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# WinFood data from Kenya and Cambodia: Constraints on field procedures

Victor O. Owino, Jutta Skau, Selina Omollo, Silvenus Konyole, John Kinyuru, Benson Estambale, Bethwel Owuor, Roos Nanna, Henrik Friis, and the WinFood Project Team

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## Abstract

**Background.** Researchers face myriad challenges in the design and implementation of randomized, controlled trials. Apart from summaries on limitations, these challenges are rarely documented in detail to inform future research projects.

**Objective.** To describe methodological challenges encountered during randomized, controlled trials (WinFood Study) designed to assess the efficacy of locally produced complementary foods based on traditional animal-source foods (edible termites and spiders) to support growth and nutritional status in Kenyan and Cambodian infants.

**Methods.** In a randomized, controlled design, infants received WinFood or corn–soy blend (CSB) for 9 months from 6 to 15 months of age. Lean mass accrual and blood nutrition indicators (lipid profile, iron and zinc status) were measured cross-sectionally at 9 and 15 months of age, respectively. Lean mass was determined by measuring deuterium oxide enrichment in saliva samples following a standard dose of deuterium solution (0.5 g/kg body weight) to infants. Blood nutrition indicators were determined following the drawing of 3 mL of blood by venipuncture.

**Results.** Challenges included rapid depletion of food rations, high rate of loss to follow-up, delayed ethical approval, lack of local food-processing capacity, low capacity among staff to draw blood, and lack of

laboratory capacity to perform both deuterium oxide and micronutrient status measurements. Spillage of deuterium oxide solution during dosing was a major challenge in the Kenya context. A high rate of morbidity among infants made some assessments very difficult, especially drawing of blood and saliva samples.

**Conclusions.** The challenges were largely contextual. Improvement of local laboratory capacity, training of staff, and sensitization of the communities and the Ethics Review Committee are highly recommended.

**Key words:** Cambodia; field challenges; Kenya; randomized, controlled trial; WinFood

## Introduction

The period of transition from breastmilk to solid food is highly critical for the development of undernutrition in food-insecure populations. Complementary foods must meet the requirements of the growing infant. Numerous studies [1–3] have reported mixed results on the effects of fortified cereal–legume blends on infant growth and micronutrient status. Few studies [4, 5] have assessed the benefits of animal-source foods to infant health in resource-poor settings. Edible insects and small fish species that are traditionally consumed in rural settings may be sources of high-quality protein and fatty acids that may enhance growth and micronutrient status during infancy.

The WinFood Study was supported by the Danish International Development Agency (DANIDA) with a broad aim of assessing food-based innovations for alleviating childhood malnutrition by utilization of traditional foods. The study was carried out in Kenya and Cambodia from 2009 to 2012 in several stages: identification of traditional foods with the potential to be used in formulation of complementary foods; formulation and optimization of complementary foods; palatability and acceptability assessment among mothers and infants; and randomized trials to assess the efficacy

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of developed complementary foods on child growth, micronutrient status, and gross motor development. In the randomized trials, the efficacy of the developed complementary food on stunting, lean mass accrual, lipid profile, hemoglobin, iron and zinc status, and gross motor development was assessed in a 9-month intervention comparing the complementary food with Corn–Soy Blend Plus (CSB+) in Kenya and Corn–Soy Blend Plus-Plus (CSB++) in Cambodia. In Kenya, the efficacy to support growth and nutritional status in Kenyan infants receiving a daily portion of either of two versions of a locally produced complementary food based on maize and germinated amaranth grains with termites (WinFood Classic) or without termites (WinFood Lite) was assessed and compared with the efficacy of a standard food aid product (CSB+).

Randomized, controlled trials are important for generating unbiased data that may influence policy. Researchers face myriad challenges in the design and implementation of randomized, controlled trials. Seldom are these challenges documented in detail to inform future research projects. In cases where challenges are documented, the narrative is restricted to a few lines addressing limitations within the discussion section. Most limitations address technical issues such as inadequacy of sample size and high attrition rates and rarely address any logistical issues. Contextual, logistical, and procurement hurdles have far more ramifications for the implementation and results of randomized, controlled trials, and there is need to document them in detail for future reference.

This paper aims to document field procedures and challenges encountered in the implementation of the WinFood Project, a multicenter study carried out in Cambodia and Kenya from 2009 to 2012.

## Methods

### Study setting

In Kenya, the WinFood Study was based at three health facilities: Makunga, Lushey, and Khaunga in Mumias Subcounty, western Kenya. Growing sugarcane is the main economic activity, with seasonal planting of food crops, mainly maize. In Cambodia, the study covered seven communes in the rural zone of Prey Veng Province.

### Study design

In Kenya, 428 infants were randomly assigned to receive for 9 months a daily portion of either of two versions of locally produced complementary foods based on maize and germinated amaranth grains with termites (WinFood Classic) or without termites (WinFood Lite) or a standard food aid product (CSB+).

## Study participants

Recruitment of study participants was carried out in three rural health facilities in Mumias District: Makunga, Lushey, and Khaunga. Mother–infant pairs were recruited to the study as they came to the health facilities for monthly clinics, growth-monitoring, and immunization. Infants 6 months old with a mid-upper-arm circumference (MUAC) > 11.5 cm, no bilateral pitting edema, no anemia (hemoglobin > 110 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot's spots), and no sign of chronic disease were enrolled in the study. Infants were enrolled regardless of breastfeeding or HIV status. Infants with MUAC < 11.5 cm or bilateral pitting edema, or with anemia (hemoglobin < 110 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot's spots) were referred for treatment and therapeutic nutritional care at the nearby Kakamega Provincial Hospital.

In Cambodia, in a randomized, single-blinded trial, 419 infants 6 months of age without severe wasting (weight-for-length z-score < -3), pitting edema, or signs of vitamin A deficiency or anemia (hemoglobin < 80 g/L) were recruited. They received daily supplementation with one of the four products (WinFood Classic with spider and fish, WinFood Lite, CSB+, or CSB++) for 9 months: 50 g/day from 6 to 8 months of age, 75 g/day from 9 to 11 months of age, and 125 g/day from 12 to 15 months of age.

### Identification, acquisition, and handling of ingredients

The rationale for selection of ingredients for the intervention foods was previously described [6]. Briefly, white winged edible termites (*Macrotermes subhyalanus*), dagaa fish (*Rastrineobola argentea*), and amaranth grains (*Amaranthus hybridus* L.) were selected for their rich protein and micronutrient (iron, zinc, and calcium) contents [7]. Additionally, termites and dagaa are widely consumed in western Kenya. Maize (*Zea mays*) was selected based on the fact that it is the main staple food consumed in Kenya.

Termites were purchased from one female supplier based at the Kakamega Municipal Market (10 km from the study site). This supplier was selected based on the high quality (free of foreign matter, well-dried, and odorless) of her termite supply. The study team collaborated with the supplier to train harvesters on the minimum quality standards required. A total of 1,300 kg of dried termites was purchased over the 9-month intervention period. The initial cost of the termites was KES 1,000 (US\$12.50) per kilogram. The supply accumulated and provided about 300 kg of termites at a given time. The termites were transported by road to Nairobi and held at -80°C at the Department of Food Science and Technology, Jomo Kenyatta University of

Agriculture and Technology prior to processing, as described previously [8]. Dagaa fish was purchased from Kisumu City on the shore of Lake Victoria and transported to Nairobi for processing. Maize and amaranth grains were supplied by farmers contracted by the East Africa Nutraceuticals Company.

In Cambodia, the intervention foods were locally produced at the small enterprise So! Nutritious Co. Ltd. in Phnom Penh by processing to a precooked semi-instant porridge. WinFood Classic contained small indigenous fish species (*Esomus longimanus* and *Paralaupeuca typus*) and edible spiders (*Haplopelma sp.*). Fish species and edible spiders were selected among a range of local foods based on screening for contents of iron and zinc (J Skau et al., unpublished data). Fish and spiders were collected fresh directly from fishermen and traders by the project staff.

### Food production and distribution

Intervention foods were processed centrally based on extrusion cooking, as detailed previously [8]. Food production was done continuously throughout the 9 months of the intervention, subject to the availability of raw materials, especially termites. Briefly, extrusion cooking, blending of micronutrient premix, packaging, and labeling were carried out at the East Africa Nutraceuticals Company using a locally fabricated extruder machine. Once produced, the foods were packed in identical 500-g plastic jars with tight lids. All packages were labeled in a similar way with the common WinFood logo. The label was marked by a unique computer-generated random number code representing the identity of the original food treatment. The assignment of the unique code was done by a food technologist not directly involved in the randomized trial. Well-packed, labeled, and coded foods were packed into 8-kg cardboard boxes that were externally marked with the code corresponding to that on the jars. The food was then transported by road from Nairobi to Mumias (a distance of about 400 km lasting 8 hours). Once in Mumias, the foods were distributed to the three health facilities from which the mothers got their monthly supplies. A buffer stock was maintained at a central store to ensure an uninterrupted supply.

### Allocation of intervention foods

Food allocation and daily dosage was based on the infant's age and the assumption that complementary foods should contribute no more than 50% of total daily energy requirements. Complementary foods should supply 200, 300, and 500 kcal for infants 6 to 8, 9 to 11, and 12 to 23 months of age, respectively [9]. In the current study, infants 6 to 8, 9 to 11, and 12 to 23 months of age were given 50, 75, and 125 g of intervention food daily, respectively. Thus, the mothers received

a supply of three, five, or eight jars of intervention food per month for infants 6 to 8, 9 to 11, and 12 to 23 months of age, respectively. To be resupplied at the end of the month, the mothers were required to bring the empty food jars back to the health center.

### Anthropometric measurements

Measurements of growth and body composition indices were made monthly in triplicate using standardized anthropometric techniques and calibrated equipment [24]. The infant's nude weight was measured to the nearest 100 g with a Salter scale. Recumbent length was measured to the nearest 0.1 cm on a portable measuring board. Triceps, biceps, subscapular, and suprailiac skinfolds were measured to the nearest 0.1 mm with Holtain skinfold calipers. Abdomen, thigh, chest, head, and mid-upper-arm circumferences were measured to the nearest 0.1 cm with a nonstretchable measuring tape.

### Determination of lean mass by the technique of deuterium dose to the infant

Deuterium oxide solution, prepared as a described above, was transported by road (8 hours) in cooler boxes maintained at about  $-20^{\circ}\text{C}$  with ice packs. All infants were given a fixed, standardized dose of deuterium-labeled water, following the guidelines of the International Atomic Energy Agency in Vienna. A pre-dose sample of 2 mL of saliva was taken from the child's mouth by using a passive cotton ball soaking collection method and marked as pre-dose ( $T_0$ ). Then 15 mL of liquid (3 g of deuterium and 12 mL of mineral water) was given to the child orally via a syringe barrel. The infants fasted for at least 15 minutes before the samples were taken. Post-dose saliva samples were taken at 2 and 3 hours. All samples were collected into tightly capped cryogenic tubes and kept in a cooler box with ice packs. On the day of collection, the samples were transported to the Lusheya Health Center where they were stored in a chest freezer at  $-20^{\circ}\text{C}$  pending transfer for analysis. The samples were transferred in dry ice packages to the Kenya Medical Research Institute laboratories in Nairobi, where analysis was done. Deuterium oxide enrichment in saliva samples was determined with a Fourier transform infrared (FTIR) spectrophotometer. In Cambodia, the saliva samples were stored at  $-20^{\circ}\text{C}$  until analysis for deuterium oxide enrichment at St. Johns Research Institute, Bangalore, India.

### Blood sampling for hemoglobin, iron status, and lipid and essential fatty acids profiles

This section describes blood sampling procedures in Kenya. All blood drawing procedures were performed by health workers based at the three study health

facilities who were routinely carrying out this role. These health workers were specifically provided with a refresher training on blood-drawing techniques in the context of the study. A venous blood sample was collected at baseline and at the 9-month follow-up visit using a field-friendly closed vacutainer system in trace-element-free tubes. The blood samples were aliquoted into equal portions, one of which was separated into serum for determination of transferrin receptor cells and ferritin; the other portion was kept whole for determination of lipid and essential fatty acid profiles. Both the serum and the whole blood portions were held at  $-20^{\circ}\text{C}$  in the field prior to transfer to Nairobi for storage at  $-80^{\circ}\text{C}$ . Hemoglobin was determined with a HemoCue machine at baseline and the 9 months follow-up visit with a control cuvet measured daily to ensure correct calibration of the HemoCue. The blood for the HemoCue test was drawn from the blood that was left in the wing-needle tube after the blood tube had been filled.

### Ethical approval

In Kenya, the study was approved by the Kenyatta National Hospital/University of Nairobi Ethics Review Committee. Written and oral information in the local language was given to the parents or guardians of all eligible children before obtaining written consent. Deuterium oxide is a naturally occurring and nonradioactive isotope of water, and there are no health concerns about its use in any age group. The foods were assessed for microbiological contamination at the Department of Food Science and Post Harvest Technology, Jomo Kenyatta University of Agriculture and Technology, and the Kenya Bureau of Standards food laboratories.

In Cambodia, all caregivers of participating infants voluntarily signed the consent form for participation and were informed that they could leave the study whenever they wanted to. The protocol was approved by the National Ethics Committee for Health Research, Ministry of Health, the Royal Government of Cambodia and consultative approval was obtained from the Danish National Committee on Biomedical Research Ethics.

### Trial registration

The trials in both Cambodia (ISRCTN19918531) and Kenya (ISRCTN30012997) were registered with [Controlled-trials.com](http://Controlled-trials.com).

## Results

This section describes the challenges and lessons learned in the implementation of the WinFood Study under the following thematic areas: planning and

logistics, implementation of the study in the field, technical procedures, and ethical issues.

### Planning and logistics stage: Approval of funds, ethical approval process

There was delayed commencement of the trial due to prolonged procedures for memoranda of understanding between collaborating institutions in Denmark, Kenya, and Cambodia. One critical issue in the Kenyan case was whether the intellectual property rights (IPR) for the foods developed belonged to the University of Copenhagen or the University of Nairobi. Upon negotiation it was agreed on equally shared IPR. Approval of funds was delayed because of stringent procurement procedures that university grant offices have to comply with. In some cases, the procurement offices purchased materials that did not suit study specifications, with the result that a fresh procurement process had to be initiated.

Maintaining a steady stock of high-quality termites was a major challenge in the Kenyan scenario, since termite abundance varies seasonally according to local rain patterns. Furthermore, termites are still gathered by traditional methods, mainly by women and children. This had a considerable impact on steady production of study foods and maintenance of buffer stock. Additionally, the highly perishable nature of termites required rapid transportation, which was limited by the need to accumulate a minimum amount before shipment. Given the bulkiness of termites and small fish, large freezing capacity was needed, with attendant constraints on the limited study budget.

Micronutrient premixes used for food fortification were imported, from South Africa in the Kenyan case with a minimum lead time of 2 months. The high import duty imposed on micronutrient premixes was another handicap.

Identification of a small-scale food processor who could produce the required amounts and quality of foods for the trial and was willing to try unique ingredients took time. Some ingredients were associated with varied organoleptic and processing modifications. In the Kenyan case, for example, the inclusion of termites in the recipe at more than 10% w/w resulted in clogging of the extruder barrel due to the high oil content. In Kenya, the fishy flavor associated with the addition of small fish reduced acceptance, whereas in Cambodia it enhanced acceptance. Researchers seldom factor in potential delays due to logistics during the planning of trials.

### Implementation of the study in the field

Since the trials in both countries were based on individual randomization, it was difficult to guarantee blinding once the food containers had been opened

by the mothers. It is possible that mothers living in close proximity were randomized to receive different food treatments. Community members participating in trials are known to share information on the nature of the treatment they are assigned. In the Kenyan case, some mothers approached the study team to change the porridge flour they were receiving to match what their neighbors were receiving. Although this was not confirmed in the current studies, it is highly possible that mothers may try out each other's treatment products. Such issues may be minimized by cluster randomization, but this approach requires very large sample sizes that may take a long time to achieve. In one case in Kenya, one mother resorted to selling the food allocation; this was detected during routine assessment of compliance by the study team.

In Kenya, the food ration was depleted by the infants long before the subsequent visit. A possible explanation for this is sharing due to frequent food insecurity. The high rate of loss to follow-up was attributed to relocations for family reunion and household conflicts. Additionally, regular attendance of the participants was limited by the long distances to the health centers. Further, unfavorable weather conditions, such as floods in the case of Cambodia, led to huge losses to follow-up.

Community misconceptions about the trials were also experienced, especially in Kenya, where intervention was associated with family planning by a section of the community. Regarding deuterium oxide, in Kenya we observed that both the Ethics Review Committee and the mothers misunderstood the concept of "special water," and it took a long time to explain it. In Cambodia, mothers were worried about the long waiting time for saliva sampling.

#### **Technical procedures: Phlebotomy challenges, difficulties with administration of deuterium oxide dose, variations in anthropometric measurements, different challenges in the age groups**

Blood assay challenges included high rates of hemolysis due to restless children and inexperienced staff, since the procedures are not routine. Nervous mothers made the situation worse. Lack of local facilities to measure iron, zinc status, and lipid profiles increased costs and delayed results.

Difficulty in maintaining the cold chain in transport and storage of deuterium and saliva samples was a challenge, due to high ambient temperatures and frequent electricity supply interruptions.

Deuterium spillage during dosing occurred mainly in the Kenyan context due to stubborn children. Older infants (15 months) spat out the deuterium solution during dosing. Deuterium spillage is one of the causes of error in body composition assessment. Saliva samples for measurement of deuterium enrichment should be clearly labeled so that they can be easily traced.

Variables to include on the label are subject identification number, date and time of dosing, and date and time of obtaining the saliva sample. Labeling of saliva and blood samples was observed as one of the most critical activities that needs attention to avoid mixing up samples. The study depended on external support for measurement of deuterium enrichment due to lack of its own FTIR machines. In the case of Kenya, samples had to be analyzed by a third party at the Kenya Medical Research Institute. In the Cambodian case, the samples had to be exported all the way to India. In both cases, it was difficult for the primary study team to ascertain that the analytical procedures were robust. This highlights the necessity for establishment of local centers where machines can be installed and local technical capacity can be developed to support similar studies. The optimal amount of saliva required for FTIR assay is about 2 ml. High morbidity among children limited saliva production, while mouth sores made sampling of saliva difficult (saliva samples were contaminated with mucus, blood, and breastmilk).

Blood assay challenges included high rates of hemolysis due to restless children and inexperienced staff, since procedures were not routinely performed prior to the study. Sometimes the amount of blood drawn was too little to enable separation. Repeat blood drawings had to be done, with the result that most mothers were scared and this could have led to failure to return for subsequent visits. To address the problem, refresher training conducted by an invited phlebotomist was organized for all study staff involved in blood drawing. Since none of the study countries had robust capacity to analyze blood micronutrient composition, lipids, and essential fatty acids, the samples had to be shipped to Canada and Germany for assay. This resulted in high costs and delay in data analysis.

## **Discussion**

This paper aimed to describe logistical and technical challenges encountered in the implementation of the WinFood Study in Kenya and Cambodia. The overall aim of the WinFood Study was to design high-quality complementary foods using traditional foodstuffs, especially rare animal-source foods that are already consumed in the target populations and to test the efficacy of the developed complementary foods on infant growth and health. Since there is a dearth of studies reporting field problems in detail, this paper may be one such source of information to inform future randomized trials targeting improvement of nutritional status of infants by food-based interventions.

The problems encountered were context-specific except for a few that were cross-cutting in both study countries. In Kenya, the food ration was depleted long before the subsequent visit due to food insecurity. Some

community members perceived that the intervention was associated with family planning. The high rate of loss to follow-up was attributed to relocations for family reunion and household conflicts. The Cambodian Ethics Review Committee had issues with the locally produced WinFood product, and hence ethical approval was delayed. Regarding deuterium oxide in Kenya, we observed that both the Ethics Review Committee and the mothers misunderstood the concept of “special water,” and it took a long time to explain it. In Cambodia, mothers were worried about the long waiting time for saliva sampling. Spillage of deuterium oxide during dosing was a major problem in Kenya due to agitated children, mainly those 15 months of age, who spat out the deuterium oxide.

## Conclusions

The challenges were largely contextual. Misconceptions about intervention and procedures, infant morbidity, and restlessness affected assessment of deuterium

oxide. Inexperienced staff and lack of local capacity for analysis of blood and deuterium oxide samples affected timelines and increased costs. Improvement of local laboratory capacity, training of staff, and sensitization of communities and the Ethics Review Committee are highly recommended.

## Conflicts of interest and other disclosures

To the best of our knowledge this is original work undertaken by the WinFood Study Group and has not been submitted elsewhere for publication. The authors have no conflicts of interest to declare.

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