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Influence of a Complementary Food on the Growth and Iron & Zinc Nutritional Status of Children 6 Months – 1 Year Old in Kilosa District, Tanzania

by

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KEYWORDS. — Anaemia; Complementary Feeding; Iron Deficiency.

SUMMARY. — A study, set up as a double-blind randomized, placebo-controlled trial, was conducted from March 2001 to March 2002 involving 309 infants who received either a processed Complementary Food (CF) or a placebo from six to twelve months of age. Both groups were comparable in baseline characteristics. The study took place in Kilosa district, Tanzania. The processed CF contained germinated, autoclaved and dried finger millet (65.2 %), kidney beans (19.1 %), roasted peanuts (8 %) and mango puree (7.7 %). The same blend, but not processed, served as placebo. Processing increased energy density for the same viscosity and solubility of iron and zinc. Mean length for age, weight for age, haemoglobin, zinc protoporphyrin and hair zinc concentration at six and twelve months were not different between the two groups. The results show that the processed food was not superior with regard to improving growth or iron and zinc nutritional status of infants when given under the study conditions. The control group consumed equal amounts of macronutrients, and the higher energy density, in this study, did not seem to have any benefits. In our study there was a very intensive follow-up with at every encounter an intensive motivation of mothers to give the required amounts and add extra lipids. In those conditions a

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well-balanced complementary food with additional lipids can cover the energy needs of young children. The observed reduction in phytates by 34 % and improved solubility of iron and zinc due to processing, might not have been enough to compensate for the rather low iron and zinc content of the complementary food.

Introduction

Growth and chance of survival at birth is to a large extent determined by the intrauterine period (MARTORELL *et al.* 1998). Exclusive breastfeeding in the first four-six months protects the child from nutritional deficiencies and decreases infection pressure. Young infants are most vulnerable at the time complementary foods are introduced. Traditional complementary foods are often bulky, have a low energy density and contain too small amounts of micronutrients, in particular iron and zinc (BENNETT *et al.* 1999, LARTEY *et al.* 1999). Complementary foods are largely cereal-based and contain considerable amounts of phytate, which affect micronutrient bioavailability greatly and so induce deficiencies in minerals. They are also not without risk of contamination (WHO 1998). Low child feeding frequency further contributes to undernutrition of children. Surveys carried out in Tanzania (UNICEF 1998) have indeed shown that most children are fed only two or three times a day.

Iron Deficiency Anaemia (IDA) is an important nutritional problem in Tanzania. It is estimated that 45 % of the children under the age of five years are suffering from nutritional anaemia (UNICEF 1999). It is more prevalent in infants and pregnant women and is usually the result of low bioavailability of dietary iron (FOX *et al.* 1998). Although the effects of iron deficiency anaemia are reversible, including impaired intellectual development, in the local suboptimal conditions this will rarely be the case. So, despite the fact that the diagnosis of anaemia is fairly simple and treatment cheap, prevalence of anaemia remains high (KOLSTEREN *et al.* 1999).

Zinc deficiency is common in developing countries (WHO 2002) where it affects mostly infants and children (FAO/WHO 2002, SALGUEIRO *et al.* 2002). Zinc is an essential component of a large number of enzymes involved in the synthesis and metabolism of nutrients in the human body (PRASAD 1996). Its role in stabilizing the molecular structure of cellular components and membranes, hence contributing to the maintenance of cell and organ integrity, is very well known. Zinc is involved in gene expression and plays a role in the central immune system (SHANKAR & PRASAD 1998). Deficiency of zinc during pregnancy may therefore lead to intrauterine growth retardation (SALGUEIRO *et al.* 2002), and as a consequence poor growth in the first years

of life. Some researchers have shown that even mild zinc deficiency may impair child growth (RIVERA *et al.* 2003).

The most frequently used strategies to correct micronutient deficiencies are food fortification and/or supplementation (STOLTZFUS *et al.* 1998). Local staples have been fortified with ferrous sulphate (MENDOZA *et al.* 2001), zinc sulphate (FAIRWEATHER-TAIT *et al.* 1995), or vitamin A and vitamin C (DAVIDSSON *et al.* 2001, ZLOTKIN *et al.* 2001, MCLAREN & FRIGG 1997). Effectiveness of large-scale fortification programmes, however, has been reduced due to factors such as cost, constant availability, timely distribution of fortificants, and compliance with the prescribed fortificant (THU *et al.* 1999). Similar experiences have been observed in many places with iron supplementation (SCHULTINK & GROSS 1996).

The above-mentioned limitations of fortification and supplementation underline the importance of preventing growth faltering and micronutrient deficiencies, such as iron deficiency anaemia, through a food-based approach (FOX *et al.* 1998, HALLBERG 1999). Food modification approaches employing natural processes such as germination to combat micronutrient deficiencies and improve growth of infants deserve more attention, since they are likely to be more sustainable in the long run (WHO 1998).

Based on the knowledge of the traditional complementary foods (CFs) in Tanzania collected by the authors earlier, a processed CF prepared from locally produced crops was formulated.

The present study aimed to compare growth, iron and zinc nutritional status of Tanzanian infants, from six to eleven months, provided processed and non-processed CF. The main hypothesis was that a processed CF with higher energy density and lower concentration in phytates and tannins would improve growth of infants and decrease iron and zinc nutritional deficiency.

Materials and Methods

The study was conducted from March 2001 to March 2002 in Kilosa District in the Morogoro Region, Tanzania. Morogoro is located about 300 km west of Dar es Salaam. Kilosa is a district with a population of ± 350,000 inhabitants. Kilosa was chosen for this study as it is among the districts in Morogoro Region which have a high prevalence of iron deficiency anaemia (Kilosa Hospital Annual Report 2000).

DESIGN OF THE STUDY

The study was set up as a double-blind randomized controlled trial in which the main investigator and the mothers were blinded with regard to the

type of food given to the infants. Processed complementary food constituted the intervention group and the non-processed food served as control. Infants were continuously enrolled when they reached the age of six months and assigned to the respective groups on the basis of their previously determined allocation. For this, all parents with children below the age of six months were contacted and invited to participate in the study. Parents of 364 children agreed to participate and were randomized. We expected a considerable change of opinion in the period between randomization and actual enrolment so increased the number to be sure to reach the minimal required number of children for the study. Allocation to the treatment or control group was determined using a bloc randomization technique. The code was broken to the main investigator at the end of the data collection. The two types of CF were distributed until the infants reached the age of twelve months. Mothers were free to give their infants any other food of their choice in addition to the CF provided during the study. Measurements were taken twice, at the age of six and twelve months with a malaria blood smear at nine months. Verbal consent was sought from mothers for their infants to participate in the trial. The Ethics Committees of the Tanzania Food and Nutrition Centre and of the University of Ghent reviewed the protocol and gave approval of the trial.

The sample size for the trial was computed to detect a haemoglobin difference of 8 $g.L^{-1}$ (equivalent to $\frac{1}{2}$ standard deviation) between the two groups with a significance level of 0.5 % and power of 95 %. A pilot study conducted in the area prior to the trial revealed that the mean Hb concentration among children aged between four-twelve months was 84.0 ± 17.0 $g.L^{-1}$. The calculated minimum sample size for the experimental and placebo groups was 117 infants (fig. 1).

The processed CF consisted of 65.2 % finger millet, 19.1 % kidney beans, 8 % peanuts and 7.7 % mango puree. Finger millet and kidney beans were washed and soaked in pre-boiled water for 2 and 7 h respectively, and germinated for 48 h at 30 °C. Later the lot was autoclaved and then solar-dried for about 6 h. Peanuts were roasted in an oven at 150 °C for 20 minutes. Mangoes were washed, peeled, sliced and puree extracted. Later the puree was dried in the solar drier for 12 h. The ingredients were mixed and milled to composite flour. The non-processed CF was a blend of finger millet, kidney beans, peanuts and dried mango puree, mixed in the same proportion as the processed CF and then milled to composite flour. The director at the production site did the packing and labelling. Packages and labels were identical and the two preparations were visually indistinguishable. The label contained preparation (cooking) instructions. Before the intervention, an acceptability trial for the CF was done involving fifty mothers with their infants.

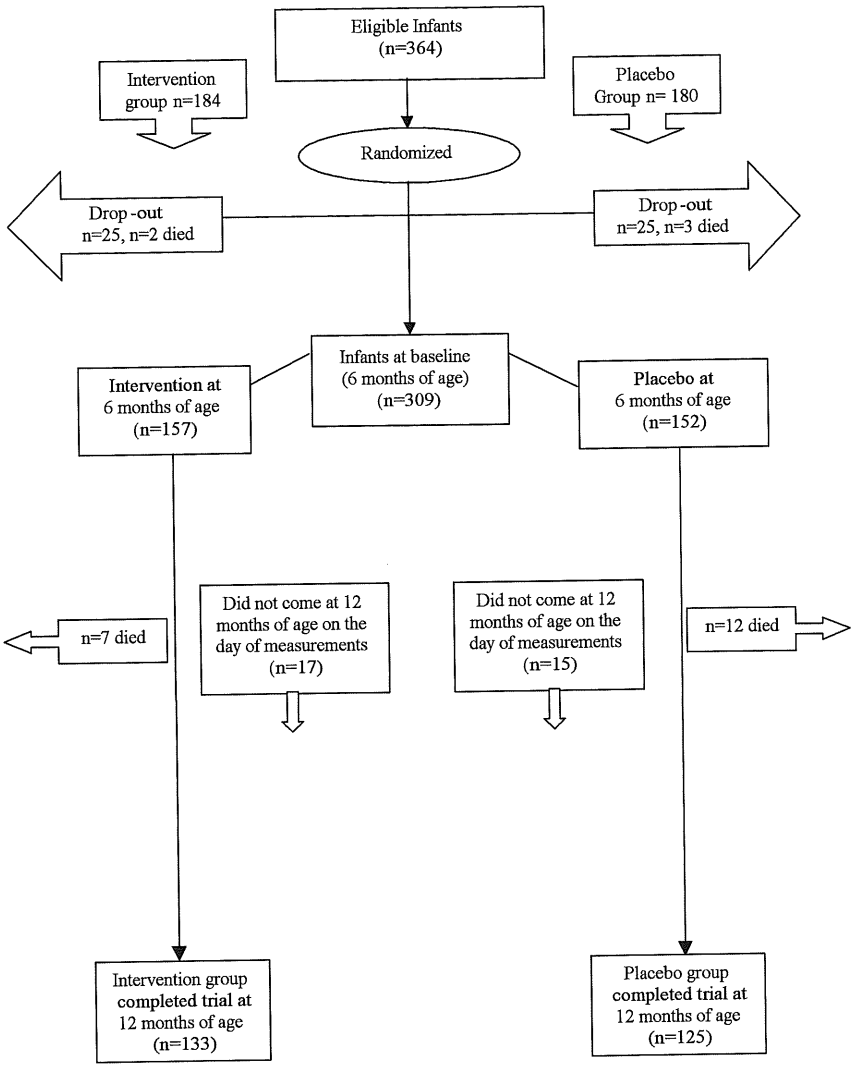

Fig. 1. — Study design. Infants (n = 309) in Tanzania received either a processed CF or an unprocessed CF (placebo) from 6-12 months of age.

Quality control was observed from purchase of raw materials through processing and distribution. For qualitative and quick checking of aflatoxin contamination an Ultra-Violet (UV) lamp (Type B-4 watt Kurtz-und Langwellig) was used. At various CF production stages samples were checked for

contamination with fumonisins (RIDASCREEN® FAST Fumonisin kit, Biopharm), aflatoxins (RIDASCREEN® FAST Aflatoxin Kit, Biopharm) and cyanides (AOAC 1995). Fumonisins and aflatoxin concentrations were less than recommended limits of 1 $mg.kg^{-1}$ and 2 $mcg.kg^{-1}$ respectively, while cyanides were not detected. Outgrowth of pathogens including *Bacillus cereus, Staphylococcus aureus* and *Clostridium perfringens* was investigated for each batch of CF following the HACCP plan (National Advisory Committee on Microbiology Criteria for Foods 1998) and found to be less than 100 $cfu.g^{-1}$ (KIMANYA *et al.* 2003).

Every two weeks, 1 and 1.6 kg of CF were allocated for infants six-eight months and nine-eleven months old, respectively. On a daily basis this means that each child was supplied with 69 g and 113 g dry matter of CF, providing 1,194 $kJ.d^{-1}$ and 1,956 $kJ.d^{-1}$ for infants aged six-eight months and nine-eleven months, respectively (DOP *et al.* 1999). The detailed formulation of the CF is described in MBITHI-MWIKYA *et al.* (2002).

Mothers came to collect the food every two weeks. The MCH nurses supervising the mothers had a list of the infants and recorded every food collection. They demonstrated to the mothers how to prepare the CF (although the packets had a self-explanatory label) and reminded them how much in local measures (tablespoons) of the CF powder to use each day. The CF for one day was prepared once. The nurses also advised the mothers to add one-two teaspoonfuls of oil to the preparation. So, at least about 1,151 $kJ.d^{-1}$ (275 kcal) and 1,883 $kJ.d^{-1}$ (450 kcal) from CF were aimed at. In case of absence, the nurse made certain that a message was sent to the responsible mother to collect her consignment on the same day. Nutrition officers from the centre followed up the mothers in their homestead at least twice a week to make sure the CF was prepared correctly.

Weight and length were recorded at enrolment (age six months) and at the end of the trial (age twelve months). Recumbent lengths of the infants were measured using an infant measuring board to the nearest 0.1 cm (Perspective Enterprises, Portage, MI). Weight of infants was measured by a Salter scale to the nearest 100 g (Model 235 6S – England) with a capacity of measuring up to 25 kg. The infant's birth weight was obtained from his/her clinic card: the MCH nurse is the one responsible to measure the child's weight immediately after birth.

Haemoglobin concentration was measured from a finger prick blood sample by the Hemocue B-Hemoglobin System (Hemocue AB Angelholm, Sweden) (VAN DEN BROEK & LETSKY 2000, PEHRSSON *et al.* 2001). Zinc protoporphyrin was determined on a drop of blood with a portable hematofluorometer (Aviv Biomedical Inc, Lakewood, NJ). The instrument was standardized using

control solutions from the same company (JACKSON & AL MOUSA 2000, ASOBAYIRE *et al.* 2001). The analyses were performed at a later date.

Blood smears, stained with Giemsa sample, were analysed within 10 h for quantitative determination of malarial parasites. Malaria parasite counts were made per two hundred white blood cells. Infants found infected with malaria and those who fell sick at any time during the trial were treated free of charge at the respective health centres.

Scalp hair was collected at baseline when the infants were six months old and at the end of the study when they were twelve months old and analysed for zinc concentration. All measures were taken to avoid external sources of adventitious contaminations during collection and preparation of the hair samples. The hair was cut with stainless steel scissors from the occipital region of the head as close to the scalp as possible. Since zinc levels vary along the length of hair (KRUSE-JARRE 2000), only the proximal 1-2 cm of the hair shaft was used. This part reflects recent trace element uptake by the follicles. The samples were collected in small clean sterile plastic envelopes with a self-adhesive mechanism. The envelopes were coded and kept in one securely closed plastic bag and transported by airfreight to the Department of Food Technology and Nutrition, Ghent University, in Belgium, for analysis.

Samples of the infants' hair were analysed by use of Atomic Absorption Spectroscopy (AAS). The wet digestion extraction for hair mineral analysis by AAS was adopted as a reference method for the determination of Zn in the infant hair samples. Selection of the wet digestion method was based on results of a preliminary recovery study which compared wet digestion and dry ashing methods and showed that the wet digestion method gives better results than the dry ashing method. All reagents used during the analysis were of ultra pure analytical grade. The glassware was washed with acid and thoroughly rinsed with deionised water.

In order to reduce measurement error, hair of six months and twelve months old infants was analysed concurrently. Because of the limited amount of hair that could be obtained from the infants, Zn analysis could not be performed in duplicate.

A twenty-four hour dietary recall was conducted on 137 randomly selected infants aged between six and eleven months, being about 50 % of the total number of infants equally distributed among the two intervention groups. A new appointment was fixed for others who were not found at home at the time of visit. Random numbers were used to select infants from each group. The dietary recall interviews were conducted at the homestead by a nutritionist accompanied by a village health worker. The mother was requested to show the type and amounts of foods the infant had actually consumed over

the last 24 h. The amount of food consumed by the infant was weighed using a digital weighing scale (Tefal scales UK) with an accuracy of 0.5 g or measured by a measuring cylinder (Pyrex-UK) with an accuracy of 0.5 ml in case of volumes. Spilled food was not estimated. Macro and micronutrient contents of the foods were calculated using the FAO food composition table (FAO 1984) with Excel Office 2000.

Samples of CF weighing 250 g from each production batch were analysed for protein (Kjedahl AOAC method 920.87 (AOAC 1995)), fat (Weibull method (EGAN *et al.* 1981)), and iron (atomic absorption spectrophotometry AOAC method 970.12 (AOAC 1995)). The nitrogen protein conversion factor for millet was 5.83 and 5.3 for beans and peanuts. Solubility of iron was determined by the method of SVANBERG & SANDBERG (1993), and phytate using the technique of HAUGH & LANTZSCH (1983).

Infants were excluded from the trial if they had received blood transfusions or presented a health condition that needed further treatment as assessed by a medical doctor at the time of enrolment. These excluded infants were referred to the local health facilities for appropriate treatment. All children were investigated for malaria at six months of age and treated accordingly. Given that all children are breastfed and that Kilosa is a malaria endemic area, anaemia is considered to be due to malaria in the standard protocols. At the end of the trial, the children were re-evaluated and treated for the most likely aetiology of anaemia by the supervising medical doctor.

STATISTICAL ANALYSIS

Data were entered in EPI-INFO (version 6.04d; Centers for Disease Control and Prevention, World Health Organization 1996), and analysis was done by using Stata 8.0 package *(Stata version 8.0; STATA, College Station, Texas)*. Z scores, weight-for-length and length-for-age were computed using EPINUT according to the National Centre for Health Statistics Standards of 1977.

Descriptive statistics were done on each variable to identify outliers and assess the normal distribution of continuous variables.

Outliers were defined from the box plot as values more extreme than three-interquatile range of the box. In the presence of outliers, a new variable was created excluding these values. However, in case of doubt, the dataset was cross-checked with original data in the rosters. All tests were done first with the original variable, and then redone with the new variable to assess influence of such outliers. Normal distribution of continuous variables was appraised by a Kolmogorov Smirnoff test. In case of severe departure from normality, the variables were log-transformed.

The α error was set at 5 % in all tests. The strategy of data analysis was set in two steps. First, a difference at twelve months of age between the two intervention groups was assessed for each primary outcome. These primary outcomes were mean zinc protoporphyrin and mean haemoglobin. Differences in anthropometric indicators, *i.e.* mean weight-for-length Z score and length-for-age Z score at twelve months of age were also looked at. A standard t-test was used for continuous variables, and a chi square test for categorical ones. Likewise, the general trend in main outcomes, between the beginning and the end of the trial, was assessed by applying a paired t-test or a McNemar test for categorical variables.

Secondly, a logistic regression analysis was applied using the EVW model (KLEINBAUM 1994). Continuous variables were transformed in categorical ones as indicated below. The dependent variables were: *i)* high zinc protoporphyrin at twelve months of age (ZP > 5 $\mu g\ g^{-1}$ Hb, coded 0/1) for the first set of models; *ii)* anaemia (Hb < 110 $g.L^{-1}$, coded 0/1) for the second set of models; *iii)* length-for-age Z score (LAZ < –2 SD, coded 0/1) for the third set of models [1]*.

The exposure variable was the type of CF received. The following covariates were inserted in the initial model because they were potential confounding factors or effect modifiers (biological or environmental): *i)* child parameters: zinc protoporphyrin > 5 $\mu g\ g^{-1}$ Hb at baseline (0 = no, 1 = yes), Hb < 110 $g.L^{-1}$ at baseline (0 = no, 1 = yes), blood smear positive for malaria at age nine months (0 = no, 1 = yes), birth weight < 2,500 g (0 = no, 1 = yes), length-for-age < –2 Z score at baseline (0 = no, 1 = yes), sex (0 = girl, 1 = boy), season at entry in the study (0 = harvest season, 1 = other season); *ii)* mother's parameters: mother's education lower than primary school (0 = no, 1 = yes), mother living alone (0 = no, 1 = yes), mother's age < 20 years (0 = no, 1 = yes), parity ≥ 3 (0 = no, 1 = yes), BMI (0 = more or equal to 18.5, 1 = under 18.5), income lower than 10,000 shillings (0 = no, 1 = yes).

All covariates were considered as potential effect modifiers and introduced in the initial model as product terms involving E (exposure to processed food). The presence of multicolinearity and other numerical problems in regression analyses was appraised by verifying the presence of high estimated standard errors for the regression estimates (HOSMER & LEMESHOW 1989). Then a hierarchical backwards elimination procedure was applied to eliminate non-significant variables.

Removal of variables was at $p > 0.05$ for the likelihood ratio test. First effect modifications were tested using a likelihood ratio test for the entire

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* The number between brackets [ ] refers to the note, p. 92.

collection of interactions term (KLEINBAUM 1994). Then variables were removed one by one according to the likelihood ratio test. The model including the remaining covariates was considered the gold standard. Then confounding was assessed by monitoring changes in the effect measure (odds ratio) for subsets of covariates. The subset of covariates included in the final model was the one allowing the best gain in precision.

Results

SUBJECTS AND COMPLIANCE

Of the 364 children randomized, 309 presented themselves for participation in the trial. 157 children started in the intervention group and 152 in the control group. At the end of the trial the respective groups had 133 and 125 participants (fig. 1). The drop-out rate did not differ significantly between the two groups.

A total of 60 infants from the intervention group and 58 from the control group did not have sufficient hair for analysis at baseline. At twelve months, 137 infants were able to provide hair samples of whom 71 were from the intervention group and 66 from the control group.

To check for compliance, nutrition officers from the centre followed up the mothers in their homestead to make sure the CF was prepared in the correct way. Compliance was regarded good if the mother collected the CF, correctly prepared and consumed it according to instructions. Surprise visits to the home were made once or twice a week. However, we were not able to monitor full compliance in the strict sense.

BASELINE CHARACTERISTICS

The characteristics of infants, mothers and household variables in both groups were similar at enrolment of the infants, aged six months (tab. 1) with the exception of WLZ (weight-for-length Z score). Mothers education ($p = 0.11$), marital status ($p = 0.36$) and family income ($p = 0.43$) was not significantly different between the intervention and control group (χ^2 test).

NUTRITIONAL CHARACTERISTICS OF THE COMPLEMENTARY FOOD

The random samples from each month's CF production unit and traditional CF had an energy, fat and protein content, which was not significantly different (tab. 2). However, the porridge prepared with the processed CF had a higher energy density, significantly higher iron and zinc solubilities and a

Table 1
Baseline characteristics

Variables	Processed food (157)	Placebo (152)	P-value
	Mean[1]	Mean[1]	
Birth weight (kg)[2]	2.9 ± 0.5	3.1 ± 0.5	0.06
Weight (kg)	6.7± 1.0	6.9 ± 1.0	0.93
Length (cm)	62.7± 3.1	62.8 ± 3.1	0.06
Haemoglobin concentration ($g.L^{-1}$)	91.4 ± 1.9	94.4 ± 21	0.27
Zinc protoporphyrin ($\mu gZP\ g^{-1}$ Hb)	9.9 ± 6.1	9.9 ± 5.8	0.97
Hair zinc concentration ($\mu g\ g^{-1}$)	250.6 ± 102.0	277.0 ± 112.5	0.05
WLZ[3]	0.45 ± 1.24	0.72 ± 1.14	0.04
LAZ[4]	– 1.53 ± 1.16	– 1.54 ± 1.11	0.96
Household size (persons)	5.8 ± 2.3	5.4 ± 2.1	0.11
Maternal Body Mass Index ($kg\ m^{-2}$)	21.9 ± 2.5	21.9 ± 2.7	0.96
Live children (persons)	2.7 ± 1.9	2.7 ± 1.8	0.86
Mother age (years)	25.6 ± 7.0	25.3 ± 6.6	0.63
Maternal parity (No. of births)	3.4 ± 2.3	3.3 ± 2.1	0.63
Sex ratio of infants (male:female)	1.01	0.95	

[1] Mean ± SD. No significant differences between the groups.
[2] Based on 280 infants, 29 infants were home deliveries.
[3] WLZ: weight-for-length Z score.
[4] LAZ: length-for-age Z score.

Table 2
Composition of the field complementary food

Parameter	Complementary food[1]		P-value
	Processed	Non-processed	
Energy (kJ/100 g DM)[2]	1731 ± 11	1731 ± 18	0.89
Protein (g/100 g DM)	12.87 ± 0.57	12.64 ± 0.56	0.33
Fat (g/100 g DM)	4.64 ± 0.52	5.08 ± 0.74	0.11
Ash (g/100 g DM)	2.38 ± 0.08	2.88 ± 0.4	0.001
Zinc (mg/100g DM)	2.52 ± .0.09	2.40 ± 0.08	0.01
Total iron (mg/100 g DM)	4.74 ± 0.41	5.89 ± 0.87	0.0002
Energy density of porridge (kJ/ml)	6.1	1.7	–
Soluble iron (%)	18.83 ± 0.72	4.76 ± 0.80	0.0001
Soluble zinc (%)	6.24 ± 2.47	2.74 ± 1.49	0.0028
Solids % (w/v) in pap at optimum viscosity	35	10	–
Phytates (% DM)	0.66 ± 0.02	1.15 ± 0.03	0.04

[1] Mean ± SD of 12 production batches.
[2] DM: Dry Matter.

lower concentration of phytates when compared to the porridge prepared with the non-processed CF. There was a small but significant reduction in total iron content in the processed CF compared to the non-processed CF probably due to leaching in the course of processing the CF (WATZKE 1998). This disadvantage was counterbalanced by the higher amount of soluble iron in the processed CF compared with non-processed CF (18.83 % versus 4.76 %), respectively.

Food consumption data from the 24 h dietary recall showed no significant difference in daily energy intake, proteins, lipids, and iron intake from the processed and non-processed CF between the two groups of children (tab. 3). The mean number of meals was, however, considerably higher in the non-processed group. The energy intake from the project CF was 1,752 kJ and 1,679 kJ in the processed and non-processed CF groups. This was increased to 1,922 kJ and 1,943 kJ in both groups, respectively due to the addition of oil. Overall the project complementary food contributed more than 50% of the total daily energy intake. The energy intake from CF exceeded the WHO recommendations. Other foods consisted of plain maize flour porridge, porridge made from family food, mixture of maize, rice, peanuts and finger millet flour and milk. Only two children received fruits (banana or mango). Other foods and breast milk contributed substantially in total daily energy, lipids and protein. Using a conservative approach and taking a low minimal breast milk contribution, the estimated total energy intake in comparison with the recommended daily energy requirements was on average 96 % and 107 % for infants six-eight months and 106 % and 103 % for infants nine-eleven months respectively (tab. 3).

EFFECT OF COMPLEMENTARY FOOD ON GROWTH

No differences could be observed between the groups at twelve months of age (tab. 4). The differences in weight gain were not significant between the two groups with an increase of 1.4 ± 0.6 kg versus 1.3 ± 0.7 kg for the supplemented and placebo group respectively ($p < 0.001$). Likewise, mean weight-for-length and length-for-age Z scores did not differ significantly between the two groups ($p = 0.12$ and $p = 0.78$ respectively). Length-for-age Z scores dropped from age six to twelve months in both groups from -1.60 Z score to -2.06 ($p < 0.001$). A similar trend was observed in weight-for-length, which declined from $+0.57$ to -0.17 ($p < 0.0001$).

EFFECT OF COMPLEMENTARY FOOD ON HAEMOGLOBIN AND ZINC PROTOPORPHYRIN

Concentrations of Hb and ZP were not significantly different between groups at twelve months of age (tab. 4). Although both groups significantly

Table 3

Twenty-four hour dietary recall of six to twelve months old infants[1] receiving processed and non-processed (placebo) complementary food

Mean daily intake	Infants receiving processed food (n = 71)	Infants receiving placebo (n = 66)	P-value
Total energy intake ($kJ.d^{-1}$)[1]	3,427 ± 915	3,426 ± 784	0.99
6-8 months infants % recommended energy intake[1,2]	95.7 ± 26.0	105.9 ± 22.7	0.20
9-12 months infants % recommended energy intake[1,2]	106.7 ± 24.0	103.4 ± 22.5	0.49
Energy from project CF + oil ($kJ.d^{-1}$) [1]	1,922 ± 793	1,943 ± 691	0.47
Energy from other CFs ($kJ.d^{-1}$) [1]	657 ± 333	636 ± 345	0.49
Total proteins (g) [1]	18.3 ± 6.3	17.9 ± 5.5	0.68
Total fats (g) [1]	29.9 ± 7.2	31.3 ± 6.4	0.24
Total iron intake (mg)	68 ± 2.7	65 ± 2.4	0.55
Total zinc intake (mg)	3.3 ± 0.12	2.9 ± 0.8	0.31
Frequency of meals day^{-1}	1-2	5-6	

[1] Mean ± SD.

[2] Based on minimum milk production by women in developing countries (4).

Table 4

Comparison of haemoglobin (Hb), zinc protoporphyrin (ZP), WLZ and LAZ of infants at twelve months of age by intervention group

Variables at age 12	Processed[1] (n = 133)	Placebo[1] (n = 125)	Mean difference	T test p-value	Overall[1] (n = 258)	T test[3] p-value
Hb ($g.L^{-1}$)[1]	96.6 ± 17.4	96.5 ± 16.2	0.1	0.96	–	–
ZP (µg g^{-1} Hb) [1,2]	5.8 ± 3.5	6.2 ± 3.1	0.4	0.34	–	–
WLZ[1,2]	– 0.27 ± 0.97	– 0.07 ± 0.98	0.19	0.12	–	–
LAZ[1]	– 2.08 ± 1.02	– 2.04 ± 1.07	0.04	0.78	–	–
Changes between age 6 and age 12						
Change Hb ($g.L^{-1}$) [1]	4.8 ± 1.8	1.5 ± 2.0	3.3	0.19	3.2 ± 1.3	0.014
Change of ZP (µg g^{-1} Hb) [1,2]	– 4.4 ± 0.5	– 3.6 ± 0.5	0.77	0.39	– 4.0 ± 0.4	< 0.0001
Change in WLZ[1]	– 0.67 ± 0.10	– 0.81 ± 0.10	0.14	0.31	– 0.74 ± 0.07	< 0.0001
Change in LAZ[1,2]	– 0.50 ± 0.06	– 0.42 ± 0.07	0.08	0.40	– 0.46 ± 0.05	< 0.0001

[1] Mean ± SD.

[2] t-test applied on zero-skewness log-transformed variables.

[3] Comparing values at age 12 with values at age 6 (baseline) by a paired-t test.

increased in Hb and decreased in ZP from six months to twelve months of age ($p < 0.014$ and $p < 0.0001$ respectively) the difference in both groups from six to twelve months was not significant (p for Hb change = 0.19 and 0.39 for ZP). The majority of infants (76 %) were still anaemic according to WHO standards at the end of the study. ZP declined significantly from a mean of 10.1 $\mu g\ g^{-1}$ Hb at the age of six months to a 6.0 $\mu g\ g^{-1}$ Hb at the age of twelve months ($p < 0.001$), but without differences in the two groups.

No significant interaction between covariates and the type of CF was detected (likelihood ratio test for set 1, p-value 0.20, likelihood ratio test for set 2, p-value 0.11, likelihood ratio test for set 3, p-value 0.11) with the logistic regression.

Effects of Complementary Food on Zinc Status

There was no significant change in hair zinc concentration from six months to twelve months of age in the processed CF group ($P = 0.53$), whereas in the unprocessed CF there was a slight significant decrease in hair zinc concentration ($p = 0.03$) (tab. 5). However, there was no significant difference in hair zinc concentration between the two groups.

Association of Zn Status with Growth

Hair zinc status was positively correlated with the length-for-age Z score (LAZ) and the weight-for-age Z score (WAZ) in both groups. However, the correlation coefficients were not statistically significant ($p > 0.05$) (tab. 6).

Discussion

The results show that the processed food was not superior with regard to improving growth, haemoglobin, and iron and zinc status of infants when given under the study conditions. Weight gain was not significantly different between the two groups, and stunting increased. The control group consumed equal amounts of macronutrients, and the higher energy density, in this study, did not seem to have any benefits. In our study there was a very intensive follow-up, with at every encounter, an intensive motivation of mothers to give the required amounts and add extra lipids. In those conditions a well-balanced complementary food with additional lipids can cover the energy needs of young children. Both groups were comparable in energy intake from complementary foods. Age specific energy and protein intake from CF was

Table 5
Mean (± SD) hair Zn concentration (μg/g) at baseline and end of the study

Timing	Intervention (n = 71)	Control (n = 66)
Baseline (6 months)	250.6 ± 102.0	277.0 ± 112.5
End of study (12 months)	241.8 ± 107.5	246.9 ± 119.8
P-value	0.53	0.03

Table 6
Correlation coefficients of hair zinc and anthropometric indices

	Processed CF		Unprocessed CF	
	Correlation	P-value	Correlation	P-value
LAZ	0.092	0.47	0.038	0.79
WAZ	0.037	0.77	0.013	0.92

according to WHO recommendations. The complementary food intake was higher during the study, compared with information obtained from a baseline study, where in the six-eight and nine-eleven age group complementary foods only covered 81 and 75 % of the recommended energy that complementary foods should provide.

We cannot exclude, however, that in less supportive conditions the energy-denser food would yield different results. Indirect evidence for this can be found in the considerable higher feeding frequency in the non-processed food. Two meals suffice to provide the necessary amount of complementary food, compared to five-six meals in the non-processed one. We introduced a bias in the study by emphasizing continuously the amount of CF to be eaten per day. In settings where time constraints of mothers limit feeding frequency, differences in total intake can be expected. The study has also certain limits: we cannot exclude with certainty that food was shared. However, there are other studies that also documented negative results. In Ghana, the effects of feeding "Weanimix" and three other locally formulated, centrally processed CFs, on nutritional status of breastfed infants (six-twelve months of age), found no significant differences between intervention groups in weight or length gain, haemoglobin and hematocrit values (LARTEY *et al.* 1999).

STEVENS & NELSON (1995) investigating the effect of feeding six months old infants a milk formula with no iron against one with 12.0 mg $Fe.L^{-1}$ for

twelve months, found no differences with respect to mean haemoglobin, ferritin concentrations and growth between the two groups of infants. Furthermore, a multicountry study, investigating the effect of supplementation with high energy density CF fortified with minerals and vitamins, on weight and linear growth of four-seven months old infants in four developing countries, found no significant difference in overall linear growth, although there was an effect of the micronutrients in stunted children but not in non-stunted children (SIMONDON *et al.* 1996).

Some studies did find a difference but they differed in a number of important aspects. In the study of CHINAMMA & GOLPALDAS (1993) two groups of children aged six – twenty-four months were either fed a high-energy low viscosity complementary food or a high-energy high viscosity one for six months. The former group increased significantly in weight and length over the latter. The difference of this study with ours is that the children were restricted to one experimental meal per day although they were allowed to eat *ad libitum* in that single meal. In a sense therefore one group was getting less experimental food than the other per day. In our study the mothers were encouraged to feed their infants the entire daily-allocated CF amount, which means they could have increased the feeding frequency up to five-six times a day.

Stunting is a very complex multicausal problem, and it is probable that other nutrients were limiting, or that the local conditions did not allow reversal of the trend or stabilization. Similar trends were observed by MARTORELL *et al.* (1998), whereby 25 % of infants at baseline (four months old) were already stunted, presumably due to intrauterine growth retardation (IUGR). They concluded that infants who experience IUGR usually never completely catch up in size to their normal birth weight peers even when raised under optimal conditions. Similar observations were made in Indonesia (MSUYA & CURTIS 1991, HURRELL 1992).

Despite an overall significant rise in mean haemoglobin, a large proportion of infants were still anaemic at the end of the study, even when low birth weight was controlled in the analysis. An important cause of anaemia is definitely malaria in the present population. At six months we found malaria parasites in 50 % of the samples. The Hb concentration was 1.29 $g.L^{-1}$ lower at month twelve in children with a positive blood smear at month six and month twelve (t-test $p < 0.0001$), while ZP concentration was 1.84 $\mu g\ g^{-1}$ Hb higher (t-test $p < 0.0001$). Mortality in the study population was also very high with nineteen deaths among the 309 (61 per thousand) supplemented children. Failure to restore haemoglobin concentrations to normal could be explained by the continuous malaria re-infection among infants despite the

treatment given when they fell sick. MSUYA & CURTIS (1991) indeed showed that in Tanzania, almost all children became re-infected within two to four weeks after effective malaria parasite clearance with sulphadoxine (Fansidar).

Iron status, as defined by erythrocyte zinc protoporphyrin, was not significantly different between the processed and non-processed feeding groups, although iron status improved in general. This was rather unexpected since both groups consumed equal amounts of complementary food and the laboratory results showed an important increase in solubility, a parameter commonly used as a proxy for bioavailability. The iron content in both processed and non-processed complementary food was low while a minimal bioavailability of 10 % is needed in them to provide the recommended daily amount of 0.6-0.7 mg absorbed iron. The observed reduction of phytates in the processed CF by 34 %, which improved iron solubility to 19%, might not have been enough to make a clinical difference. For bioavailability to improve with metabolic significance, HURRELL (1992) postulated that phytates need to be reduced by more than 95 %. Finally, infants might have covered their basic iron needs, but the food content was not adequate enough to show any significant increase with regard to covering their needs and allowing for recovery from iron deficiency (DOMELLOF 2001).

Hair zinc levels were not significantly different between the two groups after intervention, although the processed CF had improved zinc solubility (tab. 2). Moreover, the intake of Zn by the infants in both groups was found to be less than the adequate amount for normative physiological requirement of 5 mg/day (WHO 1998) (tab. 3). Probably the infants had a reduced demand for Zn compared to health children with positive linear growth, because of their small body size and possibly greater intestinal stores of endogenous Zn (LEE *et al.* 1993, YEUNDALL *et al.* 2002). The latter may have arisen as a result of an adaptation to the low content of absorbable Zn in their habitual high phytate cereal-based CF.

The correlation between hair zinc and both LAZ and WLZ for infants in both groups was not significant ($p > 0.05$). This is in contrast to a study by UMETA *et al.* (2000) who found a strong positive correlation between hair zinc concentration and growth in stunted Ethiopian infants. The difference between that study and our study is that they used zinc supplementation and not dietary modification, and that Zn was the primary growth limiting nutrient in the infants' diet. These findings emphasize that only in circumstances when Zn is the first limiting nutrient can significant relationship between hair Zn concentration and physiological functional indices of Zn status such as linear growth be expected (CAVAN *et al.* 1993, UMETA *et al.* 2000).

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NOTE

[1] Weight-for-height indicator was not considered as 99 % of the children were in the normal range (mean ± standard deviations).

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