgram, developed by the Cooper Institute for Aerobic Research in Dallas, Texas, USA, also assesses cardiovascular endurance, muscular strength and endurance, flexibility, and body composition. The criterion reference standard, based on the established relationships between fitness and health, represents a level of fitness that offers some degree of protection against diseases that result from sedentary living. This standard was used for test interpretation. It requires both scientific knowledge and measurement expertise, whereas the traditional norm reference is based on relative norms, including percentile scores [8].

Subjects and administration of tests

The study was conducted in two government schools (A and B) and two private schools (C and D). Children eight to nine years of age were randomly recruited from two or three third-grade classes in each school. The significance and objectives of the Nutrifit programme were explained to the school authorities, the children, and their parents. Written consent was obtained from the parents. Of those selected, 100% of the children in schools A, B, and D and 94% of those in school C participated. A total of 514 boys and girls participated in the study.

The Nutrifit programme was conducted in such a way that schools B and D received sporadic health-related fitness tests with intervention during the physical education period every other week, i.e., two 50-minute periods per month. Schools A and C received only the fitness tests without intervention. In the two government schools, there were 61 boys and 67 girls in school A and 70 boys and 80 girls in school B. Schools C and D, the private schools, had 126 and 110 students, respectively, all of whom were boys.

Physical fitness test

Health-related fitness was tested by the well-trained research team at the beginning of the first semester and a month later as the baseline. Each team member performed the same test throughout the study. The third measurement was taken at the end of the second semester. The fitness component included curl-up and trunk-lift for muscle strength, modified push-up for muscle endurance, back-saver sit and reach for flexibility, and one-mile walk/run for cardiovascular endurance. The test procedures are shown in table 1.

A "fitness corner" was set up in each school. Fitness test equipment included the gym mat with the measuring card for the curl-up test, the gym mat and a ruler with colour tape for the trunk-lift test, an adjustable wooden bar for the modified push-up test, and a box with ruler for the back-saver sit and reach test [9]. A bathroom scale and the microtoise were used for weight and height measurements. Posters on the procedure for measuring fitness and the cut-off points of the healthy fitness zone were displayed to reinforce achievement levels of physical training. The modified Fitnessgram included interpretation of health-related fitness based on the Fitnessgram criterion reference standard, as shown in table 1. The Thai reference standards established by the Subcommittee on Nutrition, Ministry of Public Health, were used for assessment

TABLE 1. Fitness tests and healthy fitness zones

Test	Age (yr)	Boys' zone		Girls' zone	
		Lower	Upper	Lower	Upper
Aerobic capacity					
1-mile walk/run (min/s) Record time of walk/run	8 9 10	13.00 12.00 11.30	10.00 9.30 9.00	14.00 13.00 12.30	10.30 10.00 9.30
Muscle strength, endurance, and flexibility					
Curl-up test (no. completed) Lies in a supine position Knees bent at an angle ~140° Count no. of continuous completed curl-ups	8 9 10	6 9 12	20 24 24	6 9 12	20 22 26
Trunk-lift (inches) Lies in a prone position (face down) Toes are pointed Hands are placed under the thighs Lifts the upper body off the floor Determine the distance of the child's chin from the floor	8 9 10	6 6 9	12 12 12	6 6 6	12 12 12
Modified push-up (no. completed) Lies down on the back Grasps the bar with an overhead grip Position with arms and legs straight, buttocks off the floor ~ 1–2 inches Count no. of correct push-ups performed	8 9 10	4 5 5	11 11 15	4 4 4	11 11 13
Back-saver sit and reach (inches) Sits down at the test apparatus One leg is fully extended against the end of the box The other knee is bent with the sole of the foot flat on the floor Arms are extended forward over the measuring scale Measure the maximal scales	8 9 10	8 8 8		9 9 9	

Source: ref. 9.

of nutritional status. Children with weight-for-height above the 97th percentile were classified as obese, those with weight-for height between the 90th and the 97th percentiles as overweight, and those with weight-for-height below the 10th percentile as underweight [10].

Nutrition and physical training education

Nutrition education was provided along with physical education to schools B and D. The children had 10 sessions during their physical education class that covered the concepts of proper nutrition and means of achieving it, how to plot their weight and height on the growth chart that was provided, interpretation of their growth chart, and the health benefits of maintaining their weight-for-height within the normal range. After each child had plotted his or her weight and height, the growth chart was evaluated by the research team, sent to the parents, and then kept in the classroom.

In terms of physical training and education, the children learned how to improve muscular strength, power, flexibility, and cardiovascular endurance. Instructional materials were given during physical education sessions by the research team. Children were asked to select their own buddies so that they could learn to perform the fitness test correctly as a team.

Interpretation

All children received an interpretation sheet to indicate the status of their weight and height and whether their fitness status was below, within, or above the healthy fitness zone. This interpretation also included written suggestions on how to improve their status. The results from each child were given to the child, the parents, and the school principal, with emphasis on the need to improve physical fitness through additional training and to create awareness of the optimal weight-for-height. Children who showed improvement in all of the fitness elements by the end of the seven-month pe-

riod received the interpretation sheet with a star label as a sign of recognition. This label has long been used as an effective reinforcement symbol, with which Thai children are familiar. With continuous improvement in all measures of physical fitness and nutritional status, the children were given a certificate and reward.

Statistical analysis

Descriptive statistics were used for nutritional status and the physical fitness test. The Wilcoxon matched-pairs signed-ranks test was used to compare pre- and post-intervention results. The level of statistical significance was $p < .05$.

Results

Nutritional status

Figure 1 shows the nutritional status of the children in the four schools. A larger proportion of children from government schools (70% and 71% in schools A and B, respectively, before intervention) were of normal nutritional status as compared with those from private schools (41% and 67% in schools C and D, respectively, before intervention). Thirty-nine percent of children from school C were classified as obese before intervention, whereas in schools A, B, and D, the rate of obesity ranged from 9% to 16%.

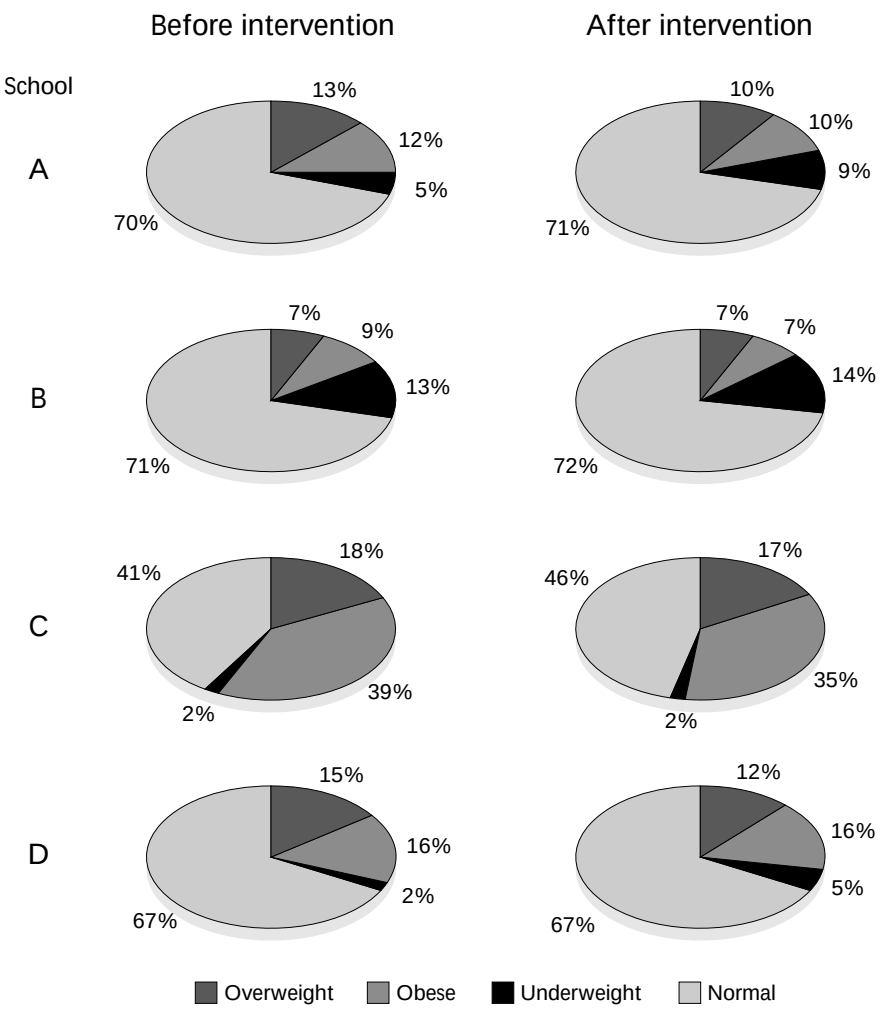

FIG. 1. Nutritional status of children in four schools before and after intervention. Schools A and B were government schools, schools C and D were private. The Thai reference standards established by the Subcommittee on Nutrition, Ministry of Public Health, were used for assessment of nutritional status. Children with weight-for-height above the 97th percentile were classified as obese, those with weight-for height between the 90th and the 97th percentiles as overweight, and those with weight-for-height below the 10th percentile as underweight [10]

Improvement of nutritional status within this seven-month period was not anticipated. However, all children received the interpretation sheet along with the individual growth chart. Nutrition guidelines were provided to overweight and obese children and their parents.

Health-related fitness

Boys and girls with Fitnessgram scores below the cut-off points of the healthy fitness zone for their sex were grouped together as "not passed." Those with scores within the fitness zone were classified as "passed," and those with scores above the fitness zone were classified as "excellent" (table 1).

The intervention using the fitness and nutrition package in school B resulted in significant improvement in muscular strength (trunk-lift and modified push-up), curl-up, and one-mile walk/run. In school D, significant improvement was observed for trunk-lift, curl-up, flexibility, and one-mile walk/run. No improvement was observed in flexibility in school B and modified push-up in school D (table 2).

Despite the lack of intervention, the authorities in school A played an important role in improving children's fitness. The names of the children who did not pass the test were listed and they were called upon to practice at the fitness corner during the school break and the physical education period. Although not all children passed the fitness test conducted by the investigator's team at the end of the second semester, there was significant improvement with regard to muscular strength (curl-up and trunk-lift) and flexibility (sit and reach), whereas modified push-up and one-mile walk/run did not show any improvement.

Fitness improvement also occurred in school C as measured by the curl-up, trunk-lift, and modified push-up. Performance was lower for aerobic capacity as measured by a one-mile walk/run. The results were partly due to the large number of overweight and obese children, who evidently had low enthusiasm for performing this endurance test. There were no changes in measurements of flexibility in this school.

A higher level of apathy existed among private school authorities and children than their government school counterparts in terms of eagerness and willingness to improve health and nutritional status. Academic achievement probably plays a more important role in private school policy, whereas exercise and play time are not stressed.

Because private schools have many obese children whose ability to pass the aerobic capacity test is low, children in these schools are more likely to be at risk. Further intervention in terms of nutrition and increase in physical activity and fitness needs to be emphasized.

Discussion

Although the nutritional status of schoolchildren is routinely measured and recorded in their health reports, it is a rather static practice, and little remedial action is taken by the school authorities. Health reports from all schools throughout the country are merely compiled to indicate the overall picture of the nutritional status of Thai children.

In this study, the nutritional education intervention enabled the children to plot their own weight and height on the growth chart. This probably increased their interest in improving themselves as compared with their peers. Nutritional guidance was given to reinforce the importance of optimal weight-for-height and ways of achieving it.

Change in the nutritional status of the children within the short period of this study was not expected. However, the children could learn about the growth chart in the Thai context. Parental viewpoints could also be obtained with reference to the Thai norm. If the growth chart of each child was plotted periodically, it would probably create awareness among children about the importance of remaining within the weight-for-height norms.

The interpretation sheet contained weight-for-height as one of the six physical fitness elements. If a child was within the zone of proper weight-for-height, one star was put on the sheet. However, for the other five elements, children received star labels only if they exceeded the cut-off points for the healthy fitness zone.

Peer pressure was observed in some children in terms of how many star labels they had achieved. They felt that they would get the recognition reward only if they received all stars in all categories. In school A, children who received this recognition were photographed with the school principal, and the picture was displayed in the classroom.

The need for recognition and star labels, however, seemed to promote performance among the children. It may be desirable to create a sense of healthy competition among peers. If they continue to improve their fitness by increased training, they may eventually learn to lead an active lifestyle.

Conclusions

The Nutrifit programme is one of the ventures proposed to create increased awareness of nutrition and fitness among young Thai schoolchildren. The health-related fitness zone can be used as an effective measure for training children to reach or exceed the fitness zone. There is need, however, to follow up on improvements in nutritional status along with fitness throughout childhood and adolescence. Eventually, children may

TABLE 2. Comparison of physical fitness of children in four schools pre- and post-intervention

School	Pre- or post-intervention	*N*	Lowest value	Highest value	Mean	SD	*Z*	*p*
1-mile walk/run								
A	Pre	122	6.50	24.28	11.60	2.49		
	Post	125	7.26	20.05	11.28	2.14	–1.73	NS
B	Pre	152	6.38	14.20	9.86	1.79		
	Post	147	6.34	12.27	9.50	1.50	–2.93	*
C	Pre	123	9.23	21.27	15.44	2.92		
	Post	109	9.09	24.38	16.05	3.72	–2.25	*
D	Pre	110	8.35	32.30	15.70	4.06		
	Post	108	7.45	24.55	14.07	3.79	–5.03	*
Curl-up								
A	Pre	126	0	36	11.36	8.75		
	Post	126	0	99	17.20	12.90	–6.24	*
B	Pre	152	0	50	13.98	10.48		
	Post	148	0	50	18.33	10.30	–5.26	*
C	Pre	126	0	56	12.24	12.19		
	Post	124	0	46	14.93	12.02	–3.97	*
D	Pre	110	0	50	14.36	12.73		
	Post	109	0	90	18.01	13.99	–3.73	*
Trunk-lift								
A	Pre	126	4.8	14.2	9.37	1.84		
	Post	126	6.9	15.5	10.81	1.76	–7.36	*
B	Pre	152	6.6	16.5	9.47	1.62		
	Post	148	6.8	18.0	11.53	2.00	–9.42	*
C	Pre	126	4.1	14.2	9.30	1.85		
	Post	124	6.5	15.8	11.05	1.72	–7.91	*
D	Pre	110	6.2	17.9	9.20	1.78		
	Post	109	3.5	17.5	10.54	2.03	–6.17	*
Push-up								
A	Pre	126	0	24	8.99	5.16		
	Post	126	0	31	8.72	4.71	–0.80	NS
B	Pre	152	1	26	9.59	4.58		
	Post	148	3	24	10.94	4.54	–4.23	*
C	Pre	126	0	21	6.59	4.80		
	Post	123	0	20	7.71	3.84	–3.00	*
D	Pre	110	1	20	7.39	3.80		
	Post	109	0	20	7.65	4.03	–0.39	NS
Flexibility								
A	Pre	126	4.6	14.6	9.64	2.16		
	Post	126	4.0	14.9	10.33	2.14	–4.12	*
B	Pre	152	6.0	14.6	10.39	1.66		
	Post	148	5.0	14.5	10.34	1.70	–0.53	NS
C	Pre	126	4.0	13.8	9.42	2.13		
	Post	124	3.0	14.5	9.42	2.42	–0.05	NS
D	Pre	110	4.5	13.5	9.12	2.00		
	Post	109	3.7	14.0	9.94	1.93	–4.60	*

* $p < .05$, Wilcoxon matched-pairs signed-ranks test.

show individual concern, and an awareness of nutrition and physical fitness may be created among them. The overall picture of fitness along with specific components should also be targeted so that physical education instructors can examine and possibly modify the existing physical education curriculum to improve fitness among their own schoolchildren.

In this study interpretation of fitness results was effective not only in encouraging children to improve their fitness status, but also in creating awareness among school administrators. This has been a preliminary attempt, and further work in this direction is necessary. Assessment and evaluation of nutrition and physical fitness should become an essential part of the physical education policy in schools. This will help to promote the emergence of a healthy and energetic Thai younger generation.

Acknowledgements

We thank Mr. Weerachat Srichan for his role in physical training and typing the manuscript, Ms. Piyanuch Visetchart for providing statistical assistance, and Dr. Lalita Bhattacharjee for editing and formatting this manuscript.

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Recommending vitamin A–rich foods in southern Thailand

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Abstract

Thailand is classified by the World Health Organization as a country with a moderate level of subclinical vitamin A deficiency. In 1992 the eruption of infant xerophthalmia in the country's lower region led to the universal distribution of vitamin A capsules among infants (50,000 IU for those < 6 months old, 100,000 IU for those 6–12 months old) and pre-school children (200,000 IU) in high-risk areas every 6 months. Since then, no new cases of xerophthalmia have arisen. The universal distribution programme covered the period 1992 to 1998. However, to control the problem and prevent it from reoccurring once capsule distribution is discontinued, a food-based strategy for the prevention of vitamin A deficiency is being undertaken in southern Thailand. This strategy has two components: recommendation of vitamin A–rich food recipes for consumption by pregnant women, and development of vitamin A–rich snacks targeted at children. Both of these components have been adopted and successfully promoted by the Health Promotion Center (Region 12) in southern Thailand as part of public special events, regular counselling, and education programmes, and as micro-level income-generation projects by community women's groups.

Introduction

The challenge of vitamin A deficiency in Thailand dates back over three decades. On the basis of community surveys and hospital reports in the 1960s and 1970s, Thailand's Fourth National Economic and Social Development Plan (NESDP) from 1977 to 1981 included vitamin A deficiency as one of the seven national nutritional problems. Since then, Thailand has launched an intensive programme nationwide to reduce poverty, improve primary health-care services, and increase household food security among rural poor families [1]. Vitamin A considerations were integrated into Thailand's health and nutrition policies, plans, and programmes along with other nutrition problems rather than forming a separate programme. These efforts led to a marked decline in clinical vitamin A deficiency among young children [2] such that in 1985 the World Health Organization (WHO) classified Thailand as a nation where vitamin A deficiency was not a public health problem, although sporadic cases might occur [3].

By the end of the Fifth NESDP (1982–1986), case reports of xerophthalmia had become rare and the country began to enter a transition in its vitamin A deficiency problem from clinical to subclinical levels [2]. This was confirmed by a 1990 prevalence survey of vitamin A deficiency in north and north-east Thailand, which indicated that approximately one-fifth of preschool children in these regions experience subclinical deficiency, as evidenced by low liver stores and abnormal conjunctival epithelium [4].

Although the problem in northern and north-eastern Thailand is marginal, the eruption of infant xerophthalmia in the lower southern region in 1992 called for the universal distribution of vitamin A capsules every 6 months to infants (50,000 IU for those < 6 months old, 100,000 IU for those 6–12 months old) and preschool children (200,000 IU) residing in the endemic areas [5]. This programme, covering the period from 1992 to 1998, resulted in no new reported cases of xerophthalmia [6]. At present, WHO has classified Thailand as a country with a moderate level of subclinical vitamin A deficiency [7].

The supplementation programme conducted in the south, however, cannot assure that the problem will not reoccur, particularly in view of the fact that it was discontinued at the end of the fiscal year 1998. As a result, the Institute of Nutrition at Mahidol University (INMU) and the Division of Nutrition, Ministry

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of Public Health, have worked closely to develop a more sustainable food-based approach for the prevention of vitamin A deficiency.

This approach draws on lessons learned from similar programmes that have been conducted in other regions of Thailand. For instance, vitamin A–rich foods such as liver, eggs, whole milk, dark-green leafy vegetables, and yellow and orange fruits and vegetables are available or can be grown in most communities throughout the country. A long-standing problem has been that these foods have been underutilized because of seasonal variation, lack of dietary appeal, or prevailing traditional food habits. However, earlier experience in social marketing of vitamin A–rich foods, such as the ivy gourd plant (*Coccinia indica*) in north-east Thailand, demonstrated that even in a relatively short time, significant changes in dietary habits can be achieved provided that foods and recipes amenable to the local culture are identified and properly promoted, not only in terms of their nutritional benefit but also in terms of other advantages (e.g., income generation) [8].

Another important factor is the effect of local preparation and preservation techniques that may cause a considerable loss of vitamin A activity. This question was addressed in a recent multicentre study involving India, Indonesia, and Thailand [9]. In Thailand the project was conducted by INMU in the north-east region. The activities consisted of identification of vitamin A–rich foods that are available all year, especially those from plant sources, followed by investigation of the loss of vitamin A activity due to local preparation and preservation techniques, and finally recommendation of the consumption of vitamin A–rich foods appropriate for young children and adults [9]. Lessons learned from this process were adapted for transfer and testing in southern Thailand.

Vitamin A recipes in the lower southern region

Southern Thailand, particularly the lower provinces, is inhabited by a Thai Muslim population who have different religious beliefs and indigenous foods than the general Thai Buddhist population. As a result, project personnel first sought an understanding of Thai Muslim dietary habits, food beliefs, and lifestyle. Initially, existing data were compiled about the consumption pattern of foods rich in vitamin A and other related factors, such as seasonal food sources and popular foods or frequently consumed foods. These data were then confirmed in focus group discussions with caretakers of pre-school children, pregnant women, lactating mothers, and community elders. These representative groups provided detailed information concerning the amount and frequency of consumption of vitamin A–rich food as well as local recipes (main dishes and desserts) containing vitamin A–rich items. Thereafter, several recipes were selected on the basis of availability and their high potential for promotion among the target groups. Local housewives also demonstrated methods of preparing these selected recipes, and data were collected concerning the ingredients (type and amount) that were used, steps in food preparation, temperature, and cooking time. Vitamin A contents were estimated by calculation from the existing database of vitamin A contents of food, taking into account the loss of vitamin A from different cooking procedures [10]. A few processed foods, desserts, and wild-grown young leaves whose vitamin A contents were unknown were analysed for retinol and β-carotene concentrations by high-performance liquid chromatography [11].

The recipes were then divided into four categories based on their vitamin A content per serving in comparison to the recommended daily allowance (RDA) for pregnant women [12](table 1). One additional strong point in favour of these local recipes is that the type and amount of ingredients for certain dishes, such as curry, can vary. Thus, ingredients that are high in vitamin A could easily replace other ingredients low in vitamin A. The local recipes were also grouped according to their vitamin A content in order to give a list of recommended recipes that could be more feasibly and practically promoted. For example, pregnant women should ideally consume group I recipes, which contain liver, once a week. On the other hand, those who are not able to consume animal food may select recipes in groups II or III, which contain eggs, vitamin A–rich vegetables, or both, along with drinking an additional one to two glasses of milk per day to meet their RDA. Those pregnant women who can consume neither liver nor milk can select group II and III recipes for regular consumption on the condition that their vitamin A content be increased through the addition of more eggs or vitamin A–rich vegetables to these recipes. A similar pattern of alternative recipes can be developed for pre-school children and schoolchildren.

Development of vitamin A–rich snacks: A food-to-food approach

A survey conducted in food shops and weekend markets in the community found that fish chips were readily available and consumed as daily snacks by people of almost all ages. They were eaten especially by people of low income because of their low cost (1 baht = US$0.05 or 5 cents per package). Therefore, fish chips were selected as a suitable vehicle for enrichment with vitamin A by adding liver. The goal was to provide at least 20% of the RDA for pre-school children or 80 retinol equivalents (RE) per package sold (four chips per package), while maintaining the texture and flavour of local chips as well as the price. Development

TABLE 1. Recommended local recipes containing animal and plant sources of vitamin A

Category	RE of vitamin A per serving (% RDA for pregnant women)[a]	Recipe group	Local recipe
I	2,400 (>100)	Liver recipes	Fried chicken liver
II	161–234 (≥20)	Recipes with eggs plus vitamin A–rich vegetables	Stir-fried Chinese swamp cabbage with eggs
			Egg drop soup with Chinese cabbage
		Yellow or orange-coloured vegetables and fruits with consumption size ≥ 100 g	Steamed pumpkin dipped in sugar
			Ripe mango, large (kaew variety)
III	77–161 (10–19)	Boiled egg menus	Thumi egg (local dish)
		Omelet with vitamin A–rich vegetables	Egg curry
		Recipes with vitamin A–rich vegetables	Omelet with maroom leaves
			Mustard green soup
			Sa-yo-eye (local dish) with pumpkin/ sweet potato (yellow)
IV	7–81 (<10)	Recipes with vegetables of moderate vitamin A content	Kale
		Mixed vegetables consumed as fresh or blanched with fermented rice noodles	Sa-yo-la-moh (local dish) with spiral-leaved pumpkin
			Young cashew nut leaves, kood, kasem leaves
		Local desserts containing eggs	Indian pennywort
			Kor-doh, ar-koh, nae-bah pee-ar-na (local desserts)

Source: ref. 12.
a. The RDA for pregnant women is 800 RE.

of the recipes containing fish and liver was based on the traditional formula for preparing fish chips, using similar ingredients and following as much as possible the same procedure as the local industry.

Both recipe development and feasibility tests showed that it was possible to add either chicken or beef liver to the locally produced fish chips. Recipe formulation also took into account the findings of a preliminary sensory test showing that chips that retained their fish flavour were more acceptable. On a production scale, the ratio of flour to animal sources was 3:2. The flour mixture consisted of sago flour and tapioca flour at a 2:1 ratio. The ratios of fish to liver were 1:1 for chips with chicken liver and 3:1, 7:3, and 5:3 for those with beef liver. Three different methods of preparation were tested: boiling and sun-drying, boiling and oven-drying, and steaming and oven-drying. The losses of vitamin A from these procedures were 90%, 70%, and 50%, respectively. The vitamin A contents of chips prepared with different formulas and procedures are shown in table 2. Chips prepared by steaming and oven drying contained 21% to 32% of the RDA of vitamin A for pre-school children per package.

When a sensory test of the chips was conducted among adult women and pre-school children two to six years of age, the acceptability scores from a five-point face scale were in the range of "slightly preferred" to "highly preferred." The acceptability was good for chips given in addition to complementary foods (rice congee or porridges) to 6- to 24-month-old children who were also being breastfed. Mothers were encouraged to feed the children up to one bag of four chips per day, which contributed about 25% to 30% of the RDA for vitamin A. Another interesting point is that over 80% of those who did not normally consume liver (regardless of cooking methods) gave a score of "satisfactory" to "like very much" to the liver-containing chips.

One problem that arose was the high production cost of the liver chips. This was resolved by reducing the size of the enriched chips by 20%, thus enabling the selling price to be comparable to that of regular fish chips (1 baht per bag). One of the considerations with the size reduction was that the end product should still contribute at least 20% of the RDA for pre-school chil-

TABLE 2. Amount of vitamin A in the fish and liver chips recipes that were adapted for commercial production according to the percentage of the RDA for pre-school children

Recipe	RE of vitamin A per package of 4 chips (%RDA for pre-school children)[a]		
	Boiled and sun-dried: 90% loss	Boiled and oven-dried: 70% loss	Steamed and oven-dried: 50% loss
Fish:chicken liver			
1:1	24 (6.0)	71 (17.8)	119 (29.8)
Fish:beef liver			
3:1	17 (4.3)	52 (12.9)	86 (21.4)
7:3	21 (5.2)	62 (15.4)	103 (25.7)
5:3	26 (6.4)	77 (19.3)	129 (32.1)

Source: ref. 12.
a. The RDA for pre-school children is 400 RE.

dren. Another problem was a loss in vitamin A activity of up to 90% due to the boiling and sun-drying process as employed locally, which reduced the vitamin A activity to 4% to 6% of the RDA for the pre-school age group. To reduce such losses, the use of a solar dryer or electric oven instead of sun-drying was recommended, which resulted in a loss of vitamin A of approximately 70%. In addition, if this process was further improved by employing steaming rather than boiling, the loss of vitamin A dropped to 50%. With these improved methods, the vitamin A concentration in a 1-baht bag of fish and liver chips could account for 20% to 30% of the RDA for pre-school children.

Conclusions and recommendations

Following the development of the vitamin A recipes and the fish and liver chips, Thailand's Health Promotion Center, Region 12, which is responsible for the lower southern provinces, with the support of the Division of Nutrition, Department of Health, Ministry of Public Health, organized several events to promote the vitamin A–rich recipes and snacks at both the provincial and the district levels. These events included recipe competitions and, more recently, a "Vitamin A Day," all of which received wide interest and participation among people living in the target provinces of Yala, Pattani, and Narathivat. District health officers are also recommending the different vitamin A recipe groups as part of their regular counselling and education programmes. In addition, the Health Promotion Center together with the Agricultural Extension Service has disseminated and promoted the fish and liver chip recipes to the public as well as providing technical assistance to women's groups to produce the chips using solar dryers. The chips are then marketed as packaged products through health posts at a compatible price (1 baht or 2.5 cents per package). Additional evaluation involving biological indicators of vitamin A status and quality-control measures will be undertaken to further assess the effectiveness of the promotion of vitamin A–rich foods in southern Thailand.

Acknowledgements

We are grateful for the assistance of the field team from the Health Promotion Center, Region 12, the community fieldworkers, and participating housewives during data collection. We would also like to thank Dr. Sangsom Sinawat and Miss Kandawasee Maleevongse for providing field support and helpful suggestions. This work was supported by the UNICEF Office for Thailand through the Department of Health, Ministry of Public Health, Thailand.

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Sustaining behavioural change to enhance micronutrient status through community- and women-based interventions in north-east Thailand: Vitamin A

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Abstract

This project was designed as a follow-up of the Social Marketing of Vitamin A–Rich Food (SM/VAF) Project in north-east Thailand. It began in 1996 to determine whether the positive changes indicated at the end of the prior project were sustained in the intervention district and to develop a process to foster the changes achieved from the earlier effort. After one year of implementation, the results showed cumulative improvement in knowledge, attitudes, and practice with respect to intake of vitamin A and fat. A sustained improvement in the consumption of vitamin A–rich foods and fat towards fulfilment of the recommended dietary allowance was clearly demonstrated among pre-school children. During the current project, serum retinol levels among schoolgirls (10–13 years of age) improved from 22.75 ± 6.96 to 33.69 ± 8.25 μg/dl in the intervention group ($p < .001$*), while no significant change was observed in the control group. The difference between intervention and control areas in the change in serum retinol before and after project implementation was highly significant (10.94 ± 9.09 and 3.24 ± 9.14,* $p < .0001$*).*

Introduction

Elimination of micronutrient deficiencies is an important concern of all developing countries in the present decade. To date, efforts to control these deficiencies have shown promising results, especially with vitamin A and iodine [1]. There have been concerns, however, about the sustainability of the changes, especially with regard to addressing the root causes of malnutrition and assisting communities to empower themselves towards the control of these preventable problems. The project was based on the Social Marketing of Vitamin A–Rich Foods (SM/VAF) Project (1989–1991) [2–12], which is now being institutionalized within the existing governmental system in Thailand [13].

It was realized that although the SM/VAF Project was successful in changing broad community behaviour on the one hand, and short-term individual behaviour on the other, sustaining behavioural change would require greater community strengthening and empowerment. Therefore, it was necessary to further develop a process that would facilitate the sustainability of behavioural change and expand the change process to control and prevent not only vitamin A deficiency, but also iron-deficiency anaemia and iodine-deficiency disorders for long-term nutritional development. This additional effort focused on using a social marketing approach to design, implement, and control activities calculated to influence community women to be actively involved in micronutrient implementation. Through empowerment, these women would not only be qualified but would also be able to motivate their communities. They would collectively create culturally appropriate community actions to overcome micronutrient deficiency.

Description of the project

Approaches used

Before the current project, a preliminary study using both quantitative and qualitative methods revealed that, in general, the vitamin A changes had persisted in the intervention district. Most local government officials and community members still had positive attitudes

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towards the SM/VAF Project; however, the level of excitement had decreased. It was therefore believed that behavioural change would be sustained if there was greater community involvement coupled with increased local governmental participation in the change process.

A process was developed to strengthen nutritional development in four selected intervention subdistricts. Three main approaches were used to guide project implementation. First, social marketing [14] was adopted as an overall approach to design, implement, and control activities to influence community women to take more action to improve not only vitamin A but iron and iodine status as well. Second, the Cornell Modified Community-Based Nutrition Monitoring (CBNM) Model [15] was selected as an approach to strengthening community organization in the nutrition problem-solving process by linking local decision makers and stakeholders to enhance their dual participatory roles. Finally, the Appreciation-Influence-Control (A-I-C) Approach [16, 17] was used to involve the main actors or a core group (women leaders) from the beginning in community planning and decision making and to prompt them to participate actively in the action plan. Later on, a modified A-I-C approach was also used to finalize these plans in the target communities.

Intervention

The project's intervention strategies were formulated on the basis of the results of formative research. Therefore, when the baseline data collection was complete, educational calendars were distributed to the target mothers first. Later, four researchers spent one week in the intervention subdistricts to conduct participatory observations in order to gain more in-depth understanding of the target populations. This activity permitted the identification of women community leaders, the collection of in-depth information on their social network and way of life, and the establishment of closer relationships between the project team and the target communities. At the same time, the project team worked with several local government officers to organize the project district advisory board and the project district and subdistrict task force.

After the basic foundation was in place, the project team, together with the district task force members, organized a three-day A-I-C workshop for 30 selected women leaders from the target communities. The aim of the workshop was to bring these women leaders together to achieve innovation, empowerment, shared vision, and collaborative action for the improvement of micronutrient status in their communities. Sixteen projects were identified by the women for community action, including horticultural activities; mother and child care; promotion of community involvement; promotion of maternal health, including iron supplementation to all women of reproductive age, provision of iodized salt for family consumption, and reduction of parasitic infestation (hookworm); and income-generating activities.

Participation from district to community levels was organized based on the framework proposed in the CBNM problem-solving model. A community advisory group was subsequently formed, and 180 people were identified by the core group. Discussion of action plans from the earlier workshop, followed by the modified A-I-C process workshop, helped to finalize the community action plan in each target community.

After the initial period, the project team provided 3,000 to 5,000 baht (approximately US$85 to $142) per community (a total of 53,000 baht for all target communities) as seed money for their interventions. Communities that showed outstanding achievements in the short term received more money as a reward. The project team also supported the core group with educational and motivational material for their community broadcasting activities, which took place approximately three times a week in each community. Direct mailings were sent to all involved in the project organizational structures as well as to all pregnant and lactating women and mothers of young children in order to report on the progress of the intervention, supply information on micronutrients, and motivate further actions.

During the project, it was observed that the women leaders took an active role in the implementation of the communication intervention activities, including operating village broadcasting and providing feedback to the mailings. They also made home visits to target populations to deliver important messages, as well as other promotional materials, such as seeds, green vegetables, iron pills, and iodized salt. They organized regular village meetings with members of their community task force group and started action programmes in the key areas identified as a result of the A-I-C process. The most outstanding action was growing green vegetables. Most target communities successfully grew green leafy vegetables, and ivy gourd was grown by almost every household as part of their home garden activities to promote the consumption of vitamin A–rich food. The women leaders of three communities organized community green vegetable gardens for village consumption and sale.

Together with district and subdistrict task forces and with the cooperation of four government sectors—health, education, agriculture, and rural development—and the project team, a mobile school nutritional education campaign was organized for all schools in the intervention area to promote micronutrient nutrition and weekly iron supplementation (60 mg) of adolescent girls. The project team also assisted community health centres in their campaigns to reduce parasitic infestation. They also organized a study tour for selected members of the core group to visit a community that successfully developed a unique integrated agriculture system.

Table 1 summarizes key community- and school-based activities of the project.

During the intervention, one field coordinator was assigned to monitor the change process and provide immediate assistance to the communities when necessary. At the middle of the intervention period, eight student volunteers spent seven days in both the intervention and control subdistricts to monitor the changes in each target community.

Methods of evaluation of the project

Project design

The project adopted a simple quasi-experimental research design in the intervention and control areas before (T_1) and after (T_2) implementation of the project. The three target groups covered were primary (pregnant and lactating mothers and pre-school children), secondary (fertile women and schoolchildren), and tertiary (influencers of the first two groups) target groups. Four subdistricts from the SM/VAF Project intervention district (Kantharom District, Si-Sa-Ket Province) were selected for the intervention area and four other subdistricts for the control area. On the basis of government information and direct observation by the project team, the two groups were found to be comparable in socio-economic status, physical facilities, levels of community leadership, and health and nutritional status.

Simple randomization was the sampling technique used for quantitative evaluation. On the basis of the previous experience of the SM/VAF Project, the sample size was determined for a confidence level of 95%, a power of 90%, a difference between means of 0.4, and a pooled variance of 1.64 (reported behaviours, points intervention). A sample size of 354 for each group (intervention and control) was thus selected.

Serum retinol levels in schoolgirls aged 10 to 13 years were determined in non-fasting venous blood samples by high-performance liquid chromatography [18]. These samples were treated, portioned, and frozen for shipment from the field to the laboratory of the Institute of Nutrition at Mahidol University, where they were stored at –20°C until analysis. Dietary intakes were determined in the primary target group and in schoolgirls by a modified 24-hour dietary recall. AKAP (Awareness, Knowledge, Attitudes, and Practices) was assessed using both quantitative and qualitative methods.

Data analysis

The SPSS/PC programme was used for statistical analysis. Nutritional intakes were calculated from 24-hour dietary recalls by the INMUCAL computer programme. Qualitative data were collected and analysed by an experienced researcher of the Institute of Social Research, Chulalongkorn University.

Values are expressed as means ± SD or percentage

TABLE 1. Key community- and school-based activities of the current project

Communication intervention activities	Action programmes
Community-based	
Village broadcasts on micronutrients by women leaders three times a week	Growing green vegetables in households, rice fields, and community gardens
Direct mail to key actors in the change process, including pregnant and lactating women	Promoting maternal health by iron pills, iodized salt, etc.
Billboards from district advisory board to promote consumption of green vegetables, iron pills, and iodized salt	Growing fruit trees in household and community gardens
Home visits to target households by women leaders and community task force groups	Promoting community participation
Interpersonal communication between women leaders and advisors and community members as well as subdistrict officials	Income-generating activities
Village meetings among community leaders and key actors	Providing nutritious foods to pre-schoolers and communities (in some communities only)
	Motivational study tour
School-based	
Mobile nutrition education campaigns	Food demonstrations focusing on micronutrient-rich foods
Billboard contest in primary schools	Improvement of school lunch menu
School broadcasting activities	Encouraging production of vegetables, poultry, and fish

of the total number of subjects. Differences in blood values and dietary intakes within each target group (preschool, schoolgirls 10 to 13 years of age, and lactating and pregnant mothers) across the two assessment periods before (T_1) and after (T_2) the intervention were tested, as well as differences between intervention and control groups. Other statistical tests included the group *t* test for KAP and dietary assessment studies, and the paired *t* test and the McNemar test for serum retinol. The association between T_1 and T_2 was determined by chi-square and Fisher's exact test.

Project results: Vitamin A

Knowledge, attitudes, and reported practices indicated a strong foundation for change

Based on the success of the Social Marketing for Vitamin A–Rich Foods (SM/VAF) Project in Kanthararom District, significant changes in KAP between 1989 and 1991 were seen, which continued markedly up to 1995 and beyond, especially with regard to knowledge and practices (table 2). The knowledge and attitudes of the target group regarding vitamin A–rich foods in both the intervention and control areas remained at a similar level at the beginning of the current project intervention. With the contribution of the new input, the knowledge scores of the intervention group increased significantly while the knowledge scores of the control group remained the same. The attitude scores in the intervention group increased slightly as compared with the control group. However, attitudinal improvements were observed in both areas. A significant increase in practice scores was observed in the intervention group but not in the control group. The input from the current project was able to strengthen knowledge, attitudes, and practices in the intervention area, which should help sustain a positive change in the target communities. Nevertheless, the positive change in attitudes and practices also observed in the control group indicated that the effects of the SM/VAF Project still generated further improvement in behaviour related to vitamin A among the target population in Kanthararom District, even without additional intervention.

Distinct achievement in changing dietary vitamin A and fat intakes

Table 3 shows vitamin A and fat intakes of the target groups of the SM/VAF Project and the current project.

TABLE 2. Changes in KAP with respect to consumption of vitamin A–rich foods in the intervention (Kanthararom) and control (Trakan Phutphon) areas before (1989) and after (1991) the SM/VAF Project, between the end of the SM/VAF Project (1991) and four years later (1995), and before (1996) and after (1997) the current intervention project[a]

		1989-91		1991–95		1996–97	
KAP profile	Area	Change	*p*	Change	*p*	Change	*p*
Knowledge	SM/VAF Project						
	Intervention	+ 0.90	< .005	+ 1.29	<0.005		
	Control	+ 0.06	NS				
	Current project						
	Intervention					+ 1.5	< .001
	Control					0.0	NS
Attitude	SM/VAF Project						
	Intervention	+ 3.14	< .005	+ 0.94	NS		
	Control	+ 1.55	< .05				
	Current project						
	Intervention					+ 3.3	<.001
	Control					+ 1.1	<.05
Practice	SM/VAF Project						
	Intervention	0.18	< .05	+ 0.23	NS		
	Control	– 0.32	< .005				
	Current project						
	Intervention				+ 0.9	1.1	< .001
	Control				+ 0.2		NS

a. The total numbers of subjects in the SM/VAF Project in 1989 were 622 in the intervention group and 426 in the control group. In 1991 the numbers were 624 and 461, respectively. Four years after the SM/VAF Project in 1995, there were 176 in the intervention group only. In the current project, there were 369 and 349 subjects in the intervention and control groups, respectively, in 1996 and 271 and 247 in 1997.

TABLE 3. Vitamin A and fat intakes of target groups in Kanthararom District (1989–1995) and before and after the current project intervention (according to 24-hour dietary recall)

Time	Pre-school children (25–60 mo)		Schoolchildren (10–13 yr)		Lactating women		Pregnant women	
	Vit A[a]	Fat[b]	Vit A[a]	Fat[b]	Vit A[a]	Fat[b]	Vit A[a]	Fat[b]
Before SM/VAF Project (1989)	45	14	35	13	29	12	–	–
After SM/VAF Project (1991)	80	13	81	14	52	11	–	–
4 yr later (1995)	98	22	60	16	65	13	–	–
Current project—mean ± SD (no. of subjects)								
Intervention								
1996	133±246 (171)	18	41±61 (69)	12	32±57 (65)	8	44±44 (41)	12
1997	142±262[c] (152)	21	72±115[d] (98)	16	71±90[d] (74)	12	89±118[c] (44)	10
Control								
1996	101±244[e] (146)	17[e]	38±35[e] (77)	13[e]	33±35[e] (58)	10[e]	48±34[e] (33)	12[e]
1997	158±296[d] (136)	22	59±79[c] (91)	14	47±65[c] (69)	8	64±94 (36)	10

a. Values are % of RDA of vitamin A.
b. Values are % of total caloric intake (recommended level is 20%–30%).
c. $p < .05$, 1996 vs. 1997, Mann-Whitney U test.
d. $p < .01$, 1996 vs. 1997, Mann-Whitney U test.
e. No significant difference between intervention and control groups in 1996.

Pre-schoolers

There was a progressive increase in vitamin A and fat consumption among the pre-school children from 1989 to 1991 (during the SM/VAF Project) and thereafter from 1991 to 1995. This population represented both the intervention and the control groups of the current project (the SM/VAF Project covered all areas in Kanthararom District). The intakes of vitamin A and fat of both intervention and control groups showed a sustained increase. The intake of vitamin A increased from about half the RDA to almost the full RDA at the end of the prior project and remained so four years later. An intake of more than 100% of the RDA was recognized before the implementation of the current project, and continuous improvement was observed in both areas at its completion. Fat intake increased from 13% of total caloric intake at the end of the SM/VAF Project to 22% four years later in the pre-school population of Kanthrararom District. In the intervention and control areas, the fat intakes dropped to 18% and 17% of total intake, respectively, before the additional intervention. Afterward they increased to 21% and 22% of total intake (20%–30% is currently recommended).

Schoolchildren

In the school-age group (10 to 13 years), the vitamin A intake more than doubled between 1989 and 1991 due to the impact of the SM/VAF Project. The intake dropped from 81% of the RDA in 1991 to 60% of the RDA in 1995 for the school-age population in the target district. At the start of the current project, the intakes were about 41% and 38% of the RDA in the intervention and control areas, respectively. By the end of the project, the respective intakes had increased to 72% and 59% of the RDA. Fat intake as a percentage of total calories increased from only 14% after the SM/VAF Project to 16% four years later. At baseline it was 12% and 13% in the intervention and control areas, respectively, and improved to 16% and 14%.

Mothers

For lactating mothers, vitamin A intake followed a similar pattern as in the school-age group. The effect of the current intervention coincided with that in the school-age group, being more pronounced in the intervention area. Follow-up data on pregnant mothers, obtained only in the current project, revealed a significant increase in vitamin A intake from 44% to 89% of the RDA in the intervention group. The fat intake of mothers did not improve significantly.

Changes in serum retinol levels in schoolchildren

After improvements had been demonstrated in knowledge, practices, and dietary intakes of vitamin A and

fat in pre-school children and schoolchildren, the effects on serum vitamin A levels were assessed in schoolchildren. A highly significant change in the mean serum retinol levels of the paired adolescent girls before and after project implementation was observed (22.75 vs. 33.69 μg/dl), whereas the change was not significant in the control group (table 4).

The difference between the intervention and control areas in the change in serum retinol before (T_1) and after (T_2) project intervention was highly significant (10.94 ± 9.09 and 3.24 ± 9.14, $p < .0001$). A significant reduction in the percentage of subjects who had inadequate vitamin A levels in the intervention group was observed (90.5% vs. 40.5%), whereas the change was not significant in the control group. There was a significant reduction in the percentage of subjects with low levels of vitamin A (10.00–20.00 μg/dl) in the intervention group from 40.5% to 2.4%, but not in the control group (table 5). The differences between 1996 and 1997 were significant according to the McNemar test (table 6).

In the intervention area, 17 subjects with low levels of serum retinol (< 20 μg/dl) in 1996 had improved retinol levels in 1997. In addition, a more marked shift in the distribution of serum retinol levels before and after project implementation was found in the intervention group than in the control group (fig. 1). The improvement in the distribution of serum retinol levels was highly significant at all cut-off points in the intervention group.

TABLE 4. Serum retinol levels (μg/dl) in schoolgirls 10–13 years of age before and after the current project intervention (paired subjects)

Year	Intervention group (N = 42)	Control group (N = 36)
1996	22.75 ± 6.96	26.51 ± 6.95[a]
1997	33.69 ± 8.25[b]	29.75 ± 6.72[a]
Change	+10.94 ± 9.09	+3.24 ± 9.14[c]

a. $p < .025$, intervention vs. control group within years, group *t* test.
b. $p < .001$, 1996 vs. 1997, paired *t* test.
c. $p < .0001$, intervention vs. control group, group *t* test.

Contribution to sustainable nutritional development

Qualitative data also indicated positive changes in the intervention area. Most of the project activities were implemented as planned. In a few communities, project implementation was reported to be less adequate because of internal conflicts, weak community leadership, lack of time of the members, and ineffective officers at the subdistrict and community level. Nevertheless, most of the community members and local government officials who recognized the benefits of the project showed willingness to foster the changes that had set in. At least

TABLE 5. Percent distribution of serum retinol levels among schoolgirls 10–13 years of age before and after the current project intervention (paired subjects)

Serum retinol (μg/dl)[a]	1996		1997	
	No.	%	No.	%
Intervention group (N=42)				
< 10.00	0	0	0	0
10.00–20.00	17	40.5	1	2.4[b]
20.01–30.00	21	50.0	16	38.1
< 30.00	38	90.5	17	40.5[c]
> 30.00	4	9.5	25	59.5
Control group (N=36)				
< 10.00	0	0	0	0
10.00–20.00	5	13.9	3	8.3
20.01–30.00	21	58.3	17	47.2
< 30.00	26	72.2	20	55.5
> 30.00	10	27.8	16	44.4

a. Serum retinol levels < 10 μg/dl are classified as deficient, levels of 10–20 μg/dl as low [19], levels of 20.01–30.00 μg/dl as marginal, levels < 30 μg/dl as inadequate, and levels > 30 μg/dl as adequate [20, 21].
b. p < .0001 (Fisher's exact test) for the comparison between 1996 and 1997 in the percentage of subjects with retinol levels ≤ 20 μg/dl (low levels).
c. p < .0001 (Fisher's exact test) for the comparison between 1996 and 1997 in the percentage of subjects with retinol levels ≤ 30 μg/dl (inadequate levels).
d. There were no statistically significant changes in the distribution of retinol levels between 1996 and 1997 in the control group.

TABLE 6. Changes in the numbers of schoolgirls 10–13 years of age classified according to different cut-off points of serum retinol in 1996 and 1997 (p values calculated by McNemar test)[a]

A. Cut-off point of 20 μg/dl (low level)

Intervention group

		1997	
		Normal	Deficient
1996	Normal	24	1
	Deficient	17	0

$p < .001$ ($N = 42$)

Control group

		1997	
		Normal	Deficient
1996	Normal	28	3
	Deficient	5	0

$p > .05$ ($N = 36$)

B. Cut-off point of 30 μg/dl (inadequate level)

Intervention group

		1997	
		Normal	Deficient
1996	Normal	4	0
	Deficient	21	17

$p < .0001$ ($N = 42$)

Control group

		1997	
		Normal	Deficient
1996	Normal	5	5
	Deficient	11	15

$p > .05$ ($N = 36$)

a. No children with deficient levels of serum retinol (< 10.0 μg/dl) were found in this study.

64 women leaders in the intervention area were motivated and actively participated in community actions. They worked with 132 other community members. At least 15 of the women leaders in the four intervention subdistricts were highly able to work towards definite nutritional improvement in their communities.

Discussion

The overall results indicated that the intervention process successfully strengthened the behavioural changes achieved by the prior social marketing project and was helpful in sustaining the positive changes in the target communities. Even without the current project, the SM/VAF Project continuously contributed to the improvement of vitamin A and fat consumption by pre-school children in Kanthararom District. This may be because changes in this group were a high priority for mothers and caretakers. Also, less effort was required to deal with both food preferences and economic resources. Sustainability through a progressively increased consumption of vitamin A and fat, approaching the RDA, was clearly observed in the pre-school children.

The behavioural changes were more complicated for the schoolchildren, because, as was general practice in the target communities, their food intake was seldom supervised by adults. The improvement observed in the intervention group could possibly be attributed to school and community education and communication as well as the school-based communication intervention activities, action programmes, and lunch programmes initiated by the current project. The diets of schoolchildren in Kanthararom depended a great deal on the school lunch programmes, and schoolteachers needed continuous encouragement to maintain the quality of such programmes. Because of a good foundation contributed by the prior project as well as the current one, positive changes could be achieved among this group within a short period.

Effects on pregnant and lactating mothers might require more time, encouragement (to counteract food aversions, especially the dislike of fat and oil among adult northeastern Thai populations), and family resources (because of the high price of fat and oil) to show satisfactory changes.

Conclusions

The cumulative efforts of the SM/VAF Project and the current project emerged as (1) a successful process of community-based nutritional development that altered nutritional factors to affect human health and quality of life positively through active participation of people as actors, and (2) an example of a women-centered approach to mobilize women as change and intervention agents, given their critical and key roles in household nutrition and decision making.

It is hoped that this experience will serve as an example to show policy makers and implementors how community- and women-based interventions to increase the production and consumption of vitamin A–rich foods can be feasibly planned and implemented within

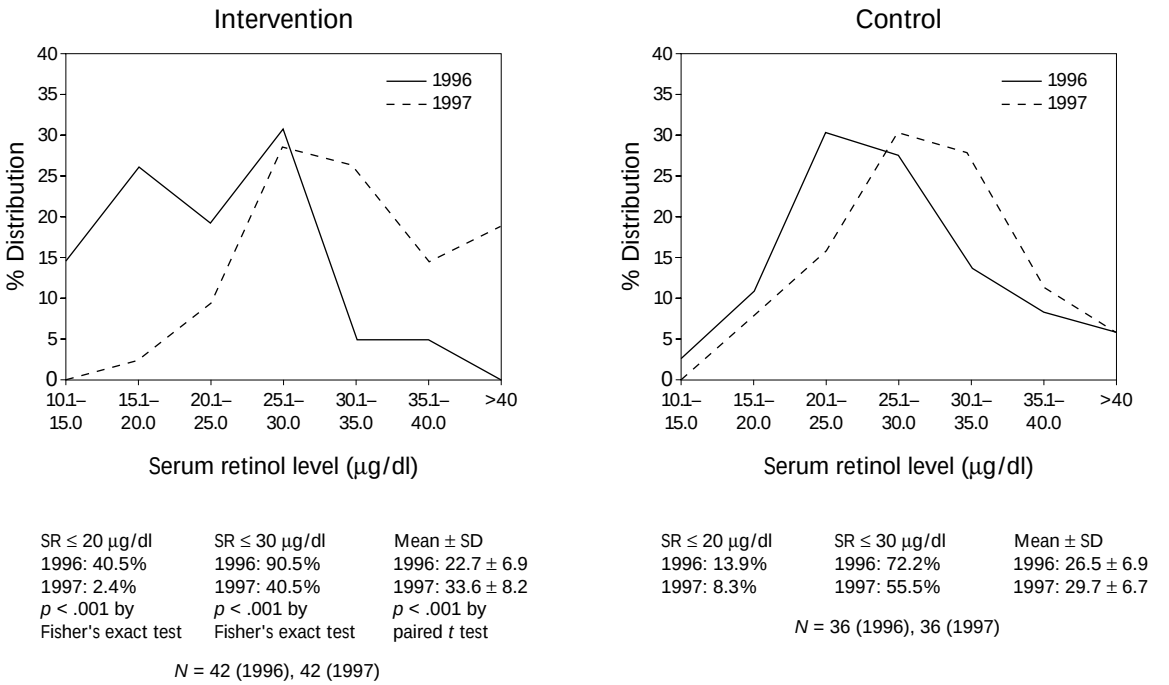

FIG. 1. Percentage distribution of serum retinol (SR) levels among schoolgirls 10–13 years of age before and after project intervention (paired subjects)

the existing community structure by working together with the community, especially women, in sustainable nutrition and health development.

Acknowledgements

Sincere appreciation goes to Dr. Frances R. Davidson of USAID, Dr. Suzanne S. Harris, and Dr. Paula Trumbo of the International Life Sciences Institute (OMNI Research), as well as Ms. Charrotte Johnson-Welch, Dr. Kathleen Kurz, and Dr. Nancy Yinger of the International Center for Research on Women for their constant support. A special note of thanks goes to Dr. Prapaisri P. Sirichakwal and Dr. Pattanee Winichagoon for their technical support, Dr. Lalita Bhattacharjee and Mr. George Attig for their editing support, and the support staff of the Institute of Nutrition at Mahidol University and the International Center for Research on Women for their administrative assistance. Deep gratitude is expressed to all members of the project committees; local government officials, especially Mr. Yongyuth Trisutt, District Officer, and Dr. Wanchai Lausathienkit, Director of the District Hospital; and community leaders in the project area. And lastly, heartfelt appreciation and respect go to all women leaders and people of Kanthrarom District. Without their committed belief in the project and untiring efforts for the improvement of nutrition and health in their communities, the intervention would not have been possible.

This project was supported by the International Center for Research on Women and the International Life Sciences Institute through OMNI Research, Office of Health and Nutrition, US Agency for International Development Cooperative Agreement HRN-5122-A-00-3046-00.

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Induction of mutation in *Drosophila melanogaster* fed a hexane extract of vegetables grown in soil contaminated with particulates from diesel engine exhaust

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Abstract

Trans-heterozygous larvae of the improved high-bioactivation cross Drosophila melanogaster (ORR;flr^3/TM3, Ser *females mated with* mwh *males) were fed with medium containing hexane extract of the edible portion of five vegetables grown in three different soil treatments for 48 hours. The wing hairs of the surviving flies were analysed for the frequency and size of single and twin spots. It was found that the clone induction frequency of the wing hairs of flies treated with a hexane extract of leaves of sacred basil and green kuang futsoi was not significantly different from that of the controls. Conclusive results were obtained when larvae were raised on the medium containing hexane extracts of lettuce and water spinach grown in contaminated soils. Interestingly, the extracts of multiply onion, grown both in the treated and in the untreated soils, induced mutation in the wing spot test. It was concluded that some plants grown in soil contaminated with diesel exhaust provoked mutagenic responses, whereas some showed negative results.*

Introduction

It is well documented that our environment contains a wide range of different types of physical and chemical contaminants. Plants, especially food plants, are exposed to a wide variety of contaminants and may be considered as a "green liver," acting as an important global sink for environmental chemicals [1] that are of public health concern. Plewa and Wagner [2] stated that plants could activate promutagens and store the products in forms that might induce mutations in organisms that consume them. Their hypothesis indicates that the function of the conjugated product of a mutagen may be to stabilize and sequester the active form in the plant and exert its mutagenicity upon organisms that consume it and metabolize it into a reactive form. It thus stimulated us to investigate whether it was possible to detect the mutagenicity of some promutagens that accumulated in our edible plants.

Benzo(a)pyrene, a promutagenic polycyclic aromatic hydrocarbon (PAH), was found in vegetables [3], and further investigations were carried out to determine the content of other PAHs in plants from various parts of the world. For instance, various Russian investigators reported the contamination of plants by PAHs. Shcherbak [4, 5] also stated that pollution of plants might occur from sedimentation of atmospheric dust and soot or by migration of the carcinogens into the plants from polluted soils, whereas Shabad and Cohan [6] concluded that the main source of contamination of soil was from air particulates. They indicated that migration or resorption of PAHs into plants was dependent on the level of PAHs in the soil and the type of plant.

Environmental PAHs are generated mainly by incomplete combustion of petroleum-derived products [7] in various types of improperly adjusted engines. Schuetzle [8] and Bartle et al. [9] stated that nearly all of the chemical species of health significance identified in diesel exhaust were PAHs. Therefore, a sample of diesel exhaust should be a suitable source of mutagenic PAHs in the model for the study of the mutagenicity of plants contaminated with these chemical compounds. In order to fulfil this requirement, the somatic mutation and recombination test (SMART) was employed to detect the mutagenicity of the contaminated plants. This assay is an efficient and versatile eukaryotic short-term *in vivo* assay, which detects various types of mutations, including mitotic recombination in cells of the wing imaginal discs of *Drosophila melanogaster* larva. In addition, the larvae possess metabolic activities that allow them to activate promutagenic PAHs and their derivatives

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Materials and methods

Chemicals and diesel exhaust

Mitomycin C was purchased from Fluka AG (Buchs, Switzerland). Yeast-glucose-agar *Drosophila* medium was prepared according to the method of Roberts [10]. Other chemicals were of laboratory grade. Diesel exhaust was collected from heavy-duty diesel engine vehicles (45 buses and 52 trucks in Bangkok) by scraping the exhaust pipes with bottle-washing brushes. The exhaust particles were ground and mixed well by a Norton pair-roller (Akron, Ohio, USA). The exhaust particulate was subdivided into two portions. The first portion (unprepared sample) was tested for mutagenicity by mixing the exhaust particulate (0.01, 0.02, or 0.05 g) with 2 ml of freshly prepared *Drosophila* culture medium in a glass tube. The second portion (1, 2, or 4 g) was stirred with 300 ml of hexane for two hours in order to extract nonpolar compounds, especially PAHs and their nitro-derivatives.

The solution was filtered through glass wool to obtain a clear solution, which was evaporated to dryness under reduced pressure (40°C). Each extract was weighed and suspended in the prescribed volume of 95% ethanol and then diluted with distilled water to obtain 4 ml of 5% ethanol suspension. The suspension was prepared for two consecutive serial dilutions (original concentration and two diluted concentrations) and subjected to the SMART assay by mixing the suspension with an equal volume of freshly prepared *Drosophila* medium.

Preparation of soil, vegetable planting, and hexane extraction

Sandy loam soil was sun-dried for five days and then loosened. Three different soil treatments were prepared in four replicates. The control soil contained no diesel exhaust, whereas the other two were mixed with two different amounts of diesel exhaust particulate to obtain 1 and 10 g of particulate per kilogram of soil. For each preparation, 1 kg (dry weight) of soil was thoroughly mixed with diesel exhaust and water and then air-dried for one day. One kilogram of treated soil was put into a clay pot (20×17 cm) with a cloth lining to prevent soil leaching.

Five vegetables were used in this study: sacred basil (*Ocimum sanctum* Linn.), lettuce (*Lactuca sativa* Linn.), water spinach (*Ipomoea aquatica* Forsk.), green kuang futsoi (*Brassica chinensis* Jusl.), and multiply onion (*Allium cepa* var. *aggregatum* Don.). Before the seeds of the first four vegetables and the bulb of the fifth one were planted, commercial chemical fertilizers (15-15-15) were added to the soil. The plants were watered twice a day until the edible portions were collected. The samples were washed with tap water and dried at 40°C.

The replicates of each vegetable were pooled and homogenized in a home-use electric blender and stored in a desiccator. Each sample was extracted with 300 ml of hexane for two hours. Solid materials were removed by filtering through glass wool. The filtrate was evaporated, weighed, and suspended in 5% ethanol. The suspension was prepared for two consecutive serial dilutions (original concentration and two diluted concentrations) and subjected to the SMART assay by mixing the suspension with an equal volume of freshly prepared *Drosophila* medium.

Mutagenicity assay

The mutagenicity assay was carried out according to the method of Graf et al. [11]. Virgin females of *ORR; flr^3/TM3, Ser* mated to males of *mwh/mwh* were used to produce larvae of improved high-bioactivation cross. The three-day- (72-hour-) old larvae were collected and washed with water and transferred, with the help of a fine artist's brush, to the glass tubes with medium containing the tested sample or the control. Mitomycin C (0.21 mg/ml) was used as a positive control. The larvae were maintained at 25 ± 1°C for 48 hours. After metamorphosis the flies, which were trans-heterozygous (*mwh flr^+/mwh^+ flr*) for the recessive wing cell marker mutations multiple wing hairs (*mwh*) and (*flr*) on the left arm of chromosome 3, were collected (between days 10 and 12 after egg laying), and their wings were mounted according to the method of Graf et al. [11].

Under the compound microscope at 400 magnification, the wings were inspected for the presence of mutant spots on the phenotypically wild-type wing surface. The following spot types could be expected: *mwh* single spots resulting from mutation, deletion, or mitotic recombination between the *mwh* and *flr* loci; rare *flr* single spots arising from point mutation or deletion; and twin spots with an *mwh* clone adjacent to an *flr* clone generated by mitotic recombination between the more proximally located *flr* locus and the centromere of chromosome 3.

For statistical analysis, a multidecision procedure was used to distinguish between positive, weakly positive, inconclusive, and negative results. The details of the calculation were explained by Frie and Wurgler [12]. Besides the null hypothesis, which assumes that there is no difference in the mutation frequency between control and the treated series, a specific alternative hypothesis was also considered. This hypothesis postulated *a priori* that the treatment results in an increased mutation frequency that is *m* (2 or 5) times the spontaneous frequency. Inconclusive results are obtained when neither hypothesis is rejected.

Results and discussion

On the basis of dry samples, for each plant there was no difference in the percentage of solid matter obtained

at each level after evaporation of hexane (table 1). The yields of hexane-soluble material were different because of species variation. Sacred basil gave the highest percentage yield, whereas green kuang futsoi gave the lowest.

The results obtained with the test compounds are shown with their concurrent solvent controls in tables 2 through 7. All experiments were chronic feeding studies (48 hours) starting with three-day-old larvae at an age of 72 hours and ending with pupation when the larvae leave the medium and stop feeding. A cross of flies with improved high-bioactivation capacity was used because it is well known that PAHs require metabolic activation to exert their genotoxicity [11, 13]. In the improved high-bioactivation cross, chromosome 2 of a DDT-resistant line of the Oregon R wild-type strain (ORR) was introduced [14]. This resistant line is characterized by an increased presence and activity of cytochrome P450 [15]. Larvae carrying the ORR second chromosome exhibit an increased sensitivity for promutagens and procarcinogens.

Mutagenicity of crude diesel exhaust

Crude diesel exhaust gave rise to clone induction frequencies of spots mostly as small single spots rather than large spots (table 2), and conclusive results were obtained with dose-response relationships. These spot types may be due to true somatic point mutations or to various types of smaller or larger chromosome aberrations. Twin spots were shown when higher concentrations of the exhaust were given to the larvae; however, the results were inconclusive and indicated that the diesel exhaust had no recombinogenic activity. Diesel exhaust was shown to be mutagenic in short-term bioassays and carcinogenic in laboratory animals [16]. The particulates contained hundreds to thousands of chemical components [8, 9]. Numerous aromatic and nitro aromatic compounds have been shown to be mutagenic for *Salmonella typhimurium* as well as carcinogenic in experimental animals [17–19]. It is likely that the effects produced by the nitro-PAHs are due to the reduction of the nitro group(s) by nitroreductase(s) [20].

Mutagenicity of hexane extract

An attempt was made to determine whether the toxic substances in the diesel exhaust could express their mutagenicity in the hexane extract fraction, since hexane was used as the extracting solvent for the plant samples. It was shown that the hexane extraction of diesel exhaust samples increased the frequency of wing hair aberrations of treated larvae (table 3). Better dose-response relationships in this trial were presented in both small and large single spots as compared with the results with the crude exhaust. Thus, hexane was a suitable solvent for plant sample extraction.

Mutagenicity of hexane extracts of plants

Tables 4 through 8 show the data obtained from the tests of five vegetables grown in three different soils (control soil, soil treated with 1 g of diesel exhaust per kilogram

TABLE 1. Yield of extracts of different plants grown in diesel exhaust-contaminated soils for mutagenicity testing

Common name	Diesel exhaust (g/kg soil)	Wet weight (g)	Dry weight (g)	Dry matter of hexane extract (g)	% yield of extract based on dry weight
Sacred basil	0	130.8	10.5	2.0	19.4
	1	99.2	8.9	1.9	22.0
	10	114.9	8.9	2.0	22.8
Green kuang futsoi	0	205.1	8.1	0.2	2.9
	1	230.2	8.2	0.2	2.8
	10	189.2	7.7	0.2	2.3
Multiply onion	0	180.9	9.4	0.9	9.6
	1	155.2	9.0	0.9	9.5
	10	129.2	8.8	0.9	9.8
Lettuce	0	250.9	8.0	0.5	6.2
	1	184.6	6.9	0.4	5.8
	10	216.2	7.0	0.4	5.9
Water spinach	0	207.3	12.2	0.9	7.2
	1	181.8	12.5	0.9	7.5
	10	204.5	12.4	0.9	7.4

TABLE 2. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of crude diesel exhaust for 48 hours

Concentration of crude diesel particulate in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
5	93	41	4	0	0.48 +
10	57	35	3	2	0.70 +
25	65	51	5	1	0.88 +
Negative control (water)	84	21	1	0	0.26
Positive control[b]	25	19	1	3	0.92 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.

b. Positive control was 0.21 mg of mitomycin C per millilitre.

TABLE 3. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of 1, 2, and 4 g diesel exhaust for 48 hours

Concentration of hexane extract in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
From 1 g diesel exhaust					
2.0	70	16	0	0	0.23 –
4.0	67	17	1	0	0.27 –
8.0	64	31	2	0	0.52 +
From 2 g diesel exhaust					
3.9	64	18	2	0	0.31 –
7.9	71	39	2	1	0.59 +
15.8	42	47	4	2	1.26 +
From 4 g diesel exhaust					
8.3	64	26	2	1	0.45 +
16.7	32	25	4	0	0.91 +
33.4	45	59	7	3	1.53 +
Negative control (5% ethanol)	112	28	3	1	0.29
Positive control[b]	25	19	1	3	0.92 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.

b. Positive control was 0.21 mg of mitomycin C per millilitre.

of soil, and soil treated with 10 g of diesel exhaust per kilogram of soil). The clone induction frequency of the wing hair of flies treated with a hexane extract of leaves of sacred basil and green kuang futsoi grown in three differently treated soils was not significantly different from that of the control (tables 4 and 5). The extract of multiply onion was the only sample that induced mutation in the wing spot test in both treated and untreated soils (table 6). The mutagenicity of the sample grown in untreated soil may be due to some naturally occurring compounds. The inherent mutagenicity of shallot (*Allium ascalonicum*), which is taxonomically very close to multiply onion (*Allium cepa* var. *aggregatum* Don.), was shown to be positive for *Salmonella typhimurium* by the mammalian microsome assay [21]. This preliminary study in purification of mutagenic extracts of shallot resulted in the isolation and identification of natural mutagens, namely, quercetin and unknown glycosides. Quercetin was also shown to be mutagenic according to SMART by Graf et al. [22].

The results obtained from the extract of multiply onion grown in diesel exhaust-treated soils showed an apparent dose-response relationship in this assay (table 6). Conclusive results were also obtained when larvae were raised on the medium containing hexane extracts of lettuce and water spinach grown in contaminated soils (tables 7 and 8). It was suggested that the extracts of vegetables grown in diesel exhaust-contaminated soil significantly increased the frequency of small single spots as compared with those of the samples grown in the control soil. It was thus postulated that some PAHs in the exhaust played an important role in increasing the mutagenicity.

Various investigators have reported the contamination of plants by PAHs. Shabad et al. [23] stated that the PAHs penetrated the soil mainly from air and spread intact across the layers into the water. They then passed into plants, fodder, and finally human food. Shcherbak [4, 5] noted that pollution of plants might occur from sedimentation of atmospheric dust and soot or by migration of the carcinogens into the plants from polluted soils. Shabad and Cohan [6] concluded that the main source of contamination of soil was from air particulates. They indicated that migration or resorption

TABLE 4. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of sacred basil (*Ocimum sanctum* Linn.) grown in control or diesel exhaust-contaminated soils for 48 hours

Concentration of hexane extract of plant in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
Control soil					
128	38	9	0	0	0.24 –
255	56	13	0	0	0.23 –
510	56	22	1	0	0.41 i
Contaminated soil I[b]					
121	30	6	0	0	0.20 –
254	42	11	0	0	0.26 –
485	31	12	0	0	0.39 i
Contaminated soil II[c]					
127	44	12	0	0	0.27 –
253	29	9	1	0	0.34 i
506	54	20	0	0	0.37 i
Negative control (5% ethanol)	81	21	2	0	0.28
Positive control[d]	22	13	1	2	0.73 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.
b. Contaminated soil I contained 1 g of diesel exhaust particulate per kilogram of soil.
c. Contaminated soil II contained 10 g of diesel exhaust particulate per kilogram of soil.
d. Positive control was 0.21 mg of mitomycin C per millilitre.

TABLE 5. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of green kuang futsoi (*Brassica chinensis* Jusl.) grown in control or diesel exhaust-contaminated soils for 48 hours

Concentration of hexane extract of plant in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
Control soil					
15	54	10	0	0	0.19 –
30	29	5	0	0	0.17 –
60	40	8	0	0	0.20 –
Contaminated soil I[b]					
14	41	8	0	0	0.19 –
28	31	6	0	0	0.19 i
57	27	3	1	0	0.15 –
Contaminated soil II[c]					
11	38	6	0	0	0.16 –
23	39	6	1	0	0.18 –
46	45	8	1	0	0.20 –
Negative control (5% ethanol)	57	12	1	0	0.23
Positive control[d]	20	11	1	1	0.65 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.
b. Contaminated soil I contained 1 g of diesel exhaust particulate per kilogram of soil.
c. Contaminated soil II contained 10 g of diesel exhaust particulate per kilogram of soil.
d. Positive control was 0.21 mg of mitomycin C per millilitre.

of PAHs into plants was dependent on the PAH levels in the soil and the type of plant.

This experiment showed that some plants grown in diesel exhaust-contaminated soil provoked a mutagenic response, whereas some showed negative results. It is likely that the mutagenicity of each plant extract may be reflected by the differences between plants. Furthermore, most of the hexane extracts contained some green pigments, which were probably chlorophylls. The weak mutagenicity or absence of mutagenicity of some samples may due to the antimutagenicity of chlorophyll against diesel exhaust mutagens absorbed by such plants or to the metabolic detoxification pathway of the plant, as suggested by others [1, 2].

Chlorophyll has been demonstrated to suppress the mutagenicity of many toxicants [24–27]. The inhibitory action of chlorophyllins, the sodium and copper salts of chlorophyll, encompasses a wide variety of chemical compounds, including nitropyrenes (airborne and diesel emission particulates) and PAHs [26]. These chlorophyll derivatives were also effective antimutagens in the *Drosophila* tests on MeIQx, benzo(a)pyrene, 2-aminofluorene, 2-aminoanthracene, 9-aminoacridine, and 4-NQO [28]. The mechanism by which chlorophyllin inhibits mutagenic activity is unknown, but it has been suggested that chlorophyllin can form complexes with planar-structured polycyclic compounds [28] and that this complex formation is responsible for the polycyclic mutagen-specific inhibition observed in the Ames test [29, 30].

TABLE 6. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of multiply onion (*Allium cepa* var. *aggregatum* Don.) grown in control or diesel exhaust-contaminated soils for 48 hours

Concentration of hexane extract of plant in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
Control soil					
56	46	10	0	0	0.22 –
113	49	9	1	2	0.24 –
225	38	19	1	4	0.63 +
Contaminated soil I[b]					
53	37	8	0	2	0.27 i
107	48	17	0	3	0.42 i
214	51	26	4	3	0.65 +
Contaminated soil II[c]					
54	46	14	2	0	0.35 i
108	45	20	2	1	0.51 +
217	35	19	1	4	0.69 +
Negative control (5% ethanol)	69	15	2	1	0.26
Positive control[d]	29	22	2	1	0.86 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.
b. Contaminated soil I contained 1 g of diesel exhaust particulate per kilogram of soil.
c. Contaminated soil II contained 10 g of diesel exhaust particulate per kilogram of soil.
d. Positive control was 0.21 mg of mitomycin C per millilitre.

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TABLE 7. Clone induction frequency after feeding 72-hour-old larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of water spinach (*Ipomoea aquatic* Forsk.) grown in control or diesel exhaust-contaminated soils for 48 hours

Concentration of hexane extract of plant in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
Control soil					
55	39	7	1	0	0.20 –
110	25	2	2	0	0.16 –
220	48	12	0	0	0.25 –
Contaminated soil I[b]					
59	34	6	1	0	0.20 –
118	38	9	0	0	0.24 –
237	33	14	1	0	0.45 +
Contaminated soil II[c]					
57	48	11	0	0	0.23 –
115	44	14	1	0	0.34 i
229	31	14	2	0	0.52 +
Negative control (5% ethanol)	92	19	3	0	0.24
Positive control[d]	29	19	2	0	0.72 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.
b. Contaminated soil I contained 1 g of diesel exhaust particulate per kilogram of soil.
c. Contaminated soil II contained 10 g of diesel exhaust particulate per kilogram of soil.
d. Positive control was 0.21 mg of mitomycin C per millilitre.

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TABLE 8. Clone induction frequency after feeding 72-hour-old-larvae of improved high-bioactivation cross with medium containing different concentrations of hexane extract of lettuce (*Lactuca sativa* Linn.) grown in control or diesel exhaust-contaminated soils for 48 hours

Concentration of hexane extract of plant in *Drosophila* medium (mg/ml)	No. of wings	Diagnosis			Clone induction frequency[a] (total spots/ wing)
		Small single spots (1–2 cells) (m = 2)	Large single spots (> 2 cells) (m = 5)	Twin spots (m = 5)	
Control soil					
31	56	13	1	1	0.27 –
63	46	11	1	1	0.28 i
125	38	9	0	0	0.24 –
Contaminated soil I[b]					
25	33	7	0	0	0.21 –
50	55	22	0	0	0.40 i
99	55	18	4	0	0.40 i
Contaminated soil II[c]					
26	38	11	2	0	0.34 i
51	48	24	1	0	0.52 +
103	45	23	2	0	0.56 +
Negative control (5% ethanol)	75	18	2	0	0.27
Positive control[d]	18	16	2	1	1.06 +

a. Statistical diagnoses using estimation of clone induction frequencies and confidence limits according to Frei and Wurgler [12]: + positive, – negative, i inconclusive, m multiplication factor. Probability levels: $\alpha = \beta = .05$. One-sided statistical tests.
b. Contaminated soil I contained 1 g of diesel exhaust particulate per kilogram of soil.
c. Contaminated soil II contained 10 g of diesel exhaust particulate per kilogram of soil.
d. Positive control was 0.21 mg of mitomycin C per millilitre.

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Improvement of dietary density by the use of germinated cereals and legumes

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Abstract

The feasibility of using germinated seeds to reduce the high viscosity of cereal- and legume-based weaning foods developed by the Institute of Nutrition at Mahidol University was investigated. The amylase-rich food (ARF) products were developed from cereals (rice, glutinous rice, and maize) and legumes (mung bean, black bean, soya bean, red kidney bean, and groundnut). The activities of α- and β-amylase were determined. The results showed that the germination processes were standardized by a four-day germination period for cereous cereal- and legume-based weaning foods.

Introduction

To prevent protein-energy malnutrition in young children, it is necessary to provide them with high-energy, nutrient-dense weaning foods that are acceptable and affordable. One of the major constraints on the acceptability of the weaning foods—particularly the starch-based types, despite their nutritional quality—is their high dietary bulk. This results in a thick, viscous food preparation that makes it difficult for young children to satisfy their nutrient requirements [1].

One possible solution is to reduce the dietary bulk without significantly changing the nutritional value of the weaning foods. Previous studies have shown that this can be done by the addition of a small amount of germinated cereal or legume to the gruels [2–9]. This is based on the principle that during germination of these grains, amylolytic enzymes are developed and activated. The enzymes, when present in the gruels, rapidly break down the starch, resulting in a decrease in its water-holding capacity. Thus, the water trapped in the gel structure is released and the mixture becomes more liquid.

Amylase-rich food (ARF) technology [5, 8, 10–13] was applied to the preparation of suitable amylase-rich products from locally available staple grains, and the properties related to consistency changes in cereal- and legume-based weaning foods were examined. The study also tested and aimed to establish the technology for producing high-density, low-viscosity weaning foods by adding locally produced amylase-rich grains and sprouted legume flour that would be feasible for use by members of low socio-economic groups.

Materials and methods

Starchy weaning food

Among several weaning food formulas developed by the Institute of Nutrition at Mahidol University (INMU) [14], a cereal- and legume-based formula was selected and suitably modified for its acceptability. The formula was prepared from locally available rice (*Oryza sativa*), mung bean (*Phaseolus aureus*), and groundnut (*Arachis hypogaea*) in the ratio 62:20:18. The grinder used was efficient and simple to operate. The mixtures were packed in small plastic bags of 100 to 500 g. The cereal-legume gruels were cooked with water for 15 minutes at several dilutions varying from 25% solid content to lower levels. Viscosity was measured by a Brookfield viscometer model DV II.

ARF product preparation

The ARF products were prepared from locally available cereals and legumes. The cereals used were paddy rice (*Oryza sativa*) var. Ko KHO 21, paddy glutinous rice (*Oryza glutinosa*) var. Ko Kho 6, and maize grain (*Zea mays*) var. Su wan 3. The legumes were black bean (*Phaseolus mungo*), soya bean (*Glycine max*), red kid-

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ney bean (*Phaseolus vulgaris*), rice bean (*Phaseolus calcaratus*), and groundnut.

The cereal grains and legume seeds were germinated to prepare the ARF products, as shown in figure 1. The seeds and grains were washed and steeped in three times their volume of tap water for 24 hours for cereal grains and 12 hours for legume seeds. The seeds were drained and germinated in a moist cloth wrapped in a black plastic bag at 30°–32°C, 70% relative humidity, for a 7-day germination period for cereal grains, and 36, 48, or 60 hours for legume seeds. The germinated seeds were dried in a hot-air oven at 60°C for 3 hours, dehulled, and devegetabled. They were ground with a Cyclotec 1090 sample mill, put in airtight bottles, and stored at –20°C. The yield of ARF powder and the amylase enzyme activity were determined.

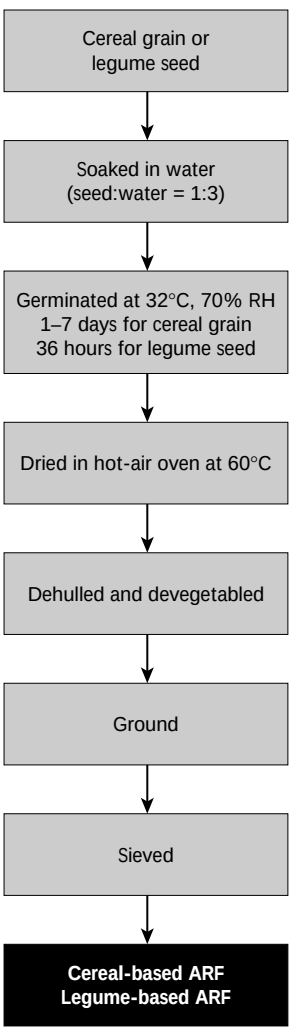

FIG. 1. Preparation of ARF products

Determination of amylase enzyme activity

The enzyme in ARF was extracted and a crude enzyme solution was determined for α- and β-amylase activity, whereas a colourimetric method [15] was modified for studying α-amylase activity. The activity of the enzyme extract was determined at 30°C by expressing it as a function of α-amylase concentration and of the velocity constant for the hydrolytic degradation of limit dextrins. β-Amylase activity was determined by the modified saccharifying method of Bernfield [16] to increase the reducing power from hydrolysis of soluble starch and determine the red-brown colour that is expressed by the reaction of reducing groups and 3,5-dinitrosalicylic acid.

Reduction of the viscosity of gruel by ARF

The viscosity-reducing effects of cereal-based and legume-based ARF were tested with the 25% rice-mung bean-groundnut weaning food formula by adding 1% cereal-based ARF or 20% legume-based ARF. The mixed formula was heated to 95°C (boiled) to inactivate the enzyme, and the viscosity was measured by a Brookfield viscometer DVII.

Results and discussion

Nutrient density and dietary bulk of cereal- and legume-based weaning food

As shown in table 1, the protein and fat contents of the formula were 3.8 and 3.7 g per 100 kcal, respectively, which meet the Thai standard requirement of 2.5 and 2 g per 100 kcal. The energy content also meets the recommended allowances for young children.

The FAO/WHO Advisory Group recommends 100 kcal per kilogram of body weight per day, which amounts to 1,000 kilocalories per day for a 12-month-old child [17]. In general, a child receives about 400 to 500 kcal per day from milk. Thus the weaning food should provide about 200 kcal per meal. As shown in table 2, the volume of gruel per meal needed to provide 200 kcal or 20% of the energy needed by a 12-month-old child doubled when the solid content of the gruel was decreased from 25% to 12.5% to make the viscosity acceptable.

According to the field experiments of Gopaldas et al. [5], the volumes of food intake of infants and toddlers were 131 and 160 ml, respectively, whereas Mosha et al. [18] reported 277 g per meal of 20% solid content for a child aged 12 to 24 months. This amount was comparable with 196 and 204 ml per meal for 25% rice-mung bean-groundnut formula and Cerelac (a commercial preparation), as shown in table 2. Therefore, from a dietary point of view, a suitable volume

TABLE 1. Nutrient contents of the cereal- and legume-based weaning food formula (rice-mung bean-groundnut)

Nutrient	Per 100 g	Per 100 kcal
Energy (kcal)	425	100
Fat (g)	16	4
Carbohydrate (g)	79	19
Protein (g)	16	4
Essential amino acids (mg)		
Isoleucine	527	124
Leucine	1,068	251
Lysine	703	165
Methionine and cysteine	283	67
Phenylalanine and tyrosine	1,086	256
Threonine	513	121
Tryptophan	165	39
Valine	733	173

of gruel for a 12 month-old-child could be estimated as 200 ml per meal.

The results showed that dietary bulk still posed a problem for the rice-mung bean-groundnut formula. The weaning food preparation of 16.6 g/100 ml at a volume of 300 ml of gruel resulted in a viscous consistency, and at a lower concentration with an acceptable viscosity, the volume of the gruel would be even larger. Furthermore, the concentration of rice-mung bean-groundnut formula at 25% solid content seemed to fulfil both the calorie density and dietary bulk, whereas its viscosity was not accepted. Hence, a reduction in viscosity of this gruel was necessary and was chosen as a parameter for the study.

TABLE 2. Comparison of the viscosity of cereal- and legume-based gruels according to dietary density

Formula and solid content (g/100 ml)	Volume (ml) per 200-kcal meal[a]	Viscosity[b]
Rice–mung bean–groundnut		
25	196	231×10^3
17	300	71×10^3
13	416	15×10^3
Cerelac		
25	204	6×10^3

a. Volume to cover 20% of the energy needs of a 12-month-old child.
b. Measured by a Brookfield viscometer model DVII spindle 3-7, speed 20 rpm, at 40°C.

Production of local ARF

Cereal-based ARF

Paddy rice, paddy glutinous rice, and maize grain used for cereal-based ARF were germinated for seven days, and the amylase activity was determined as well as the effectiveness of ARF preparation. All three cereals increased in amylase activity with longer germination periods (table 3), whereas the yield of ARF product decreased because of starch degradation during germination. The α- and β-amylase activity seemed to increase significantly after four days. Murata [19] reported that α-amylase activity declined after 10 to 13 days of germination. A germination period of four or five days is optimal for effective production of the amylase enzyme, considering the level of α-amylase activity and

TABLE 3. Effect of germination period on enzyme activity of cereal-based ARF

Type of ARF and activity	Germination (days)							
	0	1	2	3	4	5	6	7
Rice								
% yield	69.1	63.2	57.6	56.8	45.5	44.1	29.1	36.3
α-Amylase[a]	0.7	6.2	11.5	52.1	105.8	207.9	284.0	241.2
β-Amylase[b]	6.2	1.6	1.6	9.6	19.2	25.3	28.6	27.4
Glutinous rice								
% yield	69.8	63.1	55.9	53.5	46.3	46.0	32.3	29.5
α-Amylase[a]	0.4	6.9	17.8	84.0	125.3	154.5	190.4	177.6
β-Amylase[b]	0.0	1.3	4.8	24.1	29.9	48.3	51.3	50.9
Maize								
% yield	100.0	97.9	99.7	94.7	88.4	82.1	59.1	33.3
α-Amylase[a]	2.8	14.1	45.6	65.4	157.7	167.6	167.2	ND[c]
β-Amylase[b]	1.0	1.4	3.3	3.3	5.6	7.3	5.6	ND

a. A-unit.
b. $\times 10^6$ mmol maltose per gram per minute.
c. No data.

the corresponding yield of ARF produced, which were 44% to 46% yield and 105 to 207 A-units of α-amylase activity. Rice ARF has higher α-amylase activity than glutinous rice ARF or maize ARF. Since rice and wheat are common staple foods, they could serve as suitable raw materials for ARF production. Gopaldas et al. [5] developed wheat-based ARF with α-amylase enzyme activity of 39 10^6 mmol/g/min, whereas our rice ARF had 19 to 25 mmol/g/min and 105 to 207 A-units for α- and β-amylase, respectively. Moreover the amylase activity, especially the α-amylase activity, of all three cereals after four days was high enough for an effective reduction in viscosity.

Legume-based ARF

The five locally produced legumes selected for this study were black beans, soya beans, red kidney beans, rice beans, and groundnuts. Preliminary studies indicated that steeping legumes for 7 hours, followed by drying and resoaking, was not effective in increasing α-amylase activity. Therefore, the legumes were steeped for 12 hours and germinated for 36, 48, and 60 hours, then processed under the same drying and milling conditions as cereal-based ARF preparations. After germination, black bean ARF and soya bean ARF had high α-amylase activity as compared with other legume-based ARFs, which had low α- and β-amylase, resulting in ineffective reduction of viscosity (table 4). Increasing the germination time from 36 to 60 hours did not result in an increase in amylase activity. Therefore, a 36-hour germination time for legume-based ARF seemed to be sufficient for enzyme production.

TABLE 4. Effect of germination period on enzyme activity of legume-based ARF[a]

Activity and type of legume	Germination (hr)		
	36	48	60
α-Amylase activity[b]			
Mung bean	2.7	4.1	4.2
Black bean	4.6	9.6	13.6
Soya bean	0.2	0.1	0.2
Rice bean	0.6	1.6	3.7
Red kidney bean	0.7	0.9	0.5
Groundnut	0.1	0.1	0.1
β-Amylase activity[c]			
Mung bean	0.9	1.1	1.2
Black bean	1.1	1.7	2.0
Soya bean	2.1	2.2	2.2
Rice bean	0.7	0.7	0.8
Red kidney bean	0.5	0.5	0.5
Groundnut	0.3	0.3	0.5

a. Yield approximately 65%–75%.
b. A-unit.
c. $\times 10^6$ mmol maltose per gram per minute.

The amylase activity of cereal-based ARF was markedly higher (approximately 9–40 times) than that of legume-based ARF. This may be due to the mobilization of soluble carbohydrates in the endosperm of cereals during germination and the rapid growth of legumes. Furthermore, amylase activity varies with the raw material used [7], its cultivar, enzyme inhibitors, light, oxygen content, temperature, and dormancy [20, 21].

Viscosity-reduction effect

We compared the effects of ARF on the reduction in viscosity of 25% cereal- and legume-based weaning food (rice-mung bean-groundnut) by adding 1% four-day-germinated cereal-based ARF and 20% 36-hour-germinated legume-based ARF (table 5). The consistency of the gruel was suitable for children at 1% for all three cereal-based ARFs and at 20% for black bean ARF. This is in agreement with the findings of others [2–9, 11–13, 22–24], whereas optimal thinning was observed at the 4% level of wheat-based ARF. The current research found an effective viscosity-reduction power of ARF of 90% to 92%. This corresponded to the findings of Gopaldas et al. [5] of a 66% reduction in viscosity. Although soya bean ARF can produce amylase enzyme, no visible reduction in viscosity was observed because of the high fat and protein content.

In an attempt to reduce the viscosity of a thick or high solid density gruel by the liquefying action of amylase, a very small amount of an effective cereal-based ARF (0.5 g per 200 g of solid content) resulted in improper distribution of enzyme materials, giving a rigid structure to the high solid content formula and measurement difficulties. Incorporation of a starch filler or harvesting the germinated cereal materials at the optimal period is therefore suggested.

TABLE 5. Consistency changes of 25% cereal-legume gruels and viscosity reduction

Type of ARF	Viscosity ($\times 10^3$ eps)	Viscosity reduction (%)	Consistency
Cereal-based (1%)			
Rice	28	90	Drop batter
Glutinous rice	24	92	Drop batter
Corn	25	91	Drop batter
Legume-based (20%)			
Black bean	30	90	Doughlike
Soya bean	299	0	Doughlike
Rice bean	226	0	Doughlike
Red kidney bean	300	0	Doughlike
Groundnut	277	0	Doughlike
Mung bean	89	61	Thick batter

Conclusions

The procedure for preparing ARF from various cereal grains and legume seeds is effective, simple, and feasible for local production. The four-day-germinated rice had the highest concentration of amylase. The technology of creating amylase-rich flours from sprouting grains can thus be effectively applied in community-level food-processing operations. Furthermore, these cereal-based, amylase-rich flours can be used in modified traditional weaning food mixtures as developed by INMU to reduce viscosity, increase energy and nutrient density, or both. The technical feasibility can be transferred to the local level, which would result in an increased consumption by young children to fulfil their energy and protein requirements.

Acknowledgements

We are grateful to the World Health Organization Regional Office for South-East Asia for financial support and the Pathumthani Rice Research Center and Prae Rice Research Center for raw materials and equipment. Thanks are due to Prof. Sakorn Dhanamitta, Prof. Dr. Kraisid Tontisirin, and Dr. Anadi Nitththumyong for their guidance and support. Finally, thanks to Dr. Lalita Bhattacharjee for editing this paper.

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Letter to the editor

Dear Sir,

Weight-for-age and the nutritional assessment of a population

I refer to the article on the assessment of the nutritional status of children in Chobe, Botswana [1]. As Regional Medical Officer of this district from 1982 to 1986, I carried out a similar assessment with Dr. McCall in 1985 [2], in addition to regular nutritional monitoring of the children using weight-for-age. As a matter of fact, the use of weight-for-age as an indicator of malnutrition is an old public health debate, but looking at some conclusions drawn by the author on such grounds, I feel it is necessary to reopen it. Weight-for-age, usually compared with the NCHS standards, is said to reflect chronic malnutrition and can be helpful for monitoring the growth rate of individual children. However, its use in a cross-sectional survey can be very misleading, as we wrote in our article on Chobe and I confirmed in a later study in northern Uganda [3].

First of all, the "borderline" children, whose results are very close to the cut-off value (whose choice also is problematic), can constitute as many as 15% of the measured children. The decision whether or not to include them in the total number of "malnourished" (explicitly or implicitly, e.g., by mistakes in measurement) could lead to opposite epidemiological conclusions. Second, weight-for-age is influenced by many confounding factors. Height (and hence weight) can be influenced by genetic factors (although this has been shown only for Pygmy populations). Weight can also be influenced by genetic dispositions, such as distribution of muscle, fat, and bone mass, as well as by intercurrent diarrhoea (whose influence was shown in our work in 1985). Measurements of age rely on birth data, which in such contexts are sometimes approximate and left to the fieldworker's subjectivity. The weight-for-age standards are linked to breastfeeding habits and weaning procedures, and the curves proposed for monitoring do not take into account the difference between the sexes. All of these factors are potential sources of mistakes that could play a very important role by bringing a significant number of children close to the borderline mentioned above.

Therefore, all conclusions relying on weight-for-age should be interpreted quite cautiously, bearing in mind that nutrition is only one of the factors influencing this indicator. The author states (p. 43) that "ethnicity plays a very important role in the nutrition situation in Chobe," relying on the results in table 2. Besides pure epidemiological considerations, which show that the methodology used in this survey is inappropriate for drawing conclusions by ethnic groups (should one exist that would be appropriate), the Basarwa population (usually called "Bushmen" in English) accumulates all of the above-quoted sources of mistakes. Under these conditions, the use of weight-for-age will *de facto* overrepresent the Basarwa community, without discriminating whether the observed 30% of children who are under their weight-for-age are suffering from real malnutrition or are victims of confounding factors.

The description of malnutrition within a population and the analysis of its determinants must rely on a panel of several indicators, whose design has still to be reconsidered [4], and one must be very cautious in its interpretations and its conclusions, particularly when there may be serious consequences, such as designing the modalities of food aid, as in Uganda, or indirectly justifying ethnic stigmatization, as in the present survey.

Yours sincerely,

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Books received

Antioxidant status, diet, nutrition, and health. Edited by Andreas M. Papas. CRC Press, Boca Raton, Fla., USA, 1999. (ISBN 0-8493-8009-X) 650 pages, hardcover. US$99.95.

The health significance of antioxidants in foods is of increasing public health interest and concern. Forty-nine expert contributors cover the full range from the basic chemistry of free radicals to the effect of diet on the antioxidant status of humans and its significance for health and disease. Five chapters are devoted to dietary and other antioxidants and five to proposed modes of action of antioxidants. Individual chapters deal with antioxidants and cardiovascular disease, cancer, diseases of the eye, neurological diseases, ageing, and the potential health effects of tocotrienols and α-lipoic acid. For the most part, the evidence is reviewed in a balanced manner and the difficulties of distinguishing the protective effects of a diet rich in antioxidants from the effects of the antioxidants *per se* is acknowledged. In general, the effects of antioxidants on disease are neither consistent nor conclusive, except for therapeutic doses of vitamin E and heart disease. It is clear that although much is now known about dietary antioxidants and health, the final answers are elusive. Nevertheless, current knowledge of dietary antioxidants is highly suggestive of potential benefits, and there is currently no better coverage of this important topic.

The fats of life. Caroline M. Pond. Cambridge University Press, New York, 1998. (ISBN 0-521-63577-2) 337 pages, paperback. US$19.95.

This book, written by a comparative physiologist, describes where fats come from, how animals and plants handle them, and their natural roles in animal migration, mating, breeding, and living in unpredictable habitats. The multiple functions of fat in a wide range of animal species are well described. Archaeological, anthropological, and physiological evidence is marshalled to explain modern diversity in human body shape, size, and fatness. There is no conventional human nutrition in this book until the final 44-page chapter on fats and health, which in a few paragraphs for each topic presents current understanding of fat-soluble vitamins, fatty acid deficiencies, lipids and pain relief, adipose tissue and immune systems, dietary lipids and immune function, fat in arteries, heart attacks and obesity, fatty foods and the control of appetite, and slimming in the past and present. For those interested in a readable and sound account of the role of fat in animals and its implications for contemporary humans, this is an excellent book. It is good as background reading for nutritionists but not as a primary source of in-depth information on human lipid nutrition.

Folate in health and disease. Edited by Lynn B. Bailey. Marcel Dekker, New York, 1995. (ISBN 0-8247-9280-7) 469 pages, hardcover. US$179.00.

Although now four years old, this book remains an excellent and authoritative source of information on folate. This vitamin is receiving increased attention because its deficiency has multiple consequences, and deficiency occurs in industrialized as well as developing countries. In the United States NHANES II survey, 10% of the population was estimated to have low folate stores. The consequences go far beyond the traditional macrocytic anaemia and include an increase in neural tube defects, elevated levels of serum homocystine that increase the risk of atherosclerosis, and the effects of folate on the occurrence of cancer. The 16 chapters in this book cover all aspects of folate metabolism, the clinical consequences of deficiency, availability in foods, requirements and dietary recommendations, relationships to cancer, interactions with vitamin B_{12}, and other related topics. This book should be available as a reference in the library of any nutrition-related institution.

Maternal anthropometry and pregnancy outcomes: A WHO collaborative study. Supplement to volume 73 of the Bulletin of the World Health Organization, Geneva, 1997. (ISBN 92-4-068730-3) 98 pages, paperback. Sw. fr. 20.-./US$18.00.

The nutritional status of a woman before and during pregnancy is critical to both her infant's and her own health and survival. It determines her well-being and that of the foetus and child, which in turn affect the health and reproduction of the next generation of mothers. Through foetal malnutrition, as indicated by intrauterine growth retardation, it can affect the development of chronic degenerative diseases in later life. This publication is based on a workshop on the selection of maternal anthropometric indicators for screening and monitoring pregnancy and its outcome. It includes a meta-analysis of the usefulness of commonly employed indicators for selected maternal and infant outcomes as well as complications of pregnancy. It also includes reports of a pregnancy supplementation study in Keneba, Gambia; risk-care approach to anaemia in pregnancy in Hyderabad, India; predictors of infant growth retardation in Malawi; risk predictors of pregnancy outcome in Egypt; maternal anthropometry as a risk predictor of pregnancy outcome in Kenya; and a collaborative perinatal project in the United States. Each of them sheds light on factors affecting birthweight. It is a valuable publication for understanding the latter and also for the design of further studies of this important topic.

The vitamins: Fundamental aspects in nutrition and health. Second edition. Gerald F. Combs, Jr. Academic Press, San Diego, Calif., USA, 1998. (ISBN 0-12-183492-1) 618 pages, hardcover. US$59.95.

This textbook provides excellent coverage of the topic in a way that is adapted to the needs of students, as well as serving as a reference book for nutritionists. It is greatly expanded and updated from the first edition, especially in those areas in which progress has been most rapid. Each chapter begins with anchoring concepts. New terms appear in boldface and the basic concepts of the text are amplified by extensive footnotes. There are no specific references, but each chapter has a short list of recommended reading. Each vitamin is dealt with in a separate chapter following a chapter on the history of the discovery of the vitamins. A final section has chapters on the sources of vitamins, assessing vitamin status, quantifying vitamin needs, and vitamin safety. Appendices deal with examples of current vitamin research literature and the vitamin content of foods and feeds. This book makes knowledge of the vitamins accessible and greatly facilitates the teaching of this aspect of nutrition. It will be a valuable reference in the libraries of developing-country institutions as well as being useful as a textbook.

Who's hungry? And how do we know? Food shortage, poverty and deprivation. Laurie DeRose, Ellen Messer, and Sara Millman. United Nations University Press, Tokyo, 1998. (92-808-0985-7) 201 pages, paperback. US$19.95.

This book, produced by the Feinstein World Hunger Program at Brown University, utilizes the disparate disciplines of political economics, sociology, anthropology, public health and human biology, and policy organizational research. It develops a hunger typology, then distinguishes among situations of food shortage, food poverty, and food deprivation. The book argues that at a regional or national level, a food shortage may be due to political, climatic, or other socio-economic forces. Food-short or famine conditions can be distinguished from food poverty at the household level, in which people go hungry because they lack the resources to acquire food, even when food supplies are sufficient. Amartya Sen has earlier pointed out that this is the nature of modern famines [1, 2], a major reason for awarding him the 1998 Nobel Prize in Economics. Chapters deal with nutritional requirements and the measurement of hunger, the causes and reduction of food poverty, causes of food deprivation, and conflict as a cause of hunger. It observes that "the analysis of hunger situations provides guidance for policy makers to map nutritional suffering in time and space for more effective action." The message of this book is that the existence and persistence of hunger are due to political causes and that hunger situations are ultimately amenable to political solutions.

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In memoriam: David Buss

Nutrition in the United Kingdom, and food composition in particular, has lost a valued member at the untimely early age of 60. David Buss's battle against cancer was a source of inspiration to family, friends, and colleagues alike. He died in his home town in Hampshire, UK, on 29 August 1998. He was born on 26 June 1938.

From his position as Head of the Nutrition Branch at the Ministry of Agriculture, Fisheries and Food in London from 1971 to 1995, David Buss played a leading role in providing advice on all aspects of human nutrition in relation to food and diet, developing and advising on nutrition policies and monitoring their effectiveness. He will be remembered for his many contributions to government reports and to the scientific literature, his influence in numerous committees in the United Kingdom, the European Union, and internationally, and above all for his sound judgements, his friendly approachability, and his characteristic sense of humour.

Entering into government service represented a change in direction in David's career. Graduating in chemistry at the University of Bristol, and continuing there to obtain his Ph.D., he moved to the Southwest Foundation for Research and Education in San Antonio, Texas, USA. There he studied lactation in primates, including particularly the composition of baboon milk. He developed techniques to measure milk yield using isotopes, long before this field opened substantially into human studies. Returning to the United Kingdom in 1971 to take up the Ministry position, he held this influential post until taking early retirement in 1995. Becoming an independent nutrition consultant, he continued to be actively involved in advisory and project work.

One of David Buss's principal roles in the government was in ensuring that data on the nutrient intake of the UK population were reliable and constantly improved. He secured a viable future for the National Food Survey, a long-running study of household food purchases which has provided unique data on detailed trends in food and nutrient intake in the United Kingdom for over 50 years. When the fourth revised edition of the UK food composition tables, originated by McCance and Widdowson, was completed, David ensured that food composition continued to be updated and extended, and he played a leading role in the collaboration between the Ministry and the Royal Society of Chemistry in producing a complete series of supplements over a 10-year period. He was working on the proofs of the final one at the time of his death. David also acted as secretariat and observer to numerous Department of Health reports on nutrition and health, recommended dietary intakes, infant feeding, and nutritional surveillance. He was the stimulator and author of over 100 scientific papers, including many on the supply of trace and other nutrients in the British diet, and was chiefly responsible for revising the Ministry's best-selling "Manual of Nutrition." Outside the Ministry, he was a council member and held honorary posts in the United Kingdom Nutrition Society and the Royal Society of Health. In 1995 David was awarded the British Nutrition Foundation prize for his contribution to nutrition science.

As a government official, David was a frequent traveller to Brussels and other centres on EU affairs, and in 1995 he was appointed one of the UK delegates on the European Concerted Action COST 99 "Food consumption and food composition data." He was well known at FAO/WHO CODEX committees around the world. His expertise was much valued as a tutor for the International Graduate Courses on Production and Use of Food Composition Data in Wageningen, The Netherlands.

As well as his wide-ranging scientific contributions, David will be best remembered for his unfailing friendship and good humour. He had the gift of being able to see clearly a way forward through a wealth of data and thus achieve targets which may have seemed impossible.

Alison Paul
Human Nutrition Research
(Medical Research Council), UK

Editor's note

David Buss was a strong supporter of the United Nations University's International Network of Food Data Systems (INFOODS) initiative, and his contributions will be sorely missed.

Note for contributors

The editors of the *Food and Nutrition Bulletin* welcome contributions of relevance to its concerns (see the statement of editorial policy on the inside of the front cover). Submission of an article does not guarantee publication—which depends on the judgement of the editors and reviewers as to its relevance and quality. All potentially acceptable manuscripts are peer-reviewed. Contributors should examine recent issues of the *Bulletin* for content and style.

Language. Contributions may be in English, French, or Spanish. If French or Spanish is used, the author should submit an abstract in English if possible.

Format. Manuscripts should be typed or printed on a word processor, double-spaced, and with ample margins. Only an original typed copy or a photocopy of equivalent quality should be submitted; photocopies on thin or shiny paper are not acceptable.

When the manuscript has been prepared on a word processor, a diskette, either 3½- or 5¼-inch, should be included with the manuscript, with an indication of the disk format and the word-processing program used.

Length. Ordinarily contributions should not exceed 4,000 words.

Abstract. An abstract of not more that 150 words should be included with the manuscript, stating the purposes of the study or investigation, basic procedures (study subjects or experimental animals and observational and analytical methods), main findings (give specific data and their statistical significance if possible), and the principal conclusions. Emphasize new and important aspects of the study or observations. Do *not* include any information that is not given in the body of the article. Do not cite references or use abbreviations or acronyms in the abstract.

Tables and Figures. Tables and figures should be on separate pages. Tables should be typed or printed out double-spaced. Submit only original figures, original line drawings in India ink, or glossy photographs. Labels on the figures should be typed or professionally lettered or printed, not handwritten.

Photographs. Ideally photographic materials should be submitted in the form of black and white negatives or black and white glossy prints. Photographs will not be returned unless a specific request is made.

Units of measurement. Preferably all measurements should be expressed in metric units. If other units are used, their metric equivalents should be indicated.

Abbreviations. Please explain any abbreviations used unless they are immediately obvious.

References. References should be listed at the end of the article, also double-spaced. Unpublished papers should not be listed in references, nor should papers submitted for publication but not yet accepted.

Number references consecutively in the order in which they are first mentioned in the text. Identify references in the text and in tables and figure legends by arabic numerals enclosed in square brackets. References cited only in tables or figure legends should be numbered in accordance with the first mention of the relevant table or figure in the text. Be sure references are complete.

Reference citations should follow the format illustrated below.

Journal reference

—*standard journal article* (list all authors):

1. Alvarez ML, Mikasic D, Ottenberger A, Salazar ME. Caracteristicas de familias urbanas con lactante desnutrido: un análisis critico. Arch Latinoam Nutr 1979;29:220–30.

—*corporate author:*

2. Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended method for the determination of gammaglutamyltransferase in blood. Scand J Clin Lab Invest 1976;36:119–25.

Book or other monograph reference

—*personal author(s):*

3. Brozek J. Malnutrition and human behavior: experimental, clinical and community studies. New York: Van Nostrand Reinhold, 1985.

—*corporate author:*

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5. Medioni J, Boesinger E, eds. Mécanismes éthologiques de l'évolution. Paris: Masson, 1977.

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