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Crossover trial to test the acceptability of a locally produced lipid-based nutrient supplement (LNS) for children under 2 years in Cambodia: a study protocol

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ABSTRACT

Introduction The acceptability and efficacy of existing ready-to-use supplementary and therapeutic foods has been low in Cambodia, thus limiting success in preventing and treating malnutrition among Cambodian children. In that context, UNICEF and IRD have developed a locally produced, multiple micronutrient fortified lipid-based nutrient supplement. This food is innovative, in that it uses fish instead of milk as the animal source food. Very few supplementary foods have non-milk animal source foods, and in addition they have not been widely tested. This trial will assess the novel food's acceptability to children and caregivers.

Methods and analysis This is a cluster-randomised, incomplete block, 4×4 crossover design with no blinding. It will take place in four sites in a community setting in periurban Phnom Penh. Healthy children aged 9–23 months (n=100) will eat each of four foods for 3 days at a time. The amount they consume will be measured, and at the end of each 3-day set, caregivers will assess how well their child liked the food. After 12 days, caregivers themselves will do a sensory test of the 4 foods and will rank them in terms of preference.

Ethics and dissemination Ethical clearance was received from the University of Queensland Medical Research Ethics Committee (2014001070) and from Cambodia's National Ethics Committee for Health Research (03/8 NECHR).

Registration ClinicalTrials.gov, identifier: LNS-CAMB-INFANTS; NCT02257437. Pre-results.

Strengths and limitations of this study

- This trial will contribute to the literature comparing supplementary foods using animal source foods other than milk.
- It will also provide information on the kinds of supplementary foods acceptable to a Southeast Asian population.
- Testing over 3 days in an unfamiliar setting may not be an indication of how caregivers and children would accept the food over a longer period. However, should the food prove acceptable in trial, a 6-month efficacy trial will follow. The latter trial will give additional information on long-term acceptability.

BACKGROUND AND RATIONALE

It is estimated that undernutrition is implicated in some 45% of deaths in children under 5 years.[1] In Cambodia, progress in combatting malnutrition has stalled. In 2014, 32% of all children under 5 years (and 40% of children aged 3–4 years old) were stunted, 10% were wasted and 24% were underweight[2] indicating, respectively, chronic and acute malnutrition, and a combination of the two. This malnutrition may be attributed in large part to poor complementary feeding,[2] which remains inadequate for achieving optimal growth outcomes and micronutrient status.

Adequate complementary feeding can reduce and prevent malnutrition.[3] In Cambodia, the traditional weaning food is *borbor*, white rice porridge with added salt or sugar, which is low in nutrient density. Improvements to complementary feeding may be achieved with supplements, such as micronutrient powders, and supplementary foods. The latter include fortified blended products that are mixed with water to make a porridge (eg, corn-soy blend++ or CSB++, now called Supercereal Plus), biscuits that can be eaten directly (such as BP100) or ready-to-use supplementary foods (RUSFs). RUSFs are usually lipid-based nutrient supplements (LNSs), which are often pastes such as the peanut-based Plumpy'Nut. Although until fairly recently, prevention of malnutrition has relied on fortified blended products, these new LNSs are proving very effective, both as RUSFs and ready-to-use therapeutic foods (RUTFs). Compared with the existing products, LNSs are higher in energy,

have a longer shelf life and are convenient since they require no preparation.[4 5]

Regardless of how effective a product may be, it still needs to be acceptable in a given setting. In other words, children must be willing to eat the product and caregivers must be willing to feed it to them. Acceptability to children can be measured by how much they eat and how readily, while acceptability to caregivers is measured in terms of their sensory perception of the food, that is, of the smell, colour, consistency and taste.[6] Other important factors affecting acceptability are price and convenience of preparation.

In Cambodia, various supplements and supplementary or therapeutic foods, including Sprinkles micronutrient powders, CSB++, BP100 and Plumpy'Nut, have been used or trialled. However, they have met with low levels of acceptability and success, either in trial or in practice.[7–9] Moreover, they are relatively expensive to procure and ship to Cambodia. For these reasons, and due to budget constraints, the United Nations World Food Program (WFP) in Cambodia phased out CSB++ distribution in 2014. A recent study estimated that only 20% of Cambodian caregivers purchase supplementary foods for their children.[10] Hence, the Cambodian Ministry of Health sought a locally produced ready-to-use food (both therapeutic and supplementary versions) containing macronutrients and micronutrients that can be adapted for use in Cambodia. It is expected that locally produced products are more likely to be acceptable and cheaper than the imported products. They also have the advantage of contributing money and capacity to the local economy.[11]

In 2009 in Vietnam, UNICEF, the Institut de Recherche pour le Développement (IRD) and the National Institute of Nutrition had developed a supplementary food from local ingredients including rice, soy, mungbeans, sugar, milk powder, oil and multiple micronutrients.[9 12] This product proved acceptable and effective and is now widely used. Drawing on that successful experience, UNICEF and IRD created a Cambodian ready-to-use food (in both supplementary and therapeutic versions) in early 2014, using fish, rice, soy, mungbeans, oil and sugar. Based on promising initial results, the product was finalised as a micronutrient-fortified snack.

OBJECTIVES AND HYPOTHESIS

This trial aims to establish the acceptability of the locally produced Cambodian RUSF for children under 2 years and their caregivers. Its acceptability will be compared with other supplementary foods that are or have been used in Cambodia, namely CSB++ and Sprinkles micronutrient powders.

DESIGN AND METHODS

Trial design

The trial is a cluster-randomised, incomplete block, 4×4 crossover design. The allocation ratio is 1:1. This will be an open trial with no blinding, because the 4 foods will be visibly different to participants and data collectors. The trial will take place in 2 parts over 2 weeks:

1. substudy 1: acceptability by children, 3 days × 4 foods for a total of 12 days
2. substudy 2: acceptability by caregivers, 13th day.

Foods and preparation

Four foods will be tested. The RUSF in snack form, and the RUSF added to plain *borbor*, will be compared with CSB ++ porridge, and Sprinkles added to plain *borbor*.

CSB++ is the United Nations WFP's standard supplementary food to prevent malnutrition in children aged 6–23 months. Sprinkles have been promoted and distributed by the Cambodian Ministry of Health to improve the micronutrient status of children aged 6–23 months.

CSB++ contains milk and is considered to be creamy, sweet and smooth.[13] It requires 10 minutes of cooking. Sprinkles are added to food after cooking or heating and do not have a taste.[14]

Study site

The study will be conducted in periurban Phnom Penh. This population has been selected because the urban poor comprise about one quarter of the Phnom Penh's residents, or approximately one-quarter of a million people,[15] who experience high rates of child underweight and stunting (35.6% and 29.1%, respectively).[16] Furthermore, the populations are large and dense enough to yield the required sample size.

The study will be conducted in four test-feeding sites such as pagodas or health centres identified based on convenience. There will be two teams of data collectors working at two test-feeding sites each. In this way, all children at a given site will be eating the same food, which will reduce bias related to social interaction and varied responses to different foods. Children and caregivers will come at the same time each day for the 12 days, which will reduce bias related to feeding times.

The four test-feeding sites will be randomly allocated to begin on one of the foods as shown in figure 1 below, using an Excel random number table and a randomised incomplete block design. The principal researcher will generate the allocation sequence. Children will not be randomised to a food, since all children at a given test-feeding site will be eating the same food.

Study participants

Participants will be recruited by convenience from the village/s close to the four sites. Village Health Support Group members (local health volunteers) will assist with recruitment. It is expected that there will be approximately equal numbers of female and male children and that the children's caregivers will be mostly female. Caregivers and children may be recruited if they meet the following inclusion and exclusion criteria:

- To facilitate child feeding, only singletons will be eligible for inclusion.

Site	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
1	LSN + *borbor*			Sprinkles + *borbor*			CSB++			LNS snack		
2	Sprinkles + *borbor*			LSN + *borbor*			LNS snack			CSB++		
3	CSB++			LNS snack			LSN + *borbor*			Sprinkles + *borbor*		
4	LNS snack			CSB++			Sprinkles + *borbor*			LSN + *borbor*		

Figure 1 Food sequence schedule. CSB++, corn-soy blend++; LNS, lipid-based nutrient supplement.

- Children aged 9–23 months who have been eating solids for at least 3 months will be eligible for inclusion. This is to ensure that subjects are familiar with solids and will not reject the food simply because they are not yet familiar with solids. In addition, the target group for these kinds of products is children aged 6–23 months.
- Only normally nourished or moderately malnourished children (mid-upper arm circumference (MUAC) >115 mm, z-score for weight-for-height (WHZ) >−3) who have been in good health for the past 3 days will be eligible for inclusion. This is to ensure that subjects are not experiencing any loss of appetite associated with malnutrition or illness and to be able to refer sick or severely acutely malnourished children for treatment.
- Likewise, only caregivers who have no medical complications or illness will be eligible in order to avoid any associated appetite loss and to refer for treatment.
- Children who have been using Sprinkles, CSB++ or similar supplementary foods or supplements will be excluded, in order to ensure that the interventions are equally unfamiliar and that children will not be likely to reject or accept based on their unfamiliarity/familiarity with a given food.
- Children with known food intolerances will be excluded.
- Any caregivers or children who become ill during the trial will be excluded and referred for treatment.
- Only children of caregivers who have provided signed or fingerprinted consent will be eligible for inclusion.

Sample size

The main outcome of interest is the amount of food the children consume. We define acceptability as mean consumption of at least 50% of the food offered, and high acceptability as consumption of 75% or more, assume an SD of 30% and aim to detect a difference in consumption of 20%.[9 17] To ensure a precision of 0.05, power of 0.8 and p<0.05, the required sample size is 20 children. Assuming 20% attrition, we need to enrol 24 children and caregivers. This sample size is the same as a similar acceptability study, and the attrition assumed is similar.[17]

The sample size was calculated using G*Power (V.3.1.9.2). The four clusters and repeated measures were taken into account in the calculation. The four sites were purposefully chosen to represent urban poor populations and were similar. Since this is effectively a pilot study inasmuch as we have no data on the acceptability of two of four of the foods, we have no knowledge about variability within or between cluster sizes, nor of how baseline covariates would affect the sample size. Thus, baseline covariates were not taken into account in sample size calculation.

However, with such a small sample size, it may not be possible to perform regressions. Therefore, we will recruit a sample of 100 caregivers and children, which is considered a typical sample size for a hedonic test[18] and is larger than most of the samples for similar studies.[9 13 17 19 20] Attrition rates in those studies have been less than 10%; therefore, our sample size of 100 should be more than adequate. We expect to recruit 20–30 participants per cluster.

Data collection

Baseline and anthropometric data

On the day before the start of the trial, potential participants will be assessed for eligibility at the test-feeding site, using an exclusion form, and through the collection of baseline data, including demographic, anthropometric, morbidity and dietary data (breastfeeding, food frequency and dietary diversity).

Anthropometric measures include weight to the nearest 0.1 kg (with SECA scale), recumbent length to the nearest 0.1 cm (with wooden UNICEF height boards) and MUAC to the nearest 1 mm (with a UNICEF flexible insertion tape).

Substudy 1: acceptability to children

On the 12 days of substudy 1, data will be collected daily including time of arrival and of last feeding or breast feeding, and morbidity data pertaining to the previous 24 hours. Caregivers will be asked to bring their child to their designated test-feeding site. They will be asked not to feed their child for the preceding hour, if possible. The same food will be given 3 days in a row, to allow averaging of results and reduce the effect of chance findings.

Children will receive the four foods, namely the RUSF snack, RUSF added to *borbor*, CSB++ porridge and Sprinkles added to *borbor*, for 3 days each over 12 days. Children in each group will taste each food in a different sequence (to balance for carryover effects), as in figure 1 below.

A woman from each of the four sites will be hired and trained to prepare an appropriate quantity of the food each day, under the study team's supervision.

The prepared food will be served into small bowls (labelled with the child's code). Clean preweighed napkins will be given to the caregiver to clean the child's mouth and catch spits and spills. Each bowl will contain one of the following:

- 100 g of CSB++,
- 2 pieces of RUSF (approximately 32 g) added to *borbor* to make 100 g,
- Sprinkles (approximately 1 g) added to *borbor* to make 100 g,
- 2 pieces of RUSF (approximately 32 g).

The bowl, spoon (not used for RUSF snack), napkins and food will be weighed on an electronic kitchen scale to the nearest 0.1 g.

The caregivers will be asked to feed their child for 15–30 minutes or until the child refuses to eat any more. The amount of food consumed within 15–30 minutes or until the child stops eating and twice refuses attempts to feed will be recorded in grams and percentage of total.[17] The bowl with remaining food, spoon and napkins will be weighed after the child has finished eating.

Children will not be separated from their caregivers at any point. Children will not be forced to eat the foods. If they become excessively distressed, they will be given the option of taking a break or withdrawing.

After eating the food for 3 days, each caregiver will be asked to assess how he or she thinks the child liked the food, taking into account the amount eaten and the child's reactions and emotional state during feeding. Responses will be recorded by staff on a data collection form, using a five-point hedonic scale (1=disliked a lot, 2=disliked a little, 3=neither liked nor disliked, 4=liked a little and 5=liked a lot). The hedonic scale is a standard tool for measuring food acceptability, that is, how much a consumer likes or dislikes a product.[18]

Substudy 2: acceptability to caregivers

On the 13th day, caregivers will be asked to come to the test-feeding site, alone if possible. Baseline data will be collected from caregivers, including their pregnancy status, and morbidity data pertaining to the previous 3 days.

First, in a sensory test, the foods will be presented to caregivers one at a time. No weighing is necessary, and caregivers will not be expected to eat a whole bowl. Between foods, the caregiver will be asked to rinse his/her mouth out with water. Caregivers will rate them with respect to colour, consistency, smell, taste and their overall opinion. Responses will be recorded by staff on a data collection form, using the five-point hedonic scale (1=very bad, 2=bad, 3=neither bad nor good, 4=good and 5=very good). A score of 3=neither bad nor good will be considered the threshold for acceptance of the food.

Then the foods will be presented at the same time, and caregivers will be asked to rank them. Responses will be recorded by staff on a data collection form (1=best, 2=second best, 3=third best and 4=least good or worst).

Finally, a smaller number of caregivers[8–12] will be asked to stay for a focus group discussion related to infant feeding practices and more detailed reasons for preference ranking. Caregivers will be asked if they would use or buy the novel RUSF and their reasons for doing so, including the perceived benefits and value (monetary) of using such a product. The discussion will be led in the Khmer language by facilitator. A notetaker will be responsible for electronic recording, as well as taking notes, especially about non-verbal communication. The recording will be transcribed and translated into English.

Outcomes and their measurement

The main outcome of interest is how much the children consume. In the absence of clear guidelines on acceptability for supplementary food, we define acceptability as mean consumption of at least 50% (50 g of the porridges or 16 g of the snack) of the food offered in approximately 15–30 min and consumption of 75% (75 g or 24 g, respectively) or more as high acceptability. This is in keeping with similar acceptability studies.[9 17]

The secondary outcome is caregivers' assessment of their child's preference for the food. It is likely that caregivers' assessment of their child's preference is strongly correlated to the child's consumption; thus, this subjective maternal/caregiver assessment is considered an appropriate method of determining acceptability of a food to a child.[19]

A third outcome is caregivers' ranked preference for the food, as preference of the caregiver also determines in large part whether a new food will be used or not.[19 21]

These outcomes indicate how well accepted the food is by children and caregivers and how likely they would be to eat the food or feed it to their children if it were provided in the context of programming for the prevention of malnutrition.

Statistical analysis

All data will be double-entered in Excel and will be analysed in the statistical software STATA V.13.1.

Since repeated measures are being taken, the assumption of independence is not satisfied, and all statistical tests will be for dependent samples. For all tests, significance levels will be considered $p<0.05$.

Consumption: percentage and kilocalories consumed of the serving offered

The main outcome of interest is how much the children consume in terms of percentage and kilocalories. The independent variable is the food, and the dependent variable is consumption. Thus, multiple means of consumption will be compared.

The consumption data will be analysed using a mixed effects model to determine whether there is a statistically significant difference in consumption of the different foods. A mixed effects model has been chosen (in preference to analysis of variance) because it deals well with missing values in repeated measures.[22]

Preference: children

The secondary outcome is caregivers' assessment of their child's preference for the food. The independent variable is the food, and the dependent variable will be the mean of preference ratings on the hedonic scale.[1–5] The preference data will be analysed using a mixed effects model to determine whether there is a statistically significant difference in preferences for the different foods.

Ranking: caregivers

A third outcome is caregivers' ranked preference for the food. The independent variable is the food and the dependent variable will be the mean of the rankings of the foods. The ranking data will be analysed using a mixed effects model to determine whether there is a statistically significant difference in the ranking of the different foods.

Enrolment data

Enrolment data describing the characteristics of the recruited children (eg, sex, age, anthropometric measures, morbidity and breastfeeding status) and caregivers (eg, age, morbidity and breastfeeding status) will be reported as means±SD for continuous measures. Anthropometric indices will be calculated using WHO 2006 standards (ANTHRO V.3.2.2, January 2011) and expressed as z-scores for weight-for-height (WHZ), weight-for-age (WAZ) and height-for-age (HAZ).

Any missing data will be treated as 'missing at random' and accounted for using mixed model and multiple imputation. However, the immediate nature of data collection, on-site presence of a supervisor and follow-up methods should limit protocol non-adherence and missing data.

DISCUSSION

The comparison of new supplementary foods with current fortified blends and existing RUSFs in terms of their potential for preventing malnutrition responds to a need noted by various researchers.[5 6 11 23 24] It also responds to a specific need expressed by the policy makers and implementers in the Cambodian Ministry of Health. Such products need to be affordable, effective and acceptable.[20] This locally produced Cambodian RUSF attempts to respond to those needs.

The comparators chosen, CSB++ and Sprinkles, have been used in Cambodia with limited success. CSB++ proved acceptable in trials but not in practice.[7 13] Sprinkles appeared to be acceptable and did improve the micronutrient status of Cambodian children in one trial. However, there was no improvement in anthropometric measures, and the improved micronutrient status did not persist beyond the 18-month duration of supplementation.[25]

Since there is no evidence that micronutrient powders alone contribute to growth,[26–31] it was decided that the novel food should contain both macronutrients and micronutrients and be energy dense, in order to promote linear growth and weight gain as well as improved micronutrient status.[5 32] Moreover, since peanut-based RUSFs have not proved acceptable in Cambodia,[8 9] and because local production standards may not be adequate to safeguard against aflatoxin contamination,[33–35] peanut-based products will not be used.

The WHO recommends daily consumption of animal source foods for their high protein, energy and micronutrient availability and for their contribution to micronutrient status, linear growth and non-fat mass gain.[36–38] Usually, milk or whey powder is the animal source food used in supplementary foods including CSB ++ and various RUSF/RUTFs.[9 17] However, milk powder is expensive and imported. For this food, it was replaced with fish, which is inexpensive, readily available and more adapted to Cambodian tastes. While there are precedents for replacing milk in supplementary foods for cost-effectiveness,[23] until now, very few have used meat, fish or eggs, and they have generally not been tested for efficacy on a wide scale.[19 20 39–41] Not surprisingly, given the novelty of the foods, the results of the acceptability studies have concluded that although caregivers prefer their traditional food, the children consumed equal amounts of the supplementary food or liked the supplementary food.[19 20] By comparing a supplementary food with fish and one with milk (CSB++) to Sprinkles with *borbor* (a food traditionally given to infants but also consumed by the wider population), our trial will contribute much-needed data on the food preferences of Cambodian caregivers and children. This will potentially open the way for further development of locally produced supplementary foods with an animal source food other than milk.

Finally, since most studies on supplementary foods are from Africa, this trial will be an important contribution to the body of evidence from Asia.[24]

Based on WFP's experience[7] and earlier acceptability studies,[12 42] it is expected that the locally produced Cambodian RUSF will be more acceptable than CSB++ and Sprinkles. If it does prove acceptable, a 6-month efficacy trial will follow.

If the novel RUSF proves efficacious in trial, UNICEF hopes to scale up production, with the aim of producing a local product that is cheaper than imported RUSFs. A variety of distribution methods will be considered, including free distribution to malnourished children (and possibly to pregnant women) as well as commercialisation.

Contributors BB developed the original research design and refined it with FTW, SM, MG, CC and AL. BB wrote the initial draft, and all authors subsequently contributed to and commented on the manuscript and approved the final version.

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Competing interests None declared.

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Data sharing statement Data will be made available after the publication of major outputs upon request to the corresponding author.

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